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Aquaculture Science and Engineering

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Editors

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Preface

Aquaculture has the potential to contribute significantly to universal food sanctuary, but new venture is required to light expected request. In general, aquaculture's small-scale and organic growing has made it tough to normalize, contributing to an extreme level of risk for new investors. Aquaculture's major risk factors are primarily determined by water management, production intensity, and the proximity of fish farms to one another. Aquaculture now has a place of importance in fisheries administrations and private sector industries, despite decades of hesitation or outright skepticism. Though aquaculture has been a way of life for farmers in many Asian countries for centuries, its status in terms of global food production, aquatic resource management, and rural socioeconomic development has remained a point of contention until recently. With changes in global fisheries and the spectacular success of certain types of aquaculture ventures, the scenario has changed dramatically.

Aquaculture is a vital and rapidly expanding segment of global agriculture. Soon, new technological advancements and increased demand for fish as an animal protein source are expected to accelerate the industry's growth. As the industry grows, the cultivation methods have become more intensive to produce higher yields per unit area. In intensive culture operations, infectious disease is the leading cause of financial loss. Current disease treatment options in both developing and developed countries are partial to a small amount of government-approved antibiotics or chemotherapeutics that are only marginally effective. High cost, creation of antibiotic-resistant pathogens, required withdrawal period, and ecological contamination are some of the issues that arise from the use of the drugs, whether through medicated diets or water treatments. Aqua culturists are interested in developing cost-effective preventative measures to prevent outbreaks or reduce the severity of epizootics for these reasons. One such preventive measure is the development of various nutritional strategies that may reduce or eliminate diseases, which the aquaculture industry is currently investigating.

Over the past era, scientists have recognized the importance of nutrition in maintaining the health of humans and other animal species, including fish. Humans and other terrestrial animals were the focus of previous research on the links between nutrition, immune response, and disease resistance. However, attempts to conduct similar studies using fish have met with limited success in the last two decades due to a lack of understanding of the immune response in fish. Evidence from unintentional or intentional infection of fish in some of these nutritional studies suggests that most, if not all, dietary nutrients have an impact on immune role and disease resistance. A nutrient deficiency or excess could have a significant impact on fish infection and survival, owing to its effects on host defense mechanisms.

Other factors that affect fish health include nutrient bioavailability and interactions, the occurrence of immunostimulants and toxins, and feeding organization. This clearly demonstrates the potential role of nutrition in improving fish immune response and disease resistance. The book contains the chapters on nutrition, feed and feed additives, ecology, immunology, microbiology, toxicology, biochemistry, nanotechnology, pharmacology, and biotechnology, among other fields of basic and applied research. The chapters will serve as introductions to these fields and up-to-date reviews of recent research advances. This book is intended for a wide range of readers, including nutritionists, disease specialists, feed formulators, students, extension specialists, and farmers, as well as university teachers, graduates, and doctoral students in zoology, physiology, aquaculture, and biology in general.

Our sincere gratitude goes to the contributors for their insights on applications of various feed additives in aqua sector.

We sincerely thank Dr William Achauer, Director, Springer; Dr Anil Chandy, Managing Director, Springer; Ms. Emmy Lee, Associate Editor, Biomedicine & Life Science Books, for their generous assistance, constant support, and patience in finalizing this book.

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Chapter 1
Ramifications of Climate Change Induced by Anthropogenic Factors on Global Fish Population Scenario

**Suma Sarojini, Mridul Umesh, Nayana Jagannath,
Bhadrapriya Sivakumar, Anand Kaloor, and Saranya Jayaram**

Abstract One of the important consequences of climate change is its effect on the global fish population. Though not very highly pronounced each year, the effect of climate change is of cumulative nature. Global aquaculture is being affected by temperature changes of both water and air. Fluctuations in the ocean surface temperatures, ocean current patterns, wind speeds, and wave directions, all have its impact on aquaculture. Each year we see more and more incidences of extreme weather conditions in different parts of the world, be it in the form of hurricanes, heavy floods, etc. Fishes are subjected to various stress factors which in turn take a toll on its growth and development. This can lead to lower weight gain and increased mortality due to higher susceptibility to diseases. This, coupled with direct unsustainable anthropogenic activities in the oceans and rivers may lead to collapse of the marine and freshwater ecosystem. Recent studies have identified specific regions where marine aquaculture production will be positively and negatively affected. One of the sustainable ways of developing aquaculture in the coming decades would be by developing region-wise strategies to maintain or increase fish population levels and thus meet the global seafood demands even in 2050. The current review is an attempt to assess the effects of ocean warming, ocean acidification, and ocean deoxygenation on the growth, survival, and diversity of marine lifeforms and suggest ways to stop a complete collapse of marine fish population by 2050, the year for which the complete collapse is predicted based on projections.

Keywords Aquaculture · Climate change · Global warming · Ocean acidification · Fish population

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1.1 Introduction

Climate change is a phenomenon which has been consistently going on ever since the formation of planet Earth, but what has been alarming is the tremendous speed at which it is happening over the past few decades. The indications of this rapid climate change are manifested in the form of higher temperatures all across the planet—both on land and water—changing precipitation patterns, more droughts, severe cyclones, melting glaciers, shrinking sea ice, and thawing permafrost. Like many other activities, aquaculture is also facing the brunt of this unprecedented climate change. The demands of an exploding human population coupled with the dangers of unscientific fishing practices and ramifications of global climate change threaten the global fish population. Only if the countries world over adopt sustainable practices, both in the marine environment and in inland aquaculture, can we expect a steady source of this protein rich food source. The current review is an attempt to portray the causes, effects, and remedial measures of various phenomena that are negatively impacting global fisheries and aquaculture.

1.2 Ocean Warming

Ocean warming, which is a result of global warming, can be qualified as an anthropogenic factor. Oceans help a lot in making the Earth a livable planet by maintaining its temperature. Heat penetration up to 700 m could be observed in the water column, affecting horizontal thermal regimes and those in deeper parts of the ocean. The oceans have absorbed almost 90% of the increased energy, which has led to a rise in temperature of 0.11 °C every ten years between 1971 and 2010 (Reid et al. 2019).

1.2.1 Causes of Ocean Warming

One of the main causes of ocean warming is the greenhouse effect. There are several gases present in the atmosphere that prevent the Earth from freezing, thus making it habitable. These gases collectively referred to as "greenhouse gases" which include carbon dioxide, methane, nitrous oxide, etc. The difference in the Earth's ground surface temperature with and without greenhouse gases is 32 °C. So greenhouse gases are important for the existence of life on this planet. But currently, due to the burning of fossil fuels like coal, oil, and natural gas to satisfy the industrial needs of society, large quantities of greenhouse gases are released into the atmosphere. These gases affect the radiation balance of the Earth and increase the surface temperature, which causes ocean warming (Berger and Shor 2021).

El Nino is an important cause of ocean warming. This event is characterized by widespread warming of eastern and central tropical regions of the Pacific Ocean. This causes the ocean sea surface temperature (SST) to rise by several degrees (Fasullo et al. 2018). This also leads to the formation of algal blooms on the surface of the ocean, which leads to reduced dissolved oxygen levels, increased heat, hypoxia in fishes, and prompt stratification (Reid et al. 2019).

A rise in global ocean temperature can lead to major problems in aquaculture. The first one being glacier melting which leads to the second factor, *i.e.,* increasing sea level. This puts the coastal fishing communities on the front line of climate change-related disasters. Rising ocean temperature impacts the marine ecosystem and productivity, which affects the sustainability of fishes and aquaculture. In Vietnam, Mekong River delta fishermen used to produce 1000 tons of basa fish every year. However, due to rising sea levels, there is a saltwater intrusion which has reduced fish production. Rising ocean temperature can lead to both direct and indirect effects on aquaculture. Direct effects include altered growth rate and behavior, mortality, reproductive capacity, and distribution of the fishes. Indirect effects cause a change in productivity and the marine ecosystem.

The credit for being the largest active carbon pool on the planet goes to oceans. However, due to a distempered plastic-waste management system and large-scale consumption, there is more and more release of plastics into the environment. These ultimately end up getting dumped in the oceans. The effect of marine microplastics on carbon sequestration leads to enhanced release of greenhouse gases, which in turn leads to ocean warming. Several studies point out the damages posed by the marine microplastics. They can affect the growth and photosynthesis of phytoplanktons, exhibit developmental toxicity towards zooplanktons, affect stock of ocean carbon, and badly affect marine biological pumps (Shen et al. 2020).

1.2.2 Effects of Ocean Warming

Poleward displacement of the biogeographical ranges of marine life has turned out to be one of the major consequences of ocean warming. Marine life was initially seen to move either towards the North pole or the South pole, where the water was cooler. However, recently, to adapt to the increasing ocean temperatures, fishes have developed a strategy that involves vertical movement to cooler waters. The vertical movement to follow surface isotherms which leads to redistribution of organisms is three to four times lesser than the horizontal distribution. The reasons attributed for this may be the depth of seafloor and the photic layer being limiting factors of vertical migration of fishes. Limited light availability, seafloor boundaries in shallow coastal areas, restrictions on habitat and resources, oxygen minimum zones, etc. may be other reasons. However, vertical migration is only a short-term adaptation when compared to horizontal migration towards the poles. Studies prove that if ocean warming continues at this rate, by the end of the present century we can expect to reach the limits of seafloor depth and photic layer. This in turn can lead to rapid global compression of marine organisms. At this rate of vertical isotherm migration, it is projected that 50% of the biogenic diversity will be lost in the next 80–90 years (Gabriel et al. 2020).

Fluctuations in ocean temperatures indeed have a prominent effect on fish reproduction. As the temperature increases, reproductive development is stimulated in the species spawning during the spring season. As the temperature falls, reproduction is stimulated in species spawning in the Autumn season. Elevated temperatures can, however, delay the maturation and ovulation in the autumn spawning species. Temperature controls all the reproductive stages of fishes, ranging from gamete development and maturation, ovulation, spermiation, spawning, hatching, juvenile development of larvae, and its survival. Higher temperatures can affect the hypothalamo–pituitary–gonadal or HPG axis and cause a change in hormone structure, synthesis, and action (Pankhurst and Munday 2011). In *Salmo salar* (Atlantic salmon), elevated temperature inhibits P_{450} aromatase enzyme activity which is responsible for converting steroids. It also inhibits the ovarian synthesis of estradiol. This reduces the hepatic synthesis of the egg yolk precursor vitellogenin (Vtg), which results in reduced egg size, survival, and fertility (Pankhurst and King 2010).

Changes in temperature can affect the oxygen consumption of fishes. In a study, one-year-old goldfishes were grown in running water and were fed with commercial feed. They were pre-acclimatized to a particular temperature at which the experiment was to be conducted and the metabolic rates were measured by oxygen consumption. It was found that the metabolism of the fishes increased with increasing temperature until the incipient lethal temperature, which was found to be 30 °C. A further increase in temperature showed a decrease in oxygen consumption and metabolic rates. This could probably be due to denaturing of enzymes at a higher temperature (Fry and Hart 1948).

One of the important deciding factors of the rate of metabolism and growth in poikilotherms is temperature. A study with abalones was done to determine the effect of temperature on growth. They were grown in tanks and were fed with dry pellets at a rate of 4% body weight per day. These abalones were grown in different temperatures. Significant rate of weight gain was observed till a temperature of 20 °C. Above 20 °C, weight gain was seen to reduce sharply. It was also seen that protein efficiency and feed conversion rates deteriorated rapidly, and mortality rates were increased. Abalones grown at 24 °C barely gained any weight in over three months of trials. A gradual breakdown in all the physiological processes was seen (Britz et al. 1997).

Data from 273 studies showed that ocean warming led to infection of the fishes by several bacterial pathogens like *Aeromonas* spp.*,* *Flavobacterium* spp.*,* *Edwardsiella* spp.*,* *Lactococcus* spp.*,* *Streptococcus* spp.*,* *Yersinia* spp.*,* and *Vibrio* spp. Several mathematical models predicted that as the temperature increased by 1 °C in warm and temperate waters, an increase in mortality of organisms was observed at the rate of 2.82–4.12% and 3.87–6%, respectively (Reverter et al. 2020).

An increase in the water temperature was found to increase the chances of disease proliferation in fishes. Ichthyophoniasis is one of the most economically significant diseases due to the distribution, magnitude, frequency, and effects of the recurring

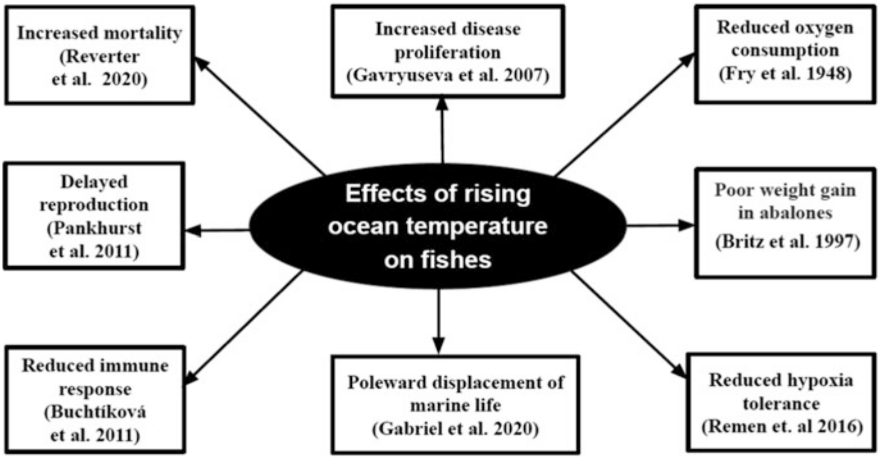

Fig. 1.1 Effects of rising ocean temperatures on fishes

epizootics. It was found to affect several species of fishes like European plaice (*Pleuronectes platessa*), yellowtail flounder (*Limanda ferruginea*), mackerel (*Scomber scrombrus*), and alewife (*Alosa pseudoharengus*) (Burge et al. 2014). These infected fishes had disturbing and foul-smelling lesions in their skeletal muscles, which rendered the fillets unmarketable. Ichthyophoniasis incidences have been shown to rise with rising water temperatures (Gavryuseva 2007).

Another effect of the increase in water temperature was the reduction of dissolved oxygen. In a study, Rainbow trouts (*Oncorhynchus mykiss*) were grown in two lakes in South California. The temperatures on the surface and deeper points of both the lakes were recorded to find the vertical and horizontal temperature gradient. The dissolved oxygen in various locations of the lake was also found out. It was found that when the temperature increased during the summers, the trouts migrated to the deeper pits of the lake, where the temperature was lower, where the dissolved oxygen was also found to be lower. The fish numbers were found to be inversely proportional to the temperature of the water and dissolved oxygen. Trouts can survive at a DO level of just above 3 mg/l. However, the DO at the lower temperature zones was found to be in the range of 1.7 mg/l–3.4 mg/l. Fishes generally avoid DO levels lesser than 5 mg/l. But when faced with a choice between lower temperature and higher dissolved oxygen, the fishes always choose lower temperatures. This induced low oxygen stress in the fishes induced low feeding rates, which affected their growth (Matthews and Berg 1997). Figure 1.1 depicts the results of various studies on effects of rising ocean temperature on fishes.

All the above effects on fishes have a profound effect on aquaculture and its economy. There is a change in harvest, as the fisherman might catch fewer fishes of one species and more fishes of another species, thus im-balancing the marine ecosystem. This leads to changes in fish production and harvest and its related employment opportunities. Due to this, the income and profits of aquaculture globally will face a severe crisis. There can also be an increase in prices and demands of certain fishes, which are rare in the markets. The fishermen community will definitely face an economic crisis and this may lead to family and community stress. There can also be changes in local and state-wide tax revenues. Aquaculture enhancement taxes, fisheries business taxes, and marketing assessments are directly linked to the value of harvests. Changes in harvests can also upset political balance in agreements over transregional or transnational fisheries (Yazdi and Shakouri 2010).

1.2.3 Remedial Measures to Control Ocean Warming

Global warming is the main cause of ocean warming since the oceans absorb almost 90% of the heat and it can penetrate 700 m into the oceans, as stated above. Therefore, the only method to control ocean warming is to have a check on global warming. During the last century, our planets mean temperature has increased by 10 °F. By the end of the present century, the average annual temperature is expected to rise globally about 4–9 °F. The mean sea level is also expected to increase between 20 and 25 cm (Guile and Pandya 2018).

There are two major ways to prevent global warming. The first option is to reduce the production of greenhouse gases. Instead of using fuels that produce the greenhouse effect, alternative sources of energy like nuclear energy, wind power, solar energy, and hydrogen fuel cells can be considered. The second option is to increase carbon sequestration. By this way, large amounts of carbon dioxide generated can be captured and stored for a long time to curb global warming rates and avoid climate change (Balajhi et al. 2017).

Several countries all over the globe have realized the threats of ocean warming and the implications it would have on the global economy. Therefore, many proposals have been made to reduce carbon emissions. These proposals vary from country to country based on the risk of warming and the benefit of mitigation efforts to the economy. In 2011, Australia had come up with a carbon trading plan, which imposes 500 worst polluters to pay a tax on every ton of carbon they emit. Since 2010, India has also started imposing carbon tax on coal. They have also set up an ambitious goal to achieve 20,000 megawatts of solar power by the year 2022. The Brazilian government has introduced a National climate change plan which gives thrust on expanding the use of biofuels and electric energy sources for the transportation industry. They are also focussing on reducing deforestation rates NPR Cookie Consent and Choices (2021).

1.3 Ocean Acidification

The natural interconnection between the atmosphere and oceans plays a vital role in global weathering. One-fourth of the one million tonnes of carbon dioxide emitted hourly into the earth's atmosphere is taken up by the oceans. This gas then reacts with the seawater to form a weak acid resulting in the decrease in the ocean's pH. This is one of the major causative factors for ocean warming. It is a global phenomenon due to the absorption of anthropogenic CO_2 emissions. When compared to the seawater acidity during the beginning of the industrial revolution, the waters are now 30% more acidic. Acidification of oceans clubbed with other climate changes such as global warming, melting icebergs, ocean deoxygenation, and coastal erosion form grave threats to the arena of aquaculture (Doney et al. 2009). These have led to reduction in ocean pH and saturation state of calcium carbonate (Ω) (Zeebe and Wolf-Gladrow 2001). Oceans are reservoirs of gases and play crucial roles in global weather patterns. Changes in ocean carbonate chemistry are hence brought about by changes in the concentrations of ratios of various inorganic carbon species (CO_2, HCO_3, $CO_3{}^{2-}$) and saturation states of calcium carbonate ($CaCO_3$), i.e. Ω (Gao et al. 2019). The value of Ω mainly depends upon the concentrations of $CO_3{}^{2-}$ since concentrations of Ca^{2+} are relatively stable (~10 mM) in seawater. Increasing concentrations of CO_2 in seawater results in corresponding increase in concentrations of dissolved CO_2, HCO^{3-}, and H^+ with a proportional decrease both in concentrations of CO^{3-} and saturation states of $CaCO_3$. Depending upon the temperature and salinity, the concentration of carbonate ions varies geographically; e.g., polar waters have a concentration of CO^{3-} of about 41% of that of tropical waters and this decreases at a greater rate than that in tropical waters under the influence of ocean acidification (Orr et al. 2005). Hence, increased CO_2 absorption by oceans and its subsequent effects on the concentrations of carbonate ions and on saturation states of calcium carbonate contribute to ocean acidification.

1.3.1 Causes of Ocean Acidification

The profound impacts of ocean acidification affect the ocean ecosystems, their biodiversity and global climates as well. These problems are inherently complex due to natural variabilities and are further worsened by difficulties like global warming, environmental changes, eutrophication, pollution, anthropogenic causes, and so on. After the onset of industrial revolution, the atmospheric levels of CO_2 have risen leading to corresponding increase in the absorption of CO_2 by oceans. Population explosion coupled with industrial revolution has thus increased ocean acidity to great proportions. Temperature has been found to be the main culprit in reducing (Arnberg et al. 2013; Hiebenthal et al. 2013; Karlberg and Wulff 2013; Dorey et al. 2013) or aggravating ocean acidification (Schalkhausser et al. 2013). Ocean warming has been found to trigger the stratification and shoaling of Upper

Mixed Layer (UML). This in turn will negatively affect the dissolution of nutrients across the depths of oceans, thereby causing the organisms to be subjected to increased levels of PAR and UV radiation exposures (Gao et al. 2012; Hutchins et al. 2017). Ocean stratification exacerbates shoaling of the UML leading to stratification that hinders upward transport of nutrients from deeper layers and reduced availability of nutrients for phytoplanktons (Orr et al. 2005). Phytoplanktons remain mostly in the upper layers of the oceans and will not be generally seen in the deeper layers. Hence these organisms living in the shallow UML get more exposed to increased amount of UV radiations (Jin et al. 2013).

Increasing temperatures decrease oxygen solubility in seawater, contributing to deoxygenation and expansion of anoxic zones in oceans. Variations in pH of oceans are influenced hugely by biotic parameters like photosynthesis and respiration that are in turn affected by fluctuating biotic and abiotic factors. These lead to highly variable pH ranges across different spatiotemporal scales (Schulz and Riebesell 2013). More specifically, release of respiratory CO_2 accompanied by the absence of photosynthetic CO_2 removal during night period contributes towards pronounced effects of ocean acidification on the dominant photoautotrophs like microalgae and cyanobacteria (Jin et al. 2013). Deoxygenation brought about by ocean acidification has deleterious effects on the species survival, biogeochemistry, and ecosystem functions in seawaters. Since oxygen is vital for the organisms to perform essential biological functions, deoxygenation leads to conditions of stressed respiration and hypoxia in organisms. More than 5% increase in the annual rate of hypoxia has been observed in coastal ecosystems primarily due to eutrophication and low oxygen levels (Vaquer-Sunyer and Duarte 2008). This could be one of the reasons for heightened levels of ocean acidification in the shallow coastal waters than the deeper waters (Cai et al. 2011; Breitburg et al. 2018). Since eutrophication enhances algal bloom, the association between eutrophication and deoxygenation is brought about by microbial breakdown of collapsed algal blooms, which in turn leads to heightened loads of organic carbon. This subsequently contributes to anoxia, promotes denitrification, and affects the ocean biogeochemical cycles.

1.3.2 Effects of Ocean Acidification on Ecology, Species Biodiversity, and Ecosystems

Research has shown that ocean acidification impacts the calcification, growth, and reproduction of calcifying oceanic species. Research data have depicted the effects of ocean acidification on the reduction of calcification of many species like coralline algae. Negative impact on the biogenic silica content in diatoms has also been observed (Milligan et al. 2004; Tatters et al. 2012; Xu et al. 2014). Adverse biological impacts of ocean acidification were proven by researches demonstrating coccolithophores becoming malformed (Riebesell et al. 2000), corals losing their ability to build skeletons (Fine and Tchernov 2007) and various threats to the sustenance of reefs and their ecosystems like the probable extinction of congregations of brittle stars that provide habitat and food for fishes in Atlantic floor, caused to the deaths of their larvae as a result of ocean acidification (Dupont et al. 2008). Recent research has also demonstrated that the upwelling of ocean waters can have many consequences. In the US west coast this has led to increased mortality rates among wild oyster populations (Barton et al. 2012). The negative effects of ocean acidification have also been seen among the macrofauna showing a 30% decline in animal biodiversity caused due to decline in pH from 8.1 to 7.8 at CO_2 seeps (Agostini et al. 2018; Fabricius et al. 2011; Hall-Spencer et al. 2008). Intolerance to hypercapnia among the reef-forming organisms has led to their degradation, chemical dissolution, and enhanced bioerosion (Fabricius et al. 2011; Enochs et al. 2015; Milazzo et al. 2014). These have exacerbated the reduction of species richness due to ocean acidification especially in biogenic reefs. The combinatorial effects of ocean acidification along with global warming have been demonstrated through various studies assessing their impacts on species physiology and biodiversity. While ocean acidification along with global warming favor the growth of picoplanktonic cyanobacterium *Synechococcus*, they have no effects on *Prochlorococcus* (Fu et al. 2007). Different experiments have shown the effects of ocean acidification and global warming on decreasing the growth rate and calcification of the coccolithophores *Coccolithus* (Schlüter et al. 2014), along with reducing the optimum growth temperature and maximum growth rate of *E. huxleyi* (Listmann et al. 2016). While ocean acidification has adverse effects on most algal communities and ecosystem composition, few benthic algal communities are tolerant to changes in ocean carbonate chemistry and benefit from this phenomenon (Hofmann et al. 2012; Hall-Spencer and Harvey 2019).

In addition to degrading biodiversity in oceans, the negative impacts of ocean acidification have also contributed towards unbalancing the species distribution and richness. CO_2 seep studies have shown that there exists greater dominance of non-calcified species, turf algae, soft corals and anemones over calcified species, coralline algae (Agostini et al. 2018) and hard corals (Inoue et al. 2013; Suggett et al. 2012). They also favor growth and survival of opportunistic organisms that can easily adapt to rapidly changing environmental conditions, thus altering the competitive interactions between species (Brown et al. 2018; Kroeker et al. 2011). For example, few coral species in the tropics grow well in acidified conditions but their habitats, comprising fewer invertebrates, fleshy macroalgae, and less calcified algae (Enochs et al. 2015; Smith et al. 2016), lack complexity and are easily prone to dissolution and bioerosion (Fabricius et al. 2011). Regions of sub-tropics also depict similar effects of ocean acidification, *i.e.,* reduction in abundance of calcified species and imbalance in species biodiversity. The reduction in the population of crustose coralline algae, increased proliferation of turf algae, diminished body size, and lower numbers of calcified animals like sea urchins—all can be the consequences of increased rates of ocean acidification in these regions (Agostini et al. 2018; Harvey et al. 2018).

Coastal ecosystem services are dependent on the retention and abundance of their keystone species (Sunday et al. 2017), maintenance of basic biotic functions

Table 1.1 Effects of increasing ocean acidification on marine life

Organism	Effect of ocean acidification	Reference
Diatoms	Reduced silica content	Xu et al. (2014)
Coccolithophores	Malformation	Riebesell et al. (2000)
Reef-forming organisms	Intolerance to hypercapnia	Enochs et al. (2015)
Sea urchins	Reduction in size and abundance	Agostini et al. (2018)
Hard corals	Reduction in species distribution and richness	Inoue et al. (2013)
Corals	Inefficiency in building skeletons	Fine and Tchernov (2007)
Wild oysters	Higher mortality rates	Barton et al. (2012)
Corals	Harbor less calcified algae	Smith et al. (2016)
Brittle stars	Extinction of congregations	Dupont et al. (2008)
Coralline algae	Reduction in species distribution and richness	Agostini et al. (2018)
Coral reef-forming species	Bioerosion	Fabricius et al. (2011)
Coccolithophores (*Coccolithus*)	Decreased rates of growth and calcification	Schlüter et al. (2014)
Coccolithophores (*E. huxleyi*)	Reduction in optimum growth temperature and maximum growth rate	Listmann et al. (2016)

(Nagelkerken and Connell 2015), and prevention of rampant spread of unwanted species (Hall-Spencer and Allen 2015). An overview of the ill effects of ocean acidification on marine life forms are depicted in Table 1.1.

Thus, regardless of the biogeographic regions, ocean services are disrupted when their habitat-forming organisms and their ecosystems deteriorate. The increased sensitivity of shallow biogenic reefs to ocean acidification has been shown by different worldwide studies on CO_2 seeps showing their degradation, decreased coastal protection, and reduced habitat provisioning for biodiversity and fisheries. These symbolize the amplified negative effects of increased carbon emissions on ecosystem services. Synergistic effects of ocean acidification and global warming also adversely impact the population of sensitive species, biological processes, and biodiversity. Economic impacts of these stressors affecting coastal biodiversity are evident from the 69% decline in provision for nursery functions and 33% decline in viable fisheries (Worm et al. 2006). The combined effects of ocean acidification along with global warming are exacerbated by anthropogenic stressors. These are particularly detrimental to industries and services reliant on marine ecosystems; as such the ecosystem goods and services worth ~375 billion USD are provided annually by coral reefs worldwide (Gattuso et al. 2014).

1.3.3 Regulation of Ocean Acidification

Ocean acidification is a global problem that is compounded by other stressors like natural variations across geographies or topologies, changes in oceanic biogeochemical cycles and anthropogenic factors. One of the ways of mitigating the problem of ocean acidification is through "climate change refugia"—comprising local microenvironments or niches which help in protection of species from looming climatic threats (Ashcroft 2010; Keppel et al. 2012; Morelli et al. 2016). Forecasts based on anthropogenic climate change data and its biological impacts help in identifying potential climate change refugia and allow investments for their regulatory management. Hence, identification of regions of less ocean acidification, *i.e.,* ocean acidification refugia (OAR), will play a beneficial role in protecting marine biodiversity and ecosystem from the repercussions of ocean acidification and associated problems, thereby preserving the values and services that we derive from these natural resources. However, the identification of OAR and planning investments into these regimes remains an unresolved issue, mainly due to the inherent complexities of ocean CO_2 chemistry, ocean weather, and ocean climate that have temporal and spatial variations. Ocean weather relates to the state of seawater chemistry, temperature, currents, and so on at a given location and time. Hence, local microclimates in oceans vary significantly from the global climate patterns, thus leading to different levels of ocean acidification rates across regions. Additionally, abiotic factors like temperature, global warming, and anthropogenic CO_2 emission along with biotic factors like rate of marine photosynthesis and biogeochemical cycles also affect ocean acidification. Hence, identification of ocean refugia and standardization of regulatory measures will depend significantly on geographical variations. These variations along with lack of standardized criteria for OAR identification renders it tough for scientists, legislators, and policy makers to invest in OAR (Kapsenberg and Cyronak 2019). Nevertheless, research has shown that regions of OAR do exist and few of their proposed examples include areas isolated from upwelling (Chan et al. 2017; Kapsenberg and Hofmann 2016), slow-flow habitats (Hurd 2015), and so on.

Marine ecosystems are threatened by major climate change associated problems like rising sea levels, global warming, and ocean acidification. Changes in carbonate chemistry of oceans have extensive effects on marine organisms and ecosystems, along with key effects in regulating global carbon cycle. Thus, protection and preservation of biodiversity and ecosystem is of great significance. OAR requires prior knowledge and understanding of the key aspects of ocean carbonate cycle that the particular species in that particular region are sensitive to and how these parameters need to be regulated (Kapsenberg and Cyronak 2019). Upon identifying these specific region-wise effects of ocean carbonate chemistry, further experiments could be conducted to identify other biotic and abiotic stressors that impact ocean biogeochemical cycles (Boyd et al. 2018). Hence, understanding and regulation of spatiotemporal variations and biotic and abiotic factors is crucial in identifying, designing, and investing in OAR. Global rates and patterns of ocean acidification are asynchronous because it is a complex problem that is interlinked with multiple parameters like temperature, salinity, oxygen, nutrient cycles, climatic changes, and so on. Hence it calls for localized management practices catering to preserve region-wise species vulnerability and micro-ecosystems. Designing preventive and precautionary measures must be coherent with ecological, environmental, and species protection. They must exhibit consistent beneficial characteristics persisting with time and external variabilities so as to prove their sustainability. Further research to understand ocean acidification and interlinking effects of different stressors that regulate it will pave the way to standardize mitigatory measures.

1.4 Ocean Deoxygenation

Deoxygenation is the loss of oxygen, and it is a significant impact factor for climate change and is supported by other events such as global change (Levin 2018). Anoxia is defined as the absence of oxygen or too low oxygen concentrations to be measured with the instruments whereas hypoxia is a state of low oxygen level where the physiological and ecological processes are weakened. In the Philippines, many of the aquaculture farms have lost many fishes due to hypoxia. When the fishes die, they release a chemical called histamines, which can poison other organisms like mollusks that consume it in higher quantities. All the aquatic organisms are at risk at low pH and low oxygen. According to a case study of Baltic Sea cod, based on surveys and otolith chemistry, the foregone biomass—amount of living matter present in a particular habitat—has not been developed from hypoxia. Hypoxia's effect on regulating several things like climate, air quality, water quality, etc. is very ambiguous, except for worsening of the water quality. Some of the traditional fisheries on coral reefs were found to be harmed by hypoxic events in some locations (Limburg et al. 2020). Some of the studies conducted in the newly discovered coral reefs in the SE Atlantic show that *Lophelia pertusa* thrives better in warm, hypoxic environments. Studies on this reef building species has thrown light on the quick adaptability of some species on the changing ocean conditions (Hebbeln et al. 2020).

Ocean deoxygenation is the decreased amount of the oxygen content in the oceans which is a sign of anthropological activities such as excess carbon dioxide emission (Stramma et al. 2008) and the deposition and the enrichment of the water bodies with nutrients. The decrease in the amount of oxygen in the oceans has been very rapid. It stands as a threat to all aerobic organisms present and people who depend on aquatic life habitat for nutrition or livelihood, which is the economic impact ocean deoxygenation will create. By the year 2100, about 4% of the oxygen in our oceans is expected to be lost, a major chunk of which is concentrated in the upper 1000 m where biodiversity is richer (Laffoley and Baxter 2019).

1.4.1 Causes of Deoxygenation

The leading causes of ocean deoxygenation are eutrophication, nitrogen release and its accumulation from the combustion of fossil fuels, and the impacts created from ocean warming, resulting in less solubility of oxygen in water. Considerable impact is created when there is a huge surge in greenhouse gas emissions to the atmosphere. This release of greenhouse gases happens primarily due to the burning of fossil fuels and deforestation. The atmospheric carbon dioxide had risen from the pre-industrial value of ~280 ppm to 412 ppm in 2020 (Naqvi 2020).

Oxygen loss due to global warming has had its negative impact on the marine oxygen reserves. These reserves have seen reductions to the tune of 2% in just half a decade from 1960 to 2010. This kind of loss of oxygen is creating changes in the cycling of various nutrients, distribution of habitats, distribution of diverse species, and marine ecosystem services. Most of the species move to an area that is more adaptable to them. On a regional scale, poor zones of oxygen and larger blooms of harmful algae are becoming more prominent and frequent (Laffoley and Baxter 2019). Water bodies near the land can be exposed to over-fertilization of water from sources such as agriculture, sewage, and the burning of fossil fuels. These low-oxygen areas are called the dead zone, but they harbor tremendously richer and more diverse microbial flora (Limburg et al. 2020). Global warming and eutrophication lead to local environmental changes, thus lowering oxygen saturation and increasing biological oxygen demands causing deoxygenation (Hughes et al. 2020).

1.4.2 Effects of Ocean Deoxygenation

Ocean deoxygenation has consequences for carbon cycling, marine habitats, nutrient cycling, and ocean productivity· Studies have been reported that oceans have already lost 1–2% of their oxygen since the middle of the twentieth century (Bopp et al. 2013). Eutrophication and global warming can reduce the saturation of oxygen and hence increase the biological oxygen demands. Coral reefs and tropical shallow waters are highly vulnerable to hypoxic conditions which can result in an increased mortality rate of the coral reef habitants. But currently most of this is not accounted for, while evaluating the abundance of coral reefs under global climatic changes (Hughes et al. 2020). Deoxygenation has a negative impact on several aspects of the ecosystem services provided by oceans (Laffoley and Baxter 2019).

Ocean deoxygenation is one of the most infamous results of climate change happening due to human activities. Climate change played an important role in ocean deoxygenation and catalyzed eutrophication, which is the increased amount of nutrients present in the ocean bodies. Ocean deoxygenation will affect the ecology, behavior, and physiology of organisms present in the aquatic bodies (McCormick and Levin 2017). In the open ocean, oxygen-depleted waters are present several hundred meters below the ocean surface, and these zones are called oxygen minimum zones (OMZ). Speedy winds and circulating waters of the oceans have the possibility of bringing the deoxygenated waters towards the surface and closer to shore. This phenomenon is called upwelling. From the mid-twentieth century onwards, 2% of the oxygen inventory has been lost by our oceans. Also more than 700 coastal water bodies have been found to have low oxygen levels (Limburg et al. 2020).

Deoxygenation in the oceans will hamper the available habitat for pelagic and mesopelagic and benthic organisms (Laffoley and Baxter 2019). Deoxygenated water sources and the OMZ increase when the sea is getting warmed, and in some parts of the earth, upwelling is creating more severely oxygen-depleted waters towards the shore (Limburg et al. 2020). In some parts of the ocean, when the oxygen level is low, aerobic organisms present will show decreased growth rate and reproduction tendencies. Those organisms that are sedentary/non-locomotive in their lifestyle will experience low oxygen-induced mortality. Exposure to low oxygen level conditions can result in changes in the food web structure, including changing organic matter flow direction to low oxygen tolerant species like the microbes. For the aerobic organisms, when there is an inadequate supply of oxygen to their tissue and the formation of a sulphatic condition, survival would be difficult whereas anaerobically respiring microbes can survive such a situation. The ocean's oxygen inventory source is only ~0.6% compared to the atmosphere, and the equilibrium created between the sea and the atmosphere is unsettled (Laffoley and Baxter 2019).

Hypoxia affects not only the physiological function but also fish behavior as well. For the social activities when fishes stay together, this is known as shoaling. When the shoal is swimming, and when they are swimming in the same direction, it is said to be schooling. Many fish species are known to practice schooling. Schooling is very much beneficial to fishes for their survival. Hypoxia creates an effect on schooling behavior. This can create many important ecological consequences and imbalance. Hypoxic effects on school dynamics and structure, along with its mechanisms, can cause an increase in the school volume, and this will lead to school disruption. The effect of hypoxia creates a number of schooling structures and school positions. It has been observed that large schools of fish can worsen the hypoxic conditions, with possible after-effects for the school size and school structure. Some models also predicted that the maximum size acquired by a fish school is directly related to the availability of oxygen. Therefore the school size can reduce when a hypoxic situation is present. Studies of the effects of hypoxia, temperature, and ocean acidification will help in better predictions of the effect of climatic stress on behavior of fishes. When exposed to hypoxic conditions, fishes show some changes in physiological conditions. These include increased oxygen uptake through the gills, increased transport rate of oxygen in the blood, and faster rate of release of oxygen in the tissues. All these effects can only relax the hypoxic condition only for a minimal time period. Some of the field studies have shown that dissolved oxygen decreases along the axis of motion in large schools due to the significant use of oxygen by the individuals swimming at the front of the school. Therefore the organisms swimming at the back of the school have a higher chance of encountering hypoxia than those swimming in the front of the school (Domenici et al. 2017).

The body temperature of cold-blooded organisms of fishes and invertebrates, also known as ectotherms, changes with the water temperature. The metabolic rate of an ectotherm is directly proportional to the external temperature. This will increase the demand for oxygen to meet the metabolic rates (Schulte 2015). Fishes have a low tolerance to habitat near the water column's surface and ventilation is done at the top layer of the water, containing higher dissolved oxygen levels. This behavior is called "aquatic surface respiration" (Kramer 1987). Microorganisms like bacteria, archaea, etc., get their energy by reducing the organic matter by aerobic respiration, which is their metabolic process. For this, they consume oxygen and produce carbon dioxide to derive energy as ATP molecules (Robinson 2019). Almost all the organic matter formed from photosynthesis becomes dissolved organic matter. During aerobic respiration of the bacteria, they take up DOM (Dissolved oxygen matter) in waters exposed to sunlight. A part of the organic matter traverses to the more deep layers of ocean, forming an aggregation called marine snow. Such aggregates which sink below are consumed by the microbes' respiration in the deeper zones (Azam and Malfatti 2007).

When the oxygen in water is consumed in the deeper layers of oceans, recharging of oxygen in these waters is possible only when it reaches the surface layers. Microbial metabolic activities which are generally inhibited by oxygen, like denitrification and anaerobic ammonium oxidation, occur when the oxygen concentrations drop below concentrations of 10 nM. Denitrification and anaerobic ammonium oxidation can lead to the loss of nitrogen in the ocean (Lam and Kuypers 2011). Decreased oxygen availability due to deoxygenation will decrease the result of many zooplankton species respiration, egg production, and food intake (Elliott et al. 2013). Zooplankton have the ability to alter distribution patterns in response to hypoxic or anoxic zones. Most of the zooplankton species have been found to avoid low oxygen zones with an aim of maintaining normal metabolic activities (Elliott et al. 2012). Some of the zooplankton, like the gelatinous zooplankton, show the ability to tolerate hypoxia because of the ability to store oxygen in between the gel, *i.e.,* the intragel regions (Thuesen et al. 2005).

In marine organisms, impairment of visual functions can occur as a result of deoxygenation. This has been happening for tens of thousands of years. Human activities played a significant role in influencing the acceleration rates of oxygen loss in the ocean through ocean deoxygenation caused by eutrophication and warming. Morphological, physiological, behavioral, and ecological changes in animals living in various oceans have been observed as a response to increased levels of ocean deoxygenation (McCormick and Levin 2017).

1.4.3 Measures to Curb Ocean Deoxygenation

Governments and industries must have policies to cut emissions of carbon dioxide emissions and limit ocean pollution in order to restore the loss of oxygen. The rules and regulations regarding these matters should be made very strict, and any form of deliberately made mistakes should be dealt with hefty fines. Reduction in greenhouse gas emissions will reduce the warming effect. When the warming impact has lessened, the solubility of the oxygen in the water increases. Leaching of nutrients from agricultural lands and both untreated and treated sewage effluent is significant, leading to eutrophication. The key to reducing the danger of these effects is by proper treatment of effluents as much as possible before the release into water bodies.

In Tampa Bay, Florida, and the Baltic Sea drainage basin, with appropriate treatment, management, and proper recovery process implications, successful restoration of the seagrass was possible. These could also bring back the balance in wildlife and the fish. Sustainable farming strategies and scientific and more efficient treatment of sewage could reduce the nutrient accumulation in basins. The oxygen saturation of the waters decreases with increasing the phosphate levels or decreasing the oxygen concentration. When the saturation levels of oxygen in water is high, there will be only very meager levels of phosphates, at a temperature of 15 °C (Ishizu and Richards 2013). Therefore if phosphorus is present, its sediments have the chance of being recycled for more years to come. The effects of ocean deoxygenation are looming large at us at the global level. To face this, adaptive planning is required. A systematic and meticulous observation of oceans and its coasts with added impetus to research and improved prediction models that uses accurate data for understanding and exposing the trends in the global water bodies must be implemented (Limburg et al. 2020).

1.5 Global Efforts to Sustain Fish Population

Climate change may affect each life stage of fish in a different manner. The response of fish populations to these changes also differs between species and is related to the adaptations of species to the environmental conditions in their respective habitats (Rijnsdorp et al. 2014). Climate change affects the range and productivity of the target fish population, its habitat, and food web and impacts the cost and productivity of fishery and livelihoods and safety of the fishing community. Changes in species interaction, shift in abundance and species distribution, growth rate and mortality rate changes, alterations in temperature extremes, precipitation and the frequency and intensity of storms, are all caused by climate change drivers (Doney et al. 2012; Kirtman et al. 2013). Adapting coping strategies climate change impacts is essential for fish population maintenance (Magawata and Ipinjolu 2014).

Climate change adaptation is a process of adjustment to actual or expected climate and its effects, including changes in processes, practices, and structures to moderate or avoid potential damages or to benefit from opportunities associated with climate change (Noble et al. 2014; UNFCCC 2018). Approaching climate change involves measures that either reduce the amounts of agents that contribute to climate change or prepare the society for the results of climate change via adaptation (Shelton 2014). The Intergovernmental Panel on Climate Change (IPCC) has incorporated consideration of resilience and vulnerability into its discussions on climate change impacts and adaptation (IPCC 2014). Adaptation demands information on risks and vulnerabilities to identify adaptation options that are context specific.

1.5.1 Adaptation

Adaptation can either be a spontaneous reaction to environmental change or planned autonomous actions based on climate-induced changes. With concern to fisheries, autonomous adaptation can change the timing or locations of fishing owing to species arrival earlier or later or shift to new areas. Planned adaptation in fisheries may be research funding for finding species resistant to salinity and temperature fluctuations for aquaculture (Shelton 2014). Based on the extent of change needed, adaptation can be incremental or transformational. Incremental adaptation deals with small adjustments to maintain the essence and integrity of the existing system of fishery and aquaculture. Transformational adaptation involves large-scale changes to the system at fundamental level and involves migrating or changing livelihoods and governance adaptation (Noble et al. 2014).

External stressors on natural systems like agricultural and urban runoff and destructive fishing exercises such as fishing with explosives and poisons can be reduced. Both climate change and upstream and downstream activities (siltation could be caused by dam building and pollution runoff could be a consequence of basin development) impact areas that play a vital role in upstream and downstream fisheries that can also act as spawning regions. Such sites need to be identified and protected. Fisheries and aquaculture sectors have to be integrated entirely into climate change adaptation and food security policies at the national level to ensure incorporation into broader development planning.

Ghost fishing is a dangerous phenomenon which is increasing in severity in the past decades. It refers to the huge quantities of fishing gear lost or abandoned in our oceans. With the increase in storm severity, it is probable that gears like lobster traps will be lost. Gears lost in this manner can cause habitat damage and mortality. Measures such as gear retrieval programs and traps with biodegradable escape panels that facilitate escape of trapped animals can reduce the damage (Shelton 2014).

Aquaculture involves partial or complete control of the life cycle and the environment. With concern to climate change, aquaculture can possibly control a certain number of changes or can moderate the amplitude of the change. On the other hand, capture fisheries are more susceptible to climate change. Hence, adaptation tools differ for activities in capture fisheries and aquaculture.

Institutional adaptation, livelihood adaptation, risk reduction and management for resilience are the three main categories of adaptation activities and the activities are not mutually exclusive. Investments in research and development on aquaculture adaptation technologies, breeding for species tolerant to specific or a combination of stressors like disease, temperature, salinity, acidification, and others are some of the tools under public policies. Legal frameworks include property rights, land tenure, access to water, standards and certification for production and resistant facilities. Institutional frameworks support cross-sectoral and inter-institutional cooperation and coordination, mainstream adaptation in food safety assurance and control. Management and planning involve better management practices including adaptation and mitigation, better feed and feed management, maintenance of water quality, use of seeds with better quality, and climate change integration in carrying capacity considerations.

Adaptation tools within the sector involve developing and encouraging new, more resilient farming systems and technologies, replacing fish meal and oil replacement, shifting to non-carnivorous species, genetic diversification and biodiversity protection, more resistant strains, resilient hatchery, and hatchery produced seeds. Livelihood diversification is an adaptation tool that can be implemented between sectors.

Risk pooling and transfer include social safety nets, social protection, and aquaculture insurance. Under early warning comes integrated monitoring, information analysis, communication and early warning of disease outbreaks and other such extreme events, development of national and local vulnerability maps, and raising risk awareness. Risk reduction techniques cover permitting adaptive movement between mariculture and inland aquaculture (recirculation aquaculture systems, aquaponics), stronger farming structures, and more resilient designs like deeper ponds (Hidalgo et al. 2018). Including fish feed to prevent massive mortality of stocks is an example of emergency assistance to avoid additional damage, loss from climate-related disasters and comes under preparedness and response (FAO 2019).

Selected project activities that relied on intensification of the coping and adaptive capacities of coastal communities include introduction of locally available species like Tilapia and Mullet and making fingerlings available for pond stocking. Farmers raising these fishes were given training and technology support such that it reduces nutritional deficiencies, reduces susceptibility to lean periods and climate variability, and provides additional income and food sources (Caritas, undated (n.d.). Managing trap ponds by building traps out of bushes and branches on seasonally flooded lands for flood periods was also practiced in some areas. Traps built using bamboo were modified with an intention to reduce catch per unit effort. The traps also provided habitat for young native species (Disaster Mitigation Programme ITDG-Bangladesh, (n.d.)). Efficient ecological forecasts of species distributions and conditions is a practice in Pacific fisheries to reduce bycatch. Also novel incentive structures for bycatch reduction are practiced in these areas (Hidalgo et al. 2018). In the North Atlantic oceans, protecting critical coastal infrastructure and building more efficient coastal structures is given due importance to sustain fish production (Peck et al. 2020).

1.5.2 The Ecosystem Approach

Protecting or rehabilitating freshwater, coastal, and marine ecosystems can facilitate climate change adaptation, climate change mitigation, and support to fisheries and aquaculture in a cost-effective manner. Ecosystem-based adaptation to climate change is a new concept that takes advantage of the ability of natural systems to assist in human adaptation to climate change. The ecosystem approach and co-management regimes are the two multiple-benefit approaches to fisheries and aquaculture management. It offers a wide range of social, environmental, and economic benefits, including support for climate change adaptation (IFAD 2014).

One of the best examples of an ecosystem approach to aquaculture is IMTA-Integrated Multi-Trophic Aquaculture. This method deals with cultivating fed species together with extractive species that use both the inorganic and organic wastes from aquaculture for biomass production. In Nha Trang Bay in Vietnam, IMTA is practiced in cages as a small-scale operation. Various aquatic species like the green mussel, snail, sandfish, seaweed, etc. form the cultivated ones in this system (DANIDA 2005). Rows of salmon cages, mussel rafts, and seaweed rafts incorporated in the Bay of Fundy, Canada, is a large commercial operation of IMTA (Chopin 2006; Barrington et al. 2009).

Conservation of wetland ecosystem for flood protection can increase fish catch. Wetlands and deep sections of shallow lakes can act as a refuge for fish populations during drought periods (Allison et al. 2007). The ecosystem approach ensures coordinated management of agriculture activities, lake fisheries and wetland as well as water resources on which they both depend. One such example is United Nations Development Programme (UNDP) project in Samoa. It helps reduce the vulnerability of fishes to rising seas and flooding by supporting the resilience of the local ecosystem through means of rehabilitation and replanting the fragile wetland. Enhanced water flow within the wetlands allows fish breeding habitats to connect with the sea (UNDP 2010).

1.5.3 Co-management

Co-management comprises the involvement of government bodies, local communities, and other stakeholders in the sustainability venture. They also commit to share both the responsibilities and benefits regarding the sustainable utilization of these natural resources. The co-management approach is beneficial in that—(1) it can be a superior mechanism to empower fish farmers in this era of changing climate and (2) they can help in assisting the stakeholders to adapt better to climate change through better governance and effective communication with the national and local authorities (Fezzardi 2001). Co-management is often helpful in increasing the plasticity of inland fisheries and aquaculture systems to drastic fluctuations in climatic conditions.

1.6 Scope of Inland Aquaculture

Broadly, aquaculture includes breeding, cultivating, and harvesting fish, shellfish, and aquatic plants. It can be simply defined as farming in water. It is a good source of food and commercial products, helps to create healthier habitats of these flora and fauna, and also serves to rebuild stocks of threatened or endangered species. Countries which cannot boast of any coastal areas can become self-sufficient in their needs of fish demand by properly planning and practicing inland aquaculture. For most parts of Asia and Africa, fisheries are significant natural resources. But these parts are also facing exponential growth of population, which in turn means that there are more mouths to feed.

Inland aquaculture, if done in a sustainable manner, can be the ultimate solution in filling the gap in seafood supply that stressed fisheries are creating. One advantage with inland fish farming is the possibility of having a close watch on the farmed fish when compared to wild fish. This allows the farmer to assert timely control over multiple variables. This is especially true considering the fact that most of the farmed fish are generally free of environmental contaminants like mercury and heavy metals. This can be attributed to the almost completely human processed food supplied to the fish in these controlled systems. There is still scope for improvement as the average commercial farm fish yield is hardly one-third of that achieved in farm trials. One of the reasons can be attributed to the higher input use in farm trials and timely documentation and interventions.

Asian countries have remained the largest aquaculture producers, accounting for 92% of the live-weight volume of animals and seaweeds in 2017 (FAO 2019). Also, Asia has the most diverse aquaculture production systems and species wealth (Bush et al. 2019). Nine out of the top ten aquaculture giants are Asian countries with China leading the list with a huge margin. China has been the world's largest producer, processor, and trader of fishes, mollusks, and crustaceans ever since the year 2000 (Crona et al. 2020).In terms of diversity of aquaculture species also, China ranks top with 86 different species of aquatic organisms (Metian et al. 2020). Aquaculture indeed has a plethora of benefits apart from meeting the fish demands of the world. Aquaculture boosts the economy of countries where it is practiced. In fact it is a multi-million dollar industry. It is a reliable source for meeting the protein demand of the population as marine resources get increasingly depleted. Thus the impact on overhunting wild stock can be reduced to some extent. Inland aquaculture relies less on fossil fuels and hence is more friendly to the environment too.

1.6.1 Effects of Floods and Sea Level Rise on Inland Aquaculture

Inland aquaculture is at the mercy of climatic conditions. In the past few decades, extreme climatic events like floods have been rampant all over the world mainly due to climate change. Heavier and untimely monsoons can cause a greater frequency of floods resulting in physical damage to aquaculture infrastructure facilities including fish farms. Preventing both the escape of cultured fishes from inland aquaculture farms and the entry of predatory fish from the wild are both highly impractical during heavy and sudden floods. Threats to cage aquaculture in rivers may also be the result of river erosion by heavy flooding.

One of the major threats of climate change resulting in sea level rise to aquaculture and agriculture is the intrusion of saline water into the freshwater ecosystems of low-lying coastal areas. Water salinity level has increased about 26% in coastal areas of Bangladesh in the last four decades (Alam et al. 2017). Significant decline has been observed both in rice and fish production due to water salinization in the coastal regions of Bangladesh (Ahmed and Diana 2015). In Vietnam, water level rise during the rainy season as well as sea level rise in the Mekong Delta has affected striped catfish farming (Nguyen et al. 2014). Frequent variations in water salinity have shown to result in increased transmission of white spot syndrome virus in shrimps (Liu et al. 2006). Saline water intrusion has been correlated with poor growth performance and hemato-biochemical parameters and altered erythrocyte structure of striped catfish, *Pangasianodon hypophthalmus* (Jahan et al. 2019). Salinity in aquatic environments was found to have an antagonistic effect on the embryonic and larval development of the freshwater species, *Ompok pabda* (Alam et al. 2020). It is predicted that the frequency of coastal flooding will be doubled by 2050 due to sea level rise (Vitousek et al. 2017). The inflow of salt water into inland aquaculture systems would hamper the biodiversity of these freshwater ecosystems as many would be sensitive to salinity. This kind of saltwater intrusion affects soil fertility and water quality. Decline in fish productivity is the net result of these events.

1.6.2 Effects of Indiscriminate Antibiotic Usage on Inland Aquaculture

Demand for increased production has invariably resulted in higher densities of fish in inland farms. Such overcrowded aquaculture can be swarming grounds for parasites and pathogens resulting in more and more disease outbreaks. More fish population translates to more waste accumulation in the water and fewer barriers to prevent infected fish from contaminating others. In such a scenario, antibiotics became a necessary disease management tool for inland fish farmers. Application of antibiotics in aquaculture paves way for a much wider environmental exposure pathway than observed in terrestrial systems. This can adversely affect multiple components in the entire ecosystem (Lulijwa et al. 2020). As the aquatic ecosystem gets disturbed, the aquatic microbiome functions also get disrupted leading to deviations in biodiversity, carbon sequestration, nutrient cycling, etc. (Kümmerer 2009).

Also, the higher amount of antibiotic residue remaining persistently in the aquatic environment may alter the environmental microbiome. Indiscriminate use of antibiotics definitely paves way for antibiotic resistance. High density aquaculture farms can act as potential reservoirs for antibacterial resistance genes. This in turn helps provide many routes for animal and human exposure to such drug resistant bacteria (Larsson et al. 2018). Although it is very difficult to prove the route of transfer, multiple studies on the route of transmission of antibacterial resistance genes coded by pathogenic mobile genetic elements in animals including human beings and the environment have pointed out that these genes indeed traverse through various elements of the food chain (Cabello et al. 2016). All these events will lead to the dangerous consequence of human pathogens becoming resistant to these antibiotics.

Probiotics may be the best bet to curb this menace of antibiotic resistance. This is attributed to the revelation of the importance of a stable microbiota in disease progression. One of the best evidence for the role of gut microbiota in disease control comes from a study comparing the disease susceptibility of germ-free animals when compared to ones with healthy gut microbiota (Collins and Carter 1978). Their study revealed that germ-free mouse can be killed with just 10 cells of *Salmonella enteritidis* whereas one million cells are required to kill the wild-type mouse with normal microflora. Hence in order to improve disease resistance and lower disease severity it is important for the cultured fish to have a robust gut microbiota.

Supplementation of aquatic animal diets with probiotics has been shown to positively influence growth and lead to better feed utilization, help in more cell proliferation under conditions of stress, help get a better gut microbiota, aid in better modulation of immune system, and give more resistance to diseases (Ringø et al. 2014; Nandi et al. 2017; Dey et al. 2018). Probiotics help in digestion too. They help in digesting some of the indigestible compounds, detoxifying compounds in the diet and increasing vitamin availability (Zorriehzahra et al. 2016). Probiotic microbes also help in reduction of harmful pathogens in the gastrointestinal tract by multiple mechanisms. These include production of siderophores, bacteriocins, peroxides, and short chain fatty acids (Hoseinifar et al. 2017). Some of the prominent probiotic genera used in aquaculture include *Lactobacillus, Bacillus, Enterococcus, Streptomyces,* etc. (Tan et al. 2016; Elshaghabee et al. 2017). During the past decade, there has been more emphasis on the use of host microbiota as probiotic sources as they have the added advantage of getting established in host defense system in a natural manner (Llewellyn et al. 2014). Today many countries are opting for probiotics in aquaculture as it goes along the way of more sustainable culture practice—it improves gut microflora offering healthier fishes and also reduces the ever-increasing menace of antibiotic resistance which looms large at us.

1.6.3 Effects of Increased Pollutant Dumping on Inland Aquaculture

The potential of inland aquaculture systems is often underestimated particularly to food security as they are seen as a less priority area majorly due to the other application of inland water resources including power generation, irrigation for agriculture, and municipal use. The aspect of increasing pollution level in inland aquaculture units is often neglected even by concerned officials and policy makers (He et al. 2017). The major source of pollutants entering inland aquaculture systems can be categorized into those directly entering the aquaculture system from industries or households and those that are indirectly entering them as a consequence of anthropogenic activities. Dumping of pollutants into inland aquaculture units affects the integrity of these production systems by changing the habitat nature, creating biotic and abiotic stress, triggering other species invasion and reduction in dissolved oxygen. The major contributing elements of pollution in the inland aquaculture system are fertilizers used in agriculture, chemicals employed in industrial production processes, and chemicals used in day-to-day life like soaps and detergents. A significant proportion of these pollutants enters these aquaculture systems through multiple methods like surface runoff, atmospheric deposition, and direct discharge (Maitland 1995). Besides these, dumping of huge amounts of slaughterhouse waste and biomedical waste is a serious threat to the aquaculture system as they are important breeding grounds for potential pathogens and antibiotic resistant organisms.

Compared to the other aquatic ecosystems, the inland aquaculture species are at higher risk associated with high bioaccumulation of these pollution. Research over the past decades reported that the pollutants entering an inland aquaculture system may not be directly fatal to fishes but may reduce their fitness and increase their susceptibility to pathogens. Organic pollutants and heavy metals are recognized as the biggest threat to inland aquaculture systems. Heavy metals enter the inland aquaculture system from industrial discharge and mineral drainage (Rai 2009). They contribute to the sedimentation load and have a direct toxic effect on benthic fauna. Apart from the direct toxic effect of pollutants on cultured aquatic organisms, they may alter the balance in aquatic organisms resulting in reproductive and developmental abnormalities.

Microplastic pollution is yet another serious threat to aquatic resources. Microplastics are commonly defined as being 5 mm or less in their longest dimension. Ingestion of microplastics is commonly observed in inland aquaculture species. They have serious implications on human health and food safety. Microplastics in addition to having toxic chemicals added during their manufacture will also absorb other toxins from the environment that adds to the pollution load of water bodies (Vázquez-Rowe et al. 2021).

1.6.4 Effect of Algal Bloom on Inland Aquaculture

Growth of algae in inland aquaculture systems is a natural phenomenon and contributes to algal bloom only when their population density increases exponentially contributing to decrease in dissolved oxygen level and alters the physicochemical and biological profile of aquaculture systems. The most common factors that trigger algal bloom in inland aquaculture units include industrial drainage-associated eutrophication, accumulation of feeds in pools, and infiltration of sewage containing appreciable amounts of minerals (Zohdi and Abbaspour 2019). Invasion of alien species and changes in environmental conditions which may be due to natural or anthropogenic activities can accelerate the rate of algal blooms in the inland aquaculture system.

Harmful algal blooms (HABs) can be broadly categorized into three types—(1) non-toxin-producing species that affect the recreational value of the sites where they proliferate, by causing discoloration of the water or extensive foaming and cause high rates of fish mortalities by drastic reduction of dissolved oxygen in water, (2) toxin-producing species that can cause a variety of neurological and gastrointestinal disorders in humans and thus pose an important threat to public health and shellfish exploitations, and (3) species that are not toxic to humans but affect fish in culture, by physically damaging the fish by obstructing their gills or by the production of ichthyotoxic chemicals (Hallegraeff 2003).

The effect of algal bloom in inland aquaculture can range from mild to extremely severe. It may cause a drastic reduction in the dissolved oxygen level by consuming the residual dissolved oxygen in water and preventing photosynthesis by aquatic plants by blocking the entry of sunlight through algal mat formation. The second and most dreadful consequence of algal blooming is the production of algal toxins that affects fishes, animals, birds, and eventually humans. Some of the algal toxins are neurotoxic in nature and have a direct effect on humans and animals by inducing asphyxiation as they target the central nervous system (Anderson et al. 2002). Some algae produces polyunsaturated fatty acids and galactolipids that results in damage to red blood cells. These hemolytic compounds were observed to cause a drastic reduction of blood flow in fishes and a dangerous reduction in the level of oxygen in blood. Indirect damage caused by algal blooms to inland aquaculture units include loss of primary productivity and formation of fetid foam with unpleasant odor due to the presence of huge amounts of decomposing bacteria (Jewel et al. 2003). Some of the ill effects of HAbs are listed in Fig. 1.2.

With the rapid advancement and research development in the aquaculture sector in the past few decades due to biotechnological interventions, constructive strategies were initiated for the effective management of algal blooms without affecting the integrity of aquatic ecosystems. This includes blocking the sources that trigger eutrophication and mineral infiltration, using limited amounts of fertilizers in agriculture and removal of algae before it reaches a threshold level. Chemical methods routinely employed include spraying with algicides like copper sulfate, potassium permanganate and silver nitrate. Biological control methods employ cyanophages

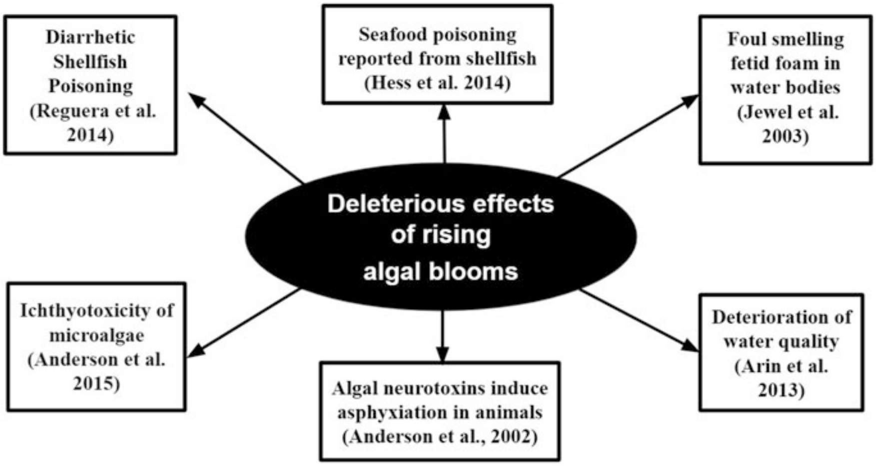

Fig. 1.2 Deleterious effects of rising algal blooms

and bacteria that affect the algae without causing a negative impact on reared aquatic species or the consumers (Anderson 2009).

1.7 Conclusion

Climate change has indeed affected the marine and inland aquaculture world over in an unprecedented fashion in the past few decades. Millions of people all over the world depend on fisheries and allied activities. The gradual but steady decrease in the global fish production and productivity will certainly affect these people. The problem is all the more grave as most of these people are from the coastal belts in developing countries and hence may face serious consequences as their very livelihoods would be at stake. Already there have been many scientific studies indicating the crash of marine fish population by 2050. The United Nations has indicated 17 SDGs or Sustainable Development Goals to be implemented globally in an attempt to reverse the harms mankind has done to our planet. The mandate of the fourteenth SDG is “Life Under Water.” Though our oceans are bountiful, the greed of humans is taking a toll even on these. A strict adherence to these goals is very important to regain or at least prevent further decrease in the biodiversity of oceans. Equally important is the sustainable practices to be judiciously carried out in future in inland aquaculture farms so as to retain these are steady sources of protein rich food. The widespread occurrence of the covid pandemic has of course brought many development activities to standstill or has lowered the pace. Though it could be detrimental to the economy of countries, for Mother nature it is a chance to recuperate from the anthropogenic damages. It is high time that we learn from our mistakes of the past and approach a less greedier and more sustainable approach in practices followed in marine and inland aquaculture. As oceans form a major chunk of this planet, there is no doubt that if sustainability issues are given due thrust by all the countries our mighty oceans would definitely be able to provide nutrient rich food for the humans and animals on this planet.

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Chapter 2
Developments in Feeds in Aquaculture Sector: Contemporary Aspects

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Abstract Aquaculture, one of the largest protein food generating sectors, greatly relies on nutrition. Up to the present time, the dependency of the aqua feed sector on fish meal and fish oil as protein and lipid sources, respectively, was too high, which led to its inadequacy and over-exploitation of marine resources. Recently, numerous researches with locally available feed ingredients have been accomplished. There is urgency for a move from fish meal to plant/terrestrial animal/microbial proteins within the aquaculture industry as over-exploitation of wild fishes has negative ecological consequences. Plant proteins cannot successfully replace fish meal due to poor protein digestibility and essential amino acids imbalance, urging feed concerns to search for cheaper and nutritious fish meal alternatives from animal origin. In order to overcome the indiscriminate use of antimicrobial drugs, the concept of probiotics in aquaculture has received firm encouragement in recent years due to their wise inhibitory mechanisms and safeness. Negative perception for synthetic antioxidants among fish farmers and their unreliable nature has resulted in the research for non-synthetic, food derived antioxidants that could encounter and neutralize the detrimental effects of free radicals. In line with this, there is a strong captivation to research on the individual or synergistic effects of protein hydrolysates, peptide fragments, and free amino acids that could innately exhibit antioxidative activity. Commercialization of economical feeds with antioxidative feed ingredients could strengthen the profits of feed processors. Recent development in the aquaculture sector propelled by the application of biotechnological methods has clearly highlighted the need for development of functional feeds through incorporation of bioactive molecules. In the last few decades, the global aqua feed industry has witnessed milestones in the development of feed ingredients from waste raw materials and sustainable alternatives to antibiotics and chemicals used to tackle the disease outbreak in aquaculture. As the health and immunity of fishes

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primarily depend on their nutritional pattern, a great research interest is extended on incorporation of biomolecules like single-cell proteins, animal proteins, plant metabolites, biopolymers, and enzymes as feed ingredients to enhance the nutritive quality and immune tolerance in fishes. Besides the focus on the feed ingredients, the modification and development of fermentation strategies for producing probiotic-based feed and enzyme-assisted bioconversion into valuable feed ingredients is also gaining more importance. This chapter deals with the recent development in the aqua feed industry with specific reference to the incorporation of non-conventional feed ingredients like animal/microbial proteins, biopolymers, enzymes, and other immunostimulatory compounds in aqua feed and their impact analysis in improving the growth profile and pathogen tolerance in fishes.

Keywords Aqua feed · Probiotics · Fish meal · Biopolymers · Polyhydroxyalkanoates · Chitosan · Cellulose · Keratin · Single-cell protein · Immunostimulants · Enzymes

2.1 Introduction

The significance of inland aquaculture becomes more pronounced as the marine resources all over the world are depleting at alarming rates. The latest Food and Agriculture Organization (FAO) report depicts a domination of Asian countries in the arena of production of farmed aquatic animals. Asia has a share of 89 percent in inland aquaculture for the last two decades. China, India, Indonesia, Vietnam, Bangladesh, Egypt, Norway, and Chile form the major contributors. 63% of the world's farmed food fish production which consists of fish farms and inland natural water sources produces 51.3 million tonnes of aquatic animals by inland aquaculture. In global aquaculture, India holds second rank (FAO 2020). Among the many reasons for low productivity of many aquaculture farms in India are poor feed conversion ratio, fragmented holdings, lack of skilled personnel, etc.

One of the ways of improving fish productivity is by scientific fish feed formulation. When cultivated in large quantities and high densities, fishes require a high quality, nutritionally balanced diet for rapid and healthy growth. In this context, the quality of fish feed assumes great importance. One of the best ways to assess the efficiency of aquaculture farms is by calculating the "feed conversion ratio" (FCR), which is the weight of feed administered over the lifetime of an animal divided by the weight gain. Apart from proteins, lipids, and carbohydrates, there should be ample amounts of essential amino acids, minerals, and vitamins in the fish feed which can contribute to a better FCR. The past decade saw the emergence of many novel components in the feed compositions for aquaculture. Some of these newer components include probiotics, enzymes, single-cell proteins (SCP), and biopolymers like chitosan, cellulose, and keratin.

One of the grave concerns in aquaculture is the increasing problem of antimicrobial resistance among fish pathogens (Laxminarayan et al. 2013; Sattanathan et al. 2020a) which in turns drive to pump more and more antibiotics in the fish feed.

Antibiotics in fish feed presents one of the best routes for environmental exposure of these chemicals as the drug distribution through water can happen at faster rates with important ecosystem health implications (Liu et al. 2020; Lulijwa et al. 2020). For these reasons, there is a global demand for alternative ways of improving the immunity of the fish population. One of the upcoming trends is the use of probiotics, the details of which would be discussed in detail. Biopolymers are also emerging as novel components in fish feed. There is more thrust given to the production of biopolymers like chitosan, keratin, etc. produced from farm wastes as these set perfect examples for recycling of organic matter in the ecosystem. For the production of biopolymers, various kinds of agricultural and industrial wastes can be used as substrates. This will have multiple advantages—prevention of excess agro by-products going to waste and chance of making waste from one sector as substrate for another sector, thereby reducing the cost for procuring fresh raw materials. Effective ways of waste valorization is an area of active research these days as it can help achieve “zero waste” targets and biopolymer production from agro wastes can be seen as a perfect example of this. The task before us is to find out means of having standardized and cost-effective methods of achieving this. In fact, if standardized and accomplished in the proper manner, biopolymer production and incorporation in fish feed would indeed cater to the sustainable goals of the United Nations. Probiotics, prebiotics, and enzymes added to the fish feed will definitely result in better feed conversion ratio by helping the proliferation of friendly microorganisms, increased digestibility of food, thereby resulting in better returns to farmers. The present article discusses the latest developments in fish feed formulation for cost-effective aquaculture and environmental sustainability. The 2018 observed all-time record of 178.5 million tonnes of global fish, mollusks, crustaceans, and other aquatic animals’ production, excluding aquatic plants, was a 3% growth compared to 2017. A 401 US billion dollar worth global fish production was estimated in the first sale out of which aquaculture contributed 250 US billion dollars. The top ten producers of aquaculture (Fig. 2.1) produced 72.8 million tonnes collectively. By quantity, this is 88.7% of the global aquaculture production of 2018 which consisted of finfish (54.3 million tonnes), mollusks (17.5 million tonnes), crustaceans (9.4 million tonnes), and other aquatic species (0.9 million tonnes) (Fig. 2.2) (Food and Agriculture Organization 2021).

Over the current estimation (2015) of 73 million tonnes, global aqua feed output is forecast to rise in 2025 by 33 percent to 101,3 million tonnes, closely aligned with the targeted 101.8 million tonnes worldwide aquaculture production (Salin et al. 2018). Feed is the single most important input in aquaculture. As a result, the expansion of aquaculture practices is dependent on the expansion of the aqua-feed industry in order to achieve the projected fish yield. Traditionally, fish meal has been used as a primary source of dietary protein in the process. However, in the past few years, a high requirement and lack of supply of the fish meal resulted in an increase of price, and an absolute reliance on fish meal is not suggested (Chakraborty et al. 2019). Modern aqua feed is a complex, inventive mix of raw materials which supply the nutritional needs to make aquaculture species more intense and productive. Commodity meals, oils, vitamins, pigments, minerals, and concentrates are

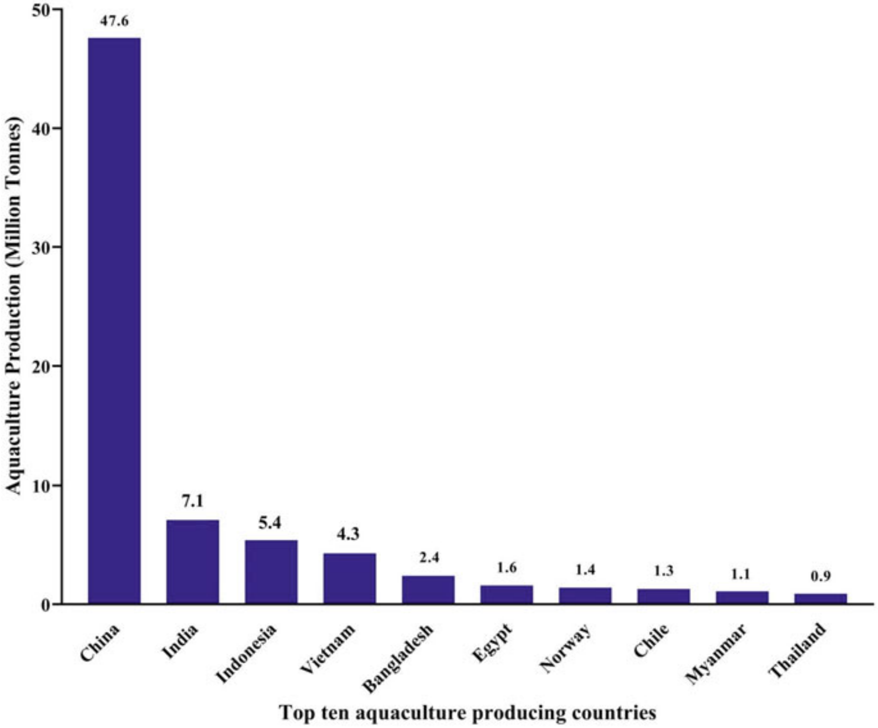

Fig. 2.1 Global aquaculture production in Million tonnes by top ten aquaculture producing countries (Food and Agriculture Organization 2021)

examples of raw ingredients that, when combined, meet an organism's macronutrient and micronutrient requirements. Furthermore, these ingredients promote rapid development, promote animal health, and, most importantly, produce a product with sensory and quality properties that meet consumer expectations (Hua et al. 2019). Over the last 20 years the fish meal and fish oil produced from forage fishes has declined steadily. The ratio of these important ingredients in the aqua feed for many crustaceans and carnivorous fishes are decreasing (Turchini et al. 2019).

2.2 Fish Meal and Its Demand

Aquaculture, one of the largest protein food generating sectors, greatly relies on nutrition. It is a process of farming aquatic organisms to promote its productivity. Fish meal (FM) could be simply defined as wild-caught fishes being used as feed for farmed fishes. It is a prime, dominant, and most expensive proteinaceous feed ingredient that provides good quality protein and essential nutrients for farmed fishes. Wild fish as FM is not only used by aquaculture feed production sectors,

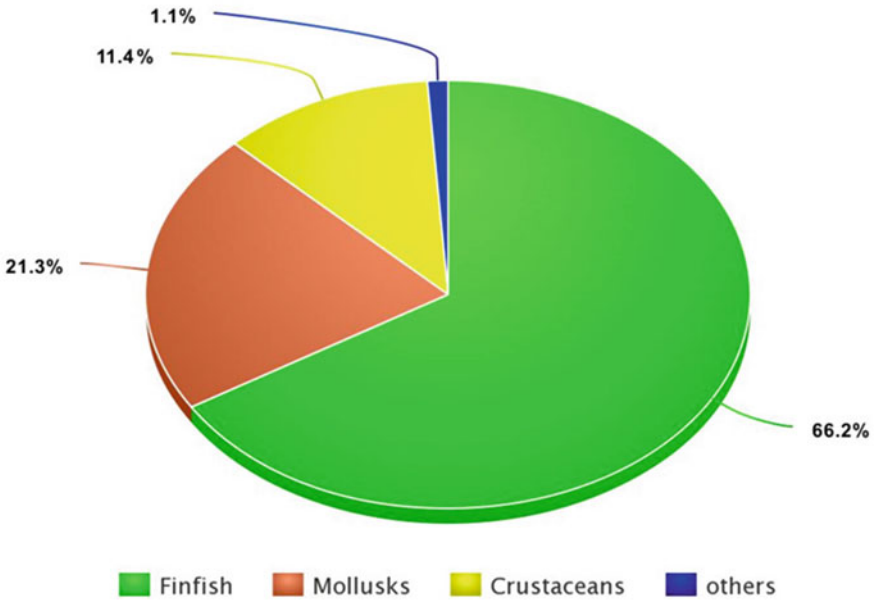

Fig. 2.2 Percentage of aquatic species contributed to global aquaculture production in 2018 (Food and Agriculture Organization 2021)

but also serves as protein source in pet animal and livestock feeds. Multi-use of these wild-caught fish for feed reduces its supply for human consumption. About 4–5 tons of whole wild fishes are required to generate 1 ton of dry FM (Allan 2004). Healthy expansion of aquaculture would not be possible if FM is relied upon as the prominent source of protein in feeds. Moreover, FM is risky due to its thiaminase activity, role as a vector of infectious diseases and rancidity at storage for their marked levels of lipid content. Hence, efforts to research substitutes for fish-derived feed ingredients (plant, animal, and microbial proteins) are highly focused in recent years.

2.2.1 Fish Meal Replacers

The suitable approach in feed formulations is to use high-quality feedstuffs that could meet the nutritional requirements of the fishes. This in turn depends on the amino acid profile in their protein. Due to the expensive nature of FM, there remains long-term scarcity for good quality proteins. Recently, numerous researches with locally available feed ingredients have been worked out. There is urgency for a move from FM to plant/terrestrial animal/microbial proteins within the aquaculture industry as over-exploitation of wild fishes has negative ecological consequences. Plant proteins cannot successfully replace FM due to poor protein digestibility and essential amino acids imbalance, urging feed concerns to search for cheaper and nutritious FM alternatives from animal origin.

FM replacers such as shrimp head waste (Oliveira Cavalheiro et al. 2007), chicken viscera and cryfish meal (Soltan and El-Laithy 2008), *Arthrospira platensis* (*Spirulina*), dried microalga (*Chlorella* spp. and *Scenedesmus* spp.) and fermented *Cladophora* (Ali et al. 2019 ; Kumar et al. 2012a ; El-Sheekh et al. 2014), fermented animal protein blend (Samaddar et al. 2015), duckweed (*Lemna minor*) meal (Ma et al. 2017), *Chlorella vulgaris* (Radhakrishnan et al. 2014), and *Arthrospira platensis* (Radhakrishnan et al. 2016); algae (Sattanathan et al. 2020b) have been researched succesfully. Similarly, animal by-product meal, feather meal, meat and bone meals, poultry meal, chicken offal meal, and poultry by-product meal have been reported to substitute FM by many researchers (Sumathi and Sekaran 2010; Thazeem et al. 2015, 2016, 2017). Move towards animal and microbe-derived proteins as FM replacers has marked appreciable encouragement among researchers, as plant proteins lack certain indispensable amino acids needed by fish for its growth and few plant metabolites are noticed to hinder absorption of nutrients into the aquatic animal's mono-gastric system. With this regard, an experimental study was conducted in which five isonitrogenous diets were formulated for *Labeo rohita,* where fermented tannery solid waste flour replaced FM by 25, 50, 75, and 100%. Appreciable growth performance, nutritional indices, and body carcass composition were evident in the fish group fed with diet formulated by replacing FM with 75% fermented tannery solid waste flour, followed by 50 and 25% replacement (Thazeem et al. 2018).

According to Hasan et al. (1997) and Thazeem et al. (2018), hydrolyzed poultry feather meal could substitute FM up to 20% in the diets of *Labeo rohita* fry without compromising growth and feed utilization. Poorest response was observed with 100% replacement. Feather meals and meat and bone meals were evaluated for their ability to replace FM in the diets of rainbow trout. Up to 15 and 24% replacement of feather meals and meat and bone meals, respectively, was evidenced with significant growth performance which proved their potency to replace FM in fish diets (Bureau et al. 2000). Protein sources such as *Leucaena* leaf meal, coffee pulp, torula yeast, and cottonseed meal have been investigated for Tilapia (Oliveira Cavalheiro et al. 2007). Possibility of substituting dietary FM with a protein combination of canola meal and corn gluten meal and to discover a substitute lipid source for milk product without affecting the fish growth rate was achieved by Umer and Ali 2009.

A 60-day feeding trial was conducted to investigate the possibility of using soybean meal protein for FM replacement for *Labeo rohita* fry (Khan et al. 2003; Jahan et al. 2012). Growth rates, feed conversion ratio, and protein efficiency ratio indicated that FM could be replaced up to 50% without supplementation of additional amino acids. Samaddar et al. (2015) concluded that up to 75% replacement of FM in the feeds by the fermented blend of fish offal and slaughterhouse blood had no negative effect on the growth of *Labeo rohita.* Moreover, muscle protein and amino acids content decreased at 75 and 100% FM replacement. Evaluation of nutritional quality and acceptability of duckweed (*Lemna minor*) meal as component in the diets of *Labeo rohita* fry up to 15% gave best results of growth in the fish; however, FM was completely non-replaceable (Ma et al. 2017).

Studies on earthworm meal as a complete replacer of FM in supplemented feeds for common carp—*Cyprinus carpio* was performed by Pucher et al. (2014). Earthworm meal acted as a partial FM replacer for major Indian carps such as *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala* (Beg et al. 2016). Nyina-wamwiza et al. (2012) studied the effects of partial and total replacement of FM with agricultural by-products and gave a significant conclusion that there were no detrimental effects on the onset of sexual maturation in African catfish. Total replacement of FM with agricultural by-product diets had positive impacts on reproduction. In the recent past, the aquaculture industries could be transformed with the advent of nanotechnology, to promote the uptake of drugs and metal nanoparticles. Muralisankar et al. (2014) reported the inclusion of zinc nanoparticles (ZnNPs) up to 60 mg kg^{-1} in the diets of freshwater prawn, *Macrobrachium rosenbergii* post larvae with superior performance in survival, growth, digestive enzymes' activities, and sound immune responses.

2.3 Probiotics in Aquaculture

The term "friendly bacteria" is quite popularly referred to as probiotic microbes (Cruz et al. 2012). Disease outbreaks due to bacterial, fungal, and viral infections in aqua farms have resulted in stock mortality due to poor sanitation, improper nutrition, and toxin production in farmed animals. As a measure of prevention, use of veterinary medicines (antibiotics) is preferentially practiced. This has led to drastic risks in terms of drug resistance among pathogens, significantly retarded growth with poor marketing, and bioaccumulation in the aquatic animals as well as the consumers (Nomoto 2005). Administration of antibiotics has led to the development of antimicrobial resistance among fish pathogens, which may be possibly due to plasmids acquisition or chromosomal mutation (Balcazar et al. 2006).

In order to overcome the indiscriminate use of antimicrobial drugs, the concept of probiotics in aquaculture has received firm encouragement in recent years due to their wise inhibitory mechanisms and safeness. Probiotic bacteria enhance the host's digestive enzymes by serving as a source of nutrients; improve the quality of water; and stimulate the immune system by the mechanism of competitive exclusion. In addition to this, they modify the intestinal microbiota, compete with pathogens for adhesion and nutrition, create antitoxins, and secrete antimicrobial compounds (Nayak 2010). They significantly regulate allergic responses and reduce/prevent cancer proliferation in mammals. Probiotic microorganisms serve as growth promoters, improve reproductive health, and support farmed animals to tolerate stress conditions.

Administration of probiotics could be accomplished in terms of oral, water, or feed additive routes. In the case of prawns, oral routes are highly suitable whereas the latter routes are widely practiced in aquaculture farms. According to Nayak (2010), several probiotic bacteria in non-viable form could potentially provoke similar effects in hosts when compared to viable probiotics, as they are commonly found in transient state and could easily expel after feed withdrawal in hydrobionts. Non-viable probionts are not only involved in gut colonization but also ultimately boost up the immune system of host due to the presence of certain microbial components (peptidoglycans, polysaccharides, and lipoteichoic acids) that act as activators of piscine immune system (Secombes et al. 2001). Lactic acid (LA) bacteria (*Lactobacillus* sp.) are the most prevalent aquaculture probiotics. Possible modes of their administration are via feed and water. They are highly preferred as they multiply rapidly and inhibit the growth of pathogens by producing beneficial antimicrobial compounds such as bacteriocins, organic acids, and hydrogen peroxide (Gatesoupe 2008). Numerous reports on the probiotic effects of *Lactobacillus* sp. on various fishes and crustaceans are available (Balcazar et al. 2006; Kesarcodi-Watson et al. 2008). Commercial fish probiotic products are mostly available in liquid and powder forms. Nowadays, increased research in bacterial fermentation and its optimization processes has led to improved functionalities of probiotic bacteria with significant results in growth performances of aquatic animals (Lacroix and Yildirim 2007). Kesarcodi-Watson et al. (2008) elaborately reviewed on the need, principles and mechanisms of action and screening processes of probiotics in aquaculture, concluding that the ever-increasing demand for aquaculture could be met with usage of probiotic formulations and this could act as substitute to hazardous antibiotics.

Nayak (2010) stated that probiotics are emerging as an important part of aquaculture practices for increasing production. *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Carnobacterium, Shewanella, Bacillus,* and *Saccharomyces* species are the probiotics which are generally used in aquaculture practices. In fishes, the participation of probiotics in nutrition, resistance to certain diseases and other enhancing activities has proven beyond any doubt. Flores (2011) has stated that the probiotics can be used as functional additives in feeds. The establishment of a strong disease prevention program, including probiotic and good management practice, can be beneficial to raise aquatic organism production.

Few of the primary reasons for the use of probiotics in aquaculture practice are due to the need for healthy growth of aquatic organisms and proper feed efficiency. The practice of using probiotics has been there since 1986 which further improves water quality and also has positive effects on controlling bacterial infection (Cruz et al. 2012). For aquatic animal growth, survival, and health, probiotics are positive promoters. More intense works on probiotics will globally provide organic aquatic products, which are needed for the safe human consumption of food and health security (Hai 2015).

2.4 Antioxidants, Protein Hydrolysates, Peptides, and Amino Acids in Fish Feeds

Formation of free radicals and their drastic effects on cells and tissues are unavoidable in living systems (Sheriff et al. 2014). With this context, synthetic antioxidants are one of the supplementary feed ingredients, as dietary antioxidants substantially promote animal health by decreasing the load of free radicals in its body. Additionally, presence of synthetic antioxidants could prevent oxidation during feed storage and could reduce the destruction of essential amino acids and vitamins, enabling maximum nutrient supply to the animal. They preserve flavor, taste, texture, and freshness of feeds. Essential criteria for selection of an antioxidative compound in feeds are—it must be efficient at lower concentrations, non-toxic, and economically viable. Ethoxyquin, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) at 150, 200, and 200 ppm, respectively, are the levels of synthetic antioxidants permitted by the U.S. Food and Drug Administration.

However, negative perception for synthetic antioxidants among fish farmers and their unreliable nature has resulted in the research for non-synthetic, food derived antioxidants that could encounter and neutralize the detrimental effects of free radicals. Disadvantageous consequences of synthetic dietary antioxidants may range from weight loss, notable unhealthy changes in liver, kidney, urinary bladder, alimentary tract, and mitochondria, lethargy, anemia, colored skin/urine, decreased survival rates, undermined immunity, condition factor fluctuation, allergy, and bioaccumulation in farmed fishes, subsequently harming the human health (Błaszczyk et al. 2013). In line with this, there is a strong interest in research on the individual or synergistic effects of protein hydrolysates, peptide fragments, and free amino acids in fermented end products that could innately exhibit antioxidative activity. Commercialization of economical feeds with antioxidative feed ingredients could strengthen the profits of feed processors.

From the viewpoint of fish nutrition, amino acids are classified as nutritionally indispensable (essential), dispensable (non-essential), and conditionally essential amino acids. Conditionally essential amino acids are provided via diets under dreadful conditions where the rate of protein synthesis and muscles build up is lower than the rate of feed utilization. Amino acids play a vital role in fish nutrition. They help in reproduction, metamorphosis, pigmentation, appetite and osmoregulation, immunity and antioxidative activity (Li et al. 2009). Major expensive component of formulated fish feeds is the dietary protein. In the recent past, attraction towards the search for feedstuffs consisting of protein hydrolysates, finer peptides, or free amino acids has gained eminence over crude intact proteins. This could differentially influence the growth of the animal as smaller hydrolysates could be feasibly digested and assimilated within the body than crude proteins. Protein hydrolysates and peptide fractions are known to exhibit antimicrobial and antioxidative properties (Balakrishnan et al. 2011). Reports on bio-functional molecules with antibacterial and antioxidant activities through microbial fermentation are abundant (Sachindra and Bhaskar 2008).

Antioxidant property of a peptide depends on the intrinsic characteristics of peptides (amino acid nature and sequence). Antioxidant activity was shown by protein hydrolysates from the shrimp wastes and the same has been attributed to the peptides and water-soluble protein seen in the material. Due to the low costs, safety, and the high nutritional values, the need for the utilization of amino acids, peptides, or proteins as antioxidants in food materials is rising. Since the intestinal absorption of farm animals appears to be more effective due to the protein hydrolysates, they are considered physiologically better than the intact protein (Kumar Rai et al. 2010).

Effective methods for the treatment of wastes generated from food industries including meat and fish along with applications of such reclaimed wastes have been recently reviewed. Microbial remediation is one of the present-day technologies recommended to reuse industrial wastes, as it is a greener technology which alleviates unsafe waste disposal methods (Umesh et al. 2021). Also, the protein hydrolysates acquired from wastes and by-products of the animal and fish processing industry are stated to show several bioactivities such as antioxidative, antihypertensive and immunomodulatory properties (Balakrishnan et al. 2011). To protect animal feed from lipid peroxidation, Ethoxyquin (EQ, 6-ethoxy-1, 2-dihydro-2, 2, 4-trimethylquinoline) is excessively used in it. For human use (except spices, e.g., chili), EQ cannot be used in any food but human beings can be revealed to this antioxidant by carrying it from feed to farmed fish, poultry, and eggs (Błaszczyk et al. 2013).

Due to the increasing interest in finding antioxidants from natural sources which may have less potential hazard than synthetic ones, research on fish protein hydrolysates exerting antioxidant activity has gained an increased interest. Antioxidants are generally employed to prevent lipid oxidation in foods in order to avoid the formation of toxic compounds and undesirable flavors and odors. In the last decade, several authors have reported a strong antioxidant activity for fish protein hydrolysates obtained from different species such as black scabbard fish (*Aphanopus carbo*), sardinelle (*Sardinella aurita*), saithe (*Pollachius virens*), yellowfin sole (*Limanda aspera*), mackerel (*Scomber austriasicus*), and herring (*Clupea harengus*). García-Moreno et al. (2013) have reported the production of antioxidant activity exhibiting fish protein hydrolysates from discarded species in the Alboran Sea.

Protein recovery from industrial solid wastes and their biotransformation into fish feed ingredients through biotechnological interventions is a promising field of interest in recent years (Basheer and Umesh 2018). In line with this, Thazeem et al. (2020) statistically optimized the fermentation medium that consisted of tannery solid waste. Through lactic acid fermentation, the underutilized tannery solid waste was efficiently bio-converted into a proteinaceous feed ingredient that exhibited potent in vitro antioxidant and antimicrobial activities.

2.5 Amino Acids and Fish Nutrition

Increasing proof from studies on both aquatic and terrestrial animals have proved that several amino acids control important metabolic pathways which are important for the maintenance, growth, reproduction, and immune responses of fishes and are called "functional amino acids." Restoring food intake and growth as dietary protein is the important and highly expensive component of formulated aqua feeds since identification and dietary supplementation of those amino acids or their biologically active metabolites are believed to counteract adverse effects of substitution of fishmeal from aqua feeds (Wilson 2003).

Recent studies shows that some amino acids and their metabolites are significant controllers of key metabolic pathways that are fundamental for support, development, feed admission, supplement usage, insusceptibility, conduct, larval transformation, multiplication, just as protection from ecological stressors and pathogenic life forms in different fishes. Amino acids assume significant and adaptable parts in fish nutrition and metabolism. These functions include cell signaling (e.g., arginine, glutamine, leucine, proline, and polyamines); appetite stimulation (e.g., alanine, glutamate, proline, and serine); growth and development regulation (e.g., arginine, glutamine, hydroxyproline, leucine); energy utilization (e.g., carnitine); immunity (e.g., arginine, glutamine, and dopamine); osmoregulation (e.g., glycine, taurine, b-alanine, and arginine); ammonia detoxification (e.g., glutamate, glutamine, and citrulline); antioxidative defense (e.g., glutathione, cysteine, glutamine, glycine, and taurine); metamorphosis (e.g., tyrosine); pigmentation (e.g., melanin); gut development (e.g., taurine, glutamine, arginine, threonine, and polyamines); neuronal development (e.g., arginine, taurine, and creatine); stress responses (e.g., tryptophan, serotonin, branched-chain amino acids and glutamine); reproduction (e.g., polyamines, arginine, melatonin, and hydroxyproline); and suppression of aggressive behavior (e.g., tryptophan and serotonin) in aquatic animals. Also, certain amino acids (glutamate, histidine, and glycine) impact taste, texture, and even post-mortem seafood quality. Advances in amino acids nutrition technologies and their application to formulate functional and environmentally oriented aqua feeds can be observed in the coming decade (Li et al. 2009).

2.6 Single-Cell Protein (SCP) as Aqua Feed Additive

Single-cell proteins are microbial cells including bacteria, fungi, molds, and microalgae that can be used as an alternative source of proteins (Umesh et al. 2019). There are many advantages of SCP over plant-based protein sources. One of these is that these microbes can grow on cheaper carbon sources without compromising on the protein content (Asmamaw and Fassil 2014). For instance, yeast could be cultivated in sugarcane bagasse-based media yielding 250 tonnes of biomass protein within 24 h. 20 tonnes of algal biomass could be harvested per year from a simple open pond culture system with minimum investment. On the other hand, plants require a considerable area and time to grow and successfully survive any diseases or environmental stress (Guedes et al. 2015). SCP are also a storehouse of essential amino acids like lysine, methionine, and threonine that cannot be supplied by plant-based proteins (Asmamaw and Fassil 2014; Sharif et al. 2021). SCP has been used as a source of food for space travellers due to their ability to provide substantial amounts of energy. The major sources of SCP are discussed below in brief.

2.6.1 Microalgae

Microalgae are single-celled autotrophic organisms that can live on both fresh water and in marine water. The common examples of microalgae routinely used as SCP include *Chlorella* sp. and *Spirulina* sp. (Radhakrishnan et al. 2014). The commercial cultivation of microalgae to serve as SCP began in the 1960s with *Chlorella* sp. being the first commercial cultivar. Presently annual dry biomass of microalgae produced globally is around 19,000 tonnes (Jacob-Lopes et al. 2019), out of which only 0.7% is available for use in aquaculture (Hua et al. 2019). This suggests that more innovations and development are needed to produce the required amount of microalgae to meet their increasing demand in aquaculture as a protein supplement. Microalgae are rich in polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). They also contain appreciable amounts of vitamins like vitamin A, B, C, E, folic acid, and pantothenic acid. They are also rich in essential amino acids, carbohydrates, and important fatty acids like linolenic acid (Jovanovic et al. 2021; Das et al. 2015; Jovanovic et al. 2021). Microalgae contain 60–70% protein and are mainly used for the production of omega-3-fatty acids (Jones et al. 2020). Cultivation of microalgae is safe and environmentally sustainable. They are known for their promising antioxidant, antimicrobial, and immunostimulatory effects which are of great significance in aquaculture. Microalgae contain pigments like carotenoids and astaxanthin which helps to bring flesh color to salmonids and other fishes (Dineshbabu et al. 2019).

2.6.2 Bacteria

Bacteria are another important source of SCP production. Bacteria contain >80% of protein in its biomass (Hua et al. 2019). It also contains important vitamins and phospholipids. The main advantage of bacterial biomass cultivation is that they can grow over cheap carbon sources and are amenable to process control strategies for increasing biomass yield. Bacteria can utilize methane, methanol, syngas, carbon dioxide, and H_2 as cheap substrates for SCP production. This offers an added advantage that along with biomass cultivation, pollutants and greenhouse gases can also be removed (Jones et al. 2020). Several bacteria including purple non-sulfur bacteria are used for SCP production. They contain a substantial amount of carotenoids and vitamins and are observed to provide pathogen resistance in hosts. Purple non-sulfur bacteria are known to contain factors that resist ammonia stress as well which is of great significance in aquaculture (Alloul et al. 2021). Hence if incorporated in fish feed, this attribute can be expected to transfer to the fishes feeding on SCP thereby enhancing their stress tolerance. The main advantage of culturing purple non-sulfur bacteria is that they could be grown in various growth conditions. They may be photoautotrophic or photoheterotrophic, anaerobic or microaerobic which grows in light conditions, or heterotrophic and aerobic under dark conditions (Chumpol et al. 2018). Another potential source of bacterial SCP is purple phototrophic bacteria. They contain >60% crude proteins and important components like polyhydroxyalkanoates (PHA). The major feature which makes bacterial biomass a good source of SCP is the digestibility of its cell wall. Due to this feature fishes can easily feed on bacterial biomass. Also, the amino acid profile of microbial protein is comparable to that of commercial fish feed (Delamare-Deboutteville et al. 2019).

2.6.3 Fungi and Yeast

Yeast and fungi are good sources of SCP (Umesh et al. 2017). They contain 30–50% protein and contain valuable amino acids and vitamin B-complex (Jones et al. 2020). The cell wall of yeast and fungi contain substantial amounts of β-glucan which was reported to provide immunity. Brewer’s yeast is the most commonly used source of yeast SCP (Andrews et al. 2011). Fungi like *Saccharomyces cerevisiae*, *Aspergillus* sp., and *Fusarium venenatum* are commercially explored for feed formulation. *Candida utilis* biomass was found to replace salmon and shrimp diet protein requirement by 40% (Jones et al. 2020). Reports suggested that 1% yeast extract when given to fingerlings of *Labeo rohita* increased their survival rate and respiratory bust activity. It also increased their total leucocyte count, serum protein level, and globulin activity which accounts for rendering immune power to the fishes (Andrews et al. 2011).

2.6.4 Challenges in Using SCP as Aqua Feed Additive

There are many challenges faced when SCP is used as a source of protein alternative in aquaculture feeds. Microalgae when used in aqua feed can be limited only to filtering mollusk and other true phytoplankton feeders. Some fishes can consume microalgae only during their larval stage (Muller-Feuga 2013). Thus despite being a potential source of SCP, microalgae are limited to certain species or certain life stages of fishes. For microalgae feeders, the next challenge could be its digestibility.

This could create problems with lower feed intake and feed conversion ability. So, while using microalgae it is important that the cell wall needs to be removed using chemical, mechanical, or enzymatic methods. Though it is a simple process it can contribute to the increase in cost of the final product. Another disadvantage with microalgae is the presence of phytic acid. Phytic acid reduces phosphate solubilization making the phosphates unavailable for consumption. A feed preparation with phytate solubilizers can solve these problems, but more research needs to be done in order to check the implications of such a feed formulation in aquaculture (Jones et al. 2020). In case of bacteria and other microbes, media preparation without contamination and maintaining culture conditions could be a rigorous task. Much care should be given in downstream processing, contributing to an increase in the production cost. Also, ethical issues need to be cleared while formulating such a diet.

2.7 Keratin in Aqua Feeds

Keratin is the second largest biopolymer after collagen and is found in all vertebrates as outer covering called integument. Keratin protein is found in skin, hair, wool, feather, quill, horn, and nails. Despite being a widespread protein, it is very difficult to digest keratin to make the proteins available in animal or fish feed. The main reason for its structural stability is the presence of disulfide bonds between cysteine amino acids present in the keratin molecule (McKittrick et al. 2012). Keratin protein is so strong that only 16% of it is digestible (Estévez et al. 2006). It is one of the richest sources of essential amino acids, containing 18–20 different types of amino acids. These amino acids are locked inside hydrophobic, intra and intermolecular disulfide bonds, ionic bonds and hydrogen bonds so that it is not easily available for consumption (Bhushan 2010; Ayutthaya et al. 2015). Even though there are a wide range of keratin sources, the main source from which keratin protein is being extracted for feed formulation is feather waste. Feathers, especially chicken feather wastes, are easily available from slaughter houses and are also a cheaper source of keratin. They contain 85–90% keratin protein (Estévez et al. 2006). In addition to being a cheaper source of keratin, feathers also have a larger surface area when compared to other keratin sources like nails and wool which make it easy for absorption and for pretreatments. Amino acid profile of feather meal matches with the amino acids needed for fish meal which adds to the value of the product. About 60,00,000 tonnes of feather meal are produced annually to incorporate into animal and aquaculture feed (Adler et al. 2018). These feather meals are prepared using different strategies. Conventional methods use a combination of steam and pressure for digesting feathers as keratin is a very stable protein. Temperature-pressure treatments, chemical hydrolysis, and steam explosion have proved to be capable of breaking down feathers to a more digestible form (Zhang et al. 2014). The common practice is steam hydrolysis under 15 psi pressure at 140 °C incorporating an acid or an alkali treatment or in the presence of a disulfide reducing agent. Even though this method is effective, they may cause a loss of important amino acids from the protein

(Łaba et al. 2015). So, the recent research focuses on using fermented feather instead of hydrolyzed feather for feed formulation experiments. Over the past few decades the fermented feather meal was recognized as more advantageous than hydrolyzed feather meal. Fermentation increases digestibility of feather from 39.09% to 48.75% (Adelina et al. 2021). For fermentation, many agents can be used, the important one being the microbes itself. Enzyme treated feathers are found to be more digestible, palatable and the amino acids released from them are more bioavailable (Mendoza et al. 2001). Rendered feather meals have been used in salmonids feed for decades (Martínez-Alvarez et al. 2015). In order to replace the conventional plant or forage fish based protein sources, many experimental trials on keratin feeding have been done in the past few decades. One such feeding experiment was done on juvenile gilthead seabream *Sparus aurata* where 25%, 35%, and 50% of the fish feed was substituted with treated chicken feather meal. It was found that 50% treated feather meal was palatable and digestible and thus opened a promising window for incorporation of keratin in aqua feed. The amino acid profiling of the treated feather meal revealed that it contained high amounts of essential amino acids and polypeptides (Al-Souti et al. 2019). The work of Psofakis et al. (2020) proved that 25% addition of keratin protein in the feed of *Sparus aurata* maintained the digestive enzyme activity and other hematological parameters in its normal level suggesting that the replacement of usual protein meal with animal by-products will not affect the metabolism or other growth parameters of fishes (Psofakis et al. 2020). 76% replacement of commercial protein with keratin in aqua feed is acceptable and is expected to give the same result as that of the commercial feed (Campos et al. 2017). One of the added benefits of using keratin protein in aquafeed is that it contains a lot of elements like calcium (0.16%), phosphorous (0.04%), potassium (0.15%), and sodium (0.15%) that helps in various metabolic processes in fishes (Chor et al. 2013).

A feeding trial done on silver pompano *Trachinotus blochii* was successful with 20% feed addition with fermented keratin protein. It was found to increase growth rate, feed efficiency and protein efficiency ratio with a satisfactory proximate composition of fish caress (Adelina et al. 2021). An experiment to ferment keratin protein with *Bacillus* sp. proved that fermentation can result in the increased protein content in fish meal. An addition of 10 ml of bacterial inoculum to ferment 2 g of feather keratin was found to be optimum with 85–90% protein recovery (Mulia et al. 2016). A feeding trial on red tilapia *Oreochromis* sp. proved that 6% addition of keratin protein in fish meal of tilapia fish is optimum to meet the protein need of tilapia. The addition of 6% keratin protein was successful as it increased the fat content and protein content in fish carcasses. The proximate analysis of fish carcass revealed the crude lipid content to be 0.83%, crude fiber content to be 2.15%, protein content to be 82.36%, ash content to be 1.49%, moisture content to be 12.33% with 64.47% carbon content, 10.41% nitrogen content and 2.46% sulfur content (Nursinatrio and Nugroho 2019; Tesfaye et al. 2017). In addition to fermentation, irradiation was done on feather meal to improve its digestibility. The experiment was a random one and it was successful. The experiment gave its findings that irradiated feather meal could replace fish meal by 180 g/kg weight of fish meal. The feeding trial was done on largemouth bass *Micropterus salmoides* (Ren et al. 2020).

The main challenge in using keratin as a protein source in aquaculture is its digestibility. Keratin is a stubborn structural protein (Ayutthaya et al. 2015) and requires a lot of energy to release the amino acids locked up inside. But fermentation has proved to be an effective method to digest keratin so that its amino acids are available in feed without degradation. The second challenge is the availability of keratin sources. Presently feather waste, especially chicken feather wastes, are being used for isolation of keratin protein. Even though there are many other sources like wool, and hog hair, they require a lot of pretreatments before keratin digestion making the process to be economically non-viable. There are fewer reports on the negative effects of using keratin protein as well. A work done on Anguilla *bicolor* showed a negative effect on the growth when feather meals were incorporated in fish feed (Thamren et al. 2018). Also, growth rate of *Sparus aurata* was found to be negatively affected as feather keratin downregulated live gene expression for growth (Psofakis et al. 2020). Research on negative effects and the way to nullify the problem need to be done so that a better protein alternative for feed addition could be formulated.

2.8 Polyhydroxyalkanoates (PHAs) as Aqua Feed Additive

Immunostimulant usage has gained importance in recent years for prophylaxis and infection control, because of its ecofriendliness and biocompatible nature in aquaculture (Umesh and Santhosh 2021). The hosts are protected from infections by their immunostimulants modulating the immune system (Bricknell and Dalmo 2005). Different groups of chemicals, biologicals, secondary metabolites from plants and microbe derived compounds have proved their effectiveness as immunomodulators in aquaculture (Sakai 1999). Different heterotrophic microbes forming biofloc were reported to promote the immunomodulatory effects in the reared organism in the aquaculture system (Crab et al. 2012). Most of the immunomodulatory methods like phage therapy and vaccinations demand high application cost and labor-intensive procedures making them no longer sustainable. This reason triggers the use of Short Chain Fatty Acids (SCFAs) and their polymers as immunostimulants in aquaculture. These SCFAs are organic fatty acids containing 1–6 carbon atoms, usually derived from oligosaccharides, polysaccharides, peptides, proteins, and glycoproteins via fermentation (Hoseinifar et al. 2017). One of such SCFA polymer families is the Polyhydroxyalkanoates, which are accumulated under nutrient imbalance conditions in many microbes as an intracellular energy reserve (Anderson and Dawes 1990). These PHAs are polymers of hydroxyalkanoates. Among the PHA family polyhydroxybutyrate (PHB) is the most widely studied (Umesh and Basheer 2018; Umesh and Preethi 2017). These compounds are fully biodegradable and fully biocompatible, which is an ideal quality to choose for packaging, drug delivery, and in other biomedical applications (Umesh et al. 2018a; Chee et al. 2019; Umesh and Thazeem 2019). But when it comes to commercialization of PHA a huge block is in place due to the high cost for fermentation and downstream processes that accelerates the final product cost (Preethi and Vineetha 2015). With the emergence of production strategy based on agro waste and environment friendly down streaming strategies, the light on PHA research is brightening (Umesh et al. 2018b). Reports of strong immunomodulatory and growth promoting effects were obtained when PHAs were used in aquaculture, by incorporating into the aqua feed (Gao et al. 2019; Suguna et al. 2014).

2.8.1 PHA Biosynthesis and Enzymology

All microbes take up the carbon source to generate energy for metabolism, the most preferred carbon source by microbes are sugar molecules (Umesh and Thazeem 2019). Sugar molecules undergo glycolysis to get converted to pyruvates. The pyruvates are converted to acetyl CoA which normally enters the Krebs cycle to produce energy molecules. As discussed earlier, PHA production happens when the microbes are in stressed condition with excess sugar, so in such PHA producers, the acetyl CoA produced from pyruvate will not enter the Krebs cycle, by arresting the β Ketothiolase enzyme activity. This will cause accumulation of acetyl CoA. When this compound is excess in the cell, PHA biosynthesis is force started (Umesh et al. 2021). Detailed molecular studies into the PHA biosynthesis pathway proved the existence of multiple biosynthesis pathways of PHA production with small differences. The most accepted and most referred PHA biosynthesis pathway is observed in *Cupriavidus necator* (Liebergesell et al. 1993). In this microbe there are 3 genes which are responsible for PHA biosynthesis, namely: *pha* A, *pha* B, and *pha* C, which codes the key PHA synthesis enzymes β-Ketoacyl-CoA thiolase, acetoacetyl-CoA dehydrogenase, and PHA synthase enzyme, respectively. β-Ketoacyl-CoA converts acetyl CoA molecules to acetoacetyl CoA. By the action of NADPH dependent acetoacetyl-CoA dehydrogenase, the acetoacetyl CoA condenses to (R)-3-hydroxybutyrate monomer unit. These monomer units are polymerized into PHAby enzyme PHA synthase (Anderson and Dawes 1990; Tsuge et al. 2005).

Among the different known applications of PHA like food processing and biomedical, its applications in the field of aquaculture are at its dawn. The major areas of research on the topic of PHA in aquaculture are PHA as feed additive and PHA in biofloc technology as a denitrification system. Due to the high growth profiles and immunological responses of PHA fed shrimps and fishes, the application of PHA in aqua feed are being focussed by many researchers during the past decades. Although many reports of high immunomodulatory effects have been reported, the actual molecular mechanism to this cause is yet to be discovered (Umesh and Santhosh 2021). The larval stage of the target organism is selected for the study of effects of different PHA on the growth performance and immunity, as larval stages are more sensitive and responsive physiologically and physically as compared to the adults. The larval stages are also prone to many diseases as they lack the adaptive immunity and these stages can give results to any exposed or fed chemicals quickly. Hence they are preferred to study the toxic effects, immunomodulatory effects of any given compounds (Castro et al. 2016).

In the studies conducted using different aquatic organisms by supplementing the different PHA molecules in the diet reported that the organism showed higher survival rate, growth rate and other growth related results. When Zoea larvae were fed with PHB enriched live feed, a significant increase in the survival rate, growth qualities and osmotic shock tolerance were observed. The reason for the growth is attributed to the additional energy provided by the digestion of PHB; the PHB enhances the absorption capability of the colonic cells by providing this extra energy (Sui et al. 2014). The supplementation of PHB in the feed can increase the crude protein and crude lipid content in organisms like *Oncorhynchus mykiss* fingerlings (Yaqoob et al. 2018). The partial or full degradation of PHAs will release β-hydroxy short chain fatty acids which will enter the lipid biosynthesis pathway (Clements et al. 1994). PHB are short chain fatty acids which will obviously change the pH in the gut of the juvenile organisms easily. This can be the reason to alter and increase the digestive enzyme activities and hematological parameters (De Schryver et al. 2010). The decrease in the pH will also arrest the infection chances of opportunistic pathogens which enters the gut. The PHA will also alter the microbial community in the gut; it will promote the growth of certain useful microbes which have the PHA degrading enzymes.

When different species like *Oreochromis mossambicus, Penaeus monodon, Liza haematocheila*, and *Labeo rohita* were fed with different PHAs, it was found that the total immune responses showed significant increase (Suguna et al. 2014; Misra et al. 2006; Laranja et al. 2017; Qiao et al. 2019). There are reports that organisms fed directly with PHA as feed and fed with live feeds enriched with PHA showed increased immune activity (Suguna et al. 2014; Laranja et al. 2017). For studying the nonspecific immune responses the total antiprotease activity, peroxidase activity, and lysozyme activity are analyzed and for understanding the total immune activity the host organisms should be challenged with virulent strains of common infectious microbes. The antiprotease activity indicates the level of total serum protease level. When pathogens invade a tissue cell, lysing enzymes are released, most of them get activated only after chelation, and the serum proteases will block the chelation and suppress the invasion. The hosts are protected from both gram-negative and gram-positive bacteria by lysozymes. In aquatic organism lysozymes are directly correlated with the phagocytic neutrophils, so an increased lysozyme activity attribute to increased number of active neutrophils (Misra et al. 2006). Other immunostimulatory effect reported by feeding PHA is that the transcriptional activities of genes controlling prophenoloxidase gene (proPO), transglutaminase gene (TGase), heat shock protein 70, penicillin-binding protein A (pbpA), aldehyde oxidase (AOX), interleukin-8 (IL-8), and major histocompatibility complex class II (MHC II) are upregulated (Morimoto 1998; Amparyup et al. 2013; Maningas et al. 2008; Ishida et al. 2006; Baggiolini et al. 1993; Wright et al. 1997; Ryhänen et al. 1991). Another immunological activity which showed a significant increase is the antioxidant enzyme activity. The main antioxidant enzymes which play a crucial role in the immune system’s first line of defense are superoxide dismutase, catalase, and total antioxidant capacity. Superoxide dismutase removes the excess of reactive oxygen species, thus reducing the chances of adverse reactions (Meng et al. 2013). Catalases prevent the hydroxyl radical toxicity and convert peroxides into water and oxygen (Bagnyukova et al. 2005). As discussed above PHA can change the pH and microbial community in the gut. Before the Soiny mullets were fed with PHA the microbial community present in the gut were *Lactococcus, Bacillus, Carnobacterium, Achromobacter,* and *Delftia.* After feeding with PHA there was a significant increase in the population of intestinal *Bacillus* spp. Furthermore when analyzed with Kyoto Encyclopedia of Genes and Genomes (KEGG) it was found that the genes which regulate some secondary metabolites biosynthesis, major metabolism pathways, signalling molecules and interaction, immune system, and other downregulated genes involved in disease pathways, were significantly upregulated in PHB fed organisms (Semova et al. 2012). Table 2.1 shows the effect of PHA supplementation in aquaculture.

By the use of PHA as immunostimulant and growth enhancer, it tends to replace the traditional antibiotic administration for resisting pathogens. More research is needed on a global basis to fully understand how the administration of PHA is upregulating the immune system and growth. The non-toxic, fully degrading nature of PHA in the host should be also given importance in research. PHAs may become an alternative to the antibiotics and other disinfectants used in aquaculture in the near future if proper studies are done.

2.9 Chitosan in Aquaculture

Chitosan is a biopolymer which is extracted by the deacetylation of chitin. Chitosan is a highly basic polysaccharide which exhibits various properties such as solubility in different media, mucoadhesive property, polyoxy salt formation, viscosity, polyelectrolyte behavior, potential to form films, metal chelating property, optical and structural characteristics (Shukla et al. 2013). Chitosan is also known for its antimicrobial properties and free-radical scavenging property, which increases its scope in pharmaceutical applications (Shariatinia 2019). These properties increase the potential of chitosan to be applied in different fields such as cosmetics, agriculture, food industry, textile industry, bioimaging, tissue engineering, and in several other biotechnological applications (Lodhi et al. 2014). In natural conditions, chitosan is present in the cell walls of fungi, green algae, yeast, and protozoa and also in the exoskeletons of crustaceans and cuticles of insects (Alishahi and Aïder 2012). Chitosan consists of glucosamine and acetyl-glucosamine units which are accessible in various grades depending upon the degree of acetylated moieties and the degree of acetylation controls several properties of chitosan (Sorlier et al. 2002). Deacetylation is a process which contains elimination of acetyl groups from the molecular chain of chitin in order to obtain chitosan.

Chitosan is a part of a linear polysaccharides family which is a linear copolymer of β-(1–4)-linked N-acetyl-2-amino-2-deoxy-d-glucose (acetylated, A-unit) and

Table 2.1 Effect of PHA supplementation in aquaculture

Scientific name	Common name of the host	Effects of PHA supplementation on the organism	References
Mytilus edulis	Blue mussel	Increased survival and improved growth and development	Van Hung et al. (2015)
		Enhanced larval development and survival	Thai et al. (2014))
Litopenaeus vannamei	White leg shrimp	When fed with PHB along with glucose, an increased survival rate and final weight was observed	Luo et al. (2019)
		Improved growth, survival and robustness of the larvae when exposed and not exposed to pathogenic *Vibrio anguillarum.*	Gao et al. (2019)
Artemia franciscana	Brine shrimp	Survival of the starved nauplii were increased when fed with PHA were challenged with *Vibrio campbellii*	Defoirdt et al. (2007)
		Remarkable survival was observed in *Artemia* fed with 100 mg l^{-1}, when challenged with *Vibrio campbellii*	Baruah et al. (2015)
		Better survival when challenged with *Vibrio campbellii*	Halet et al. (2007)
Oreochromis niloticus	Nile tilapia	Increased serum lysozyme activity, serum peroxidase activity and immune response as host showed resistance towards virulent *Aeromonas hydrophila* strain when feed was added with 5% PHB	Suguna et al. (2014)
		Increased survival rate by 20% and increased lipase activity was observed when the larvae fed with PHB was challenged with *Edwardsiella ictaluri* gly09R	Situmorang et al. (2016)
Dicentrarchus labrax	European sea bass	Survival was enhanced when fed with 2%, 5% and 10%, the maximum weight gain was observed when 5% PHB was fed and overall decrease in the pH was also observed.	De Schryver et al. (2010)
Oncorhynchus mykiss	Rainbow trout	When fed with 1% PHB, increased specific growth rate, weight gain and remarkably higher specific activity of the total protease and amylase were seen. Enhanced immunostimulation and survival when challenged with *Yersinia ruckeri.*	Najdegerami (2020)
Acipenser baerii	Siberian sturgeon	Accumulation of more whole-body lipid content and pepsin activity when fed with PHB and PHB-HUFA	Najdegerami et al. (2015)
		Enhanced specific growth rate, survival and weight gain when fed with 2% PHB	Najdegerami et al. (2012)
Penaeus monodon	Giant tiger prawn	Increased growth and survival of the larvae which were exposed and not exposed to pathogens. High survival was observed when exposed with ammonium chloride	Laranja et al. (2014)

(continued)

Table 2.1 (continued)

Scientific name	Common name of the host	Effects of PHA supplementation on the organism	References
Eriocheir sinensis	Chinese mitten crab	Increased osmotic stress tolerance, developmental rate, and survival.	Sui et al. (2014)
		Growth and survival were enhanced when challenged and not challenged with *Vibrio anguillarum*	Sui et al. (2012)

2-amino-2-deoxy-d-glucose (deacetylated, D-units) (Kaur and Dhillon 2014). This arrangement in chitosan ends up in showing a rigid crystalline structure through inter- and intramolecular hydrogen bonding (Roberts 1992). On every glycosidic residue of chitosan, there is one –NH2 group and two –OH groups which provide various chemical as well as biological properties to chitosan. Due to these properties chitosan is majorly used in pharmaceutical applications. Drug delivery systems which use chitosan are gaining interest as they act as carriers and are able to release contents such as small active molecules, proteins, peptides, vaccines, genes, and oligonucleotides which are released at specific rate and location in the body (Vatanparast and Shariatinia 2018). Chitosan also shows wound healing properties (Zhang et al. 2018) and several other applications in the pharmaceutical sector such as in making contact lenses (Anirudhan et al. 2016), bioimaging (Agrawal et al. 2010), etc.

The extraction of chitosan is majorly done by chemical and biological methods where the chemical methods involve removal of calcium carbonates and proteins, using strong acids and bases, demineralization and deproteinization in biological methods involves bacteria which produces lactic acid and proteases from bacteria and the process of deacetylation is performed using enzymatic methods by chitin deacetylase (El Knidri et al. 2018). Obtaining industrial scale chitosan with completely removed organic salts is the major advantage of using chemical methods of extraction along with the short processing time. Biological methods of extraction on the other hand are environmentally safe but have longer processing time than chemical methods. The three main steps involved in the extraction process of chitosan are demineralization, deproteinization, and deacetylation, where demineralization is the process of eliminating the calcium carbonate and calcium chloride, deproteinization is the step where proteins are removed, and deacetylation is the process where chitin is converted to chitosan by removal of acetyl groups (Table 2.2).

In recent years the commercial use of chitosan has been increasing and demanding. Due to the versatile feature and property of chitosan, research based on its use in the seafood industry and as an immunostimulant is showing an exponential growth. Chitosan has been studied for its antimicrobial activity; this property of the substance has been explored for its application to extend the shelf life of marine-based food products. Chitosan can be used as an antibacterial food additive in the different food industries (Cao et al. 2009). Researchers have studied the effect of chitosan as

Table 2.2 Comparison of the degree of deacetylation of various aquaculture substrates and different extraction process

Raw materials	Extraction methods	Deacetylation degree%	References
Shrimp shells	Demineralization: 1 M HCl, ratio 1:15, at optimal temperature Deproteinization: 1 M NaOH, ratio 1:15, at 100 °C for 8 h Deacetylation: 50% NaOH, ratio 1:15, at 100 °C for 8 h	74	Marei et al. (2016)
Squid gladius (*Loligo vulgaris*)	Demineralization: 1.5 M HCl, ratio 1:10, at 50 °C for 8 h, Enzyme/substrate 10 U/mg, 3 h at pH 8 at 50 °C using Alcalase Deacetylation: 50% NaOH, at 120 °C for 4 h	71	Abdelmalek et al. (2017)
Squid pens (*Doryteuthis* spp.)	Ultrasound-assisted deacetylation (USAD) of β-chitin in 40% NaOH with ratio 1:10, for 50 min at 60 °C. The process was performed twice.	95.7	Fiamingo et al. (2016)
Larvae (*Zophobas morio*)	Demineralization: 1 M HCl, ratio 1:20, 35 °C water bath for 30 min Deproteinization: 2 M NaOH, ratio 1:20, 80 °C water bath for 20 h Deacetylation: 50% NaOH, ratio 1:20, 90 °C water bath for 30 h	74.14	Soon et al. (2018)
Catharsius molossus residue	Demineralization: 1.3 M HCl, at 80 °C for 30 min, kept 12 h at room temperature. Deproteinization: 4 M NaOH, at 90 °C for 6 h Deacetylation: Chitin was soaked in 18 M NaOH, at room temperature for 24 h. Then heated at 90 °C for 7 h and the alkali solution was respectively replaced at 3, 5, 7 h	94.9	Ma et al. (2015)
Fish scales (*Labeo rohita*)	Demineralization: 1% HCl, 36 h at room temperature Deproteinization: 0.5 N NaOH, 18 h at room temperature Deacetylation: 50% NaOH, 80 °C in oil bath for 2 h	–	Kumari and Rath (2014)
Antarctic krill (*Euphausia superba*)	Demineralization: 1.7 M HCl, 6 h at ambient temperature Deacetylation: 2.5 M NaOH at 75 °C for 1 h	11.28	Wang et al. (2013)
Cephalothorax (*Macrobrachium rosenbergii*)	USAD of α-chitin in 40% NaOH, ratio 1: 44, alternation of irradiation and non-irradiation periods with total time 45 min and 30 min respectively	77.9	Birolli et al. (2016)

(continued)

Table 2.2 (continued)

Raw materials	Extraction methods	Deacetylation degree%	References
Cuttlefish pens (*Sepia* spp.)	Demineralization: 1.0 M HCl, ratio 1:40, at optimum temperature for 3 h Deproteinization: 1.0 M NaOH, ratio 1: 20, at 70 °C for 24 h Deacetylation: 45% NaOH in the ratio 1: 15, at 600 W for 15 minutes	93	Sagheer et al. (2009)
Metapenaeus stebbingi shells	Demineralization: 2.5 N NaOH, at 65 °C for 6 h Deproteinization: 1.7 N HCl, at 25 °C for 6 h Deacetylation: 50% NaOH, at 120 °C	92.19	Kucukgulmez et al. (2011)

food additives and it was observed that chitosan increased the shelf life of fish balls (Kok and Park 2007) . Fernandez-Saiz et al. on the other hand studied its effect on fish soup and reported a reduction in the growth of many bacteria when chitosan was used as a preserve (Fernandez-Saiz et al. 2010) . Chitosan possesses the property of an antioxidant which further assists with the preservation process. The seafood industry has used and researched on the various aspects of the application of chitosan for film-forming ability, gel enhancement, encapsulation, a tissue engineering scaffold, and more in order to improve seafood quality.

2.9.1 Biomedical Applications in Aquaculture

The use of nanotechnology has shown tremendous growth in recent years and its use for the creation of chitosan nanoparticles has been recently studied. Chitosan nanoparticle and its application in the biomedical and aquaculture field has shown potential advancement. The chitosan polysaccharide and its derivatives are widely used in the areas of fish biotechnology, fish genetics, fish reproduction, aquatic health, etc. (Sharma and Ahmad 2013). The potential application of chitosan on aquatics has been widely researched which can be utilized for animal health, production, prevention, and treatment of diseases. There are properties of the chitosan structure such as the NH2 and hydroxyl group which make it suitable for certain specific chemical reactions for its biomedical uses. Chitosan has been studied for its antimicrobial activity against gram-positive and -negative bacteria along with certain fungal fish pathogen as well (Luo et al. 2011). The antimicrobial activity contributes to its use in various other industries such as for water disinfection of fish farms, certain food processing industry, and other medical areas. Chitosan-based products have biomedical applications which extend from their use as a dietary supplement for the freshwater fish species. The study conducted by Cha et al. shows the effect of using chitosan coated diet supplements for olive flounder which further showed to improve the water quality as well (Cha et al. 2008). The use of these chitosan-based supplements provides an enhanced survival rate, better growth, and improved meat quality. One of the other applications of chitosan is its use as a vaccine for fishes (Ferosekhan et al. 2014). There are studies conducted that show the use of chitosan as a drug oral vaccine delivery, the primary reason for it to be used as a delivery system is due to its high solubility, bioavailability, and its ability to penetrate through tissues (Shi et al. 2010). The proprietary use of nanoencapsulation technology for the release of bioactive ingredients has also incorporated the use of chitosan as a carrier. The most common encapsulated ingredients that have been studied are vitamins (Alishahi et al. 2011), hormones (Wisdom et al. 2018), enzymes, bioactive ingredients, and more. Researchers like Fernandes et al. have studied the use of chitosan as a fish disease diagnostic method. *Aeromonas* spp. which is a fish pathogen can be detected using chitosan-based nanoparticles (Fernandes et al. 2015). Thus, although there are few areas in the biomedical application that need to be studied thoroughly the use of chitosan in fish medicine has shown tremendous potential with the promising application as carriers, dietary supplements, antimicrobial activities, disease diagnosis, and more.

2.10 Cellulose as Aqua Feed Additive

With the advent of plant components to the fish feed have consequently made the addition of fiber inevitable (Sun et al. 2019). Cellulose is one such fiber that is available in abundance. It is generally found in the cell wall of plants, particularly in the stalk, trunk, and woody portion of the plant tissue (O'sullivan 1997). It is a straight chain polymer composed of glucose molecules linked together by β 1–4 glycosidic bonds (Hansen and Storebakken 2007). Cellulose from plants can be isolated in a series of steps, which includes dewaxing (Floros et al. 1987), alkali treatment (García et al. 2013), and bleaching (Rehman et al. 2018). It can be recovered in different forms such as microcrystals, nanofibers, and so on. There are even numerous applications of cellulose in various sectors. In aquaculture, cellulose is also utilized as an aerogel for wastewater treatments other than being an aquafeed additive (Darabitabar et al. 2020). However, most fishes lack the ability to digest cellulose due to the absence of enzyme cellulase (Sun et al. 2019). Due to its inability to digest, cellulose in fishes is even considered to be non-nutritional if the addition of cellulose in feed exceeds 7%. Despite this fact, there is adequate other research that suggests cellulose in fish can be above 7% and the capability varies within the fish species. The studies moreover showed that the cellulose fed to the fishes have shown a positive influence in growth rate (Ashraf et al. 2014). Therefore cellulose can be utilized as a successful aqua feed additive to an extent.

2.10.1 Effect of Cellulose in Fishes

The expansion of intensive aquaculture is an effective tool to meet the human consumption rate. Compound feed is one of the bases for intensive aquaculture. However, the high price for aqua feed makes it unaffordable for some fish farmers. In this regard, a compound feed with balanced nutrition at an affordable rate has to be assessed for fulfilling the aquaculture requirements. Concerning this aspect, addition of fiber can be included to the feed from naturally available plants, since addition of plant components in fish feed formulations is progressing. With reference to plant fiber, cellulose is one of the major plant components that are widely available. It is a polysaccharide composed of glucose and accounts for more than 50% of carbon content (Sinha et al. 2011). Also, the current research suggests that the addition of cellulose in feed improves feed stickiness, stimulates digestive tract peristalsis, and promotes digestion and absorption (Sun et al. 2019). Therefore, an optimal amount of cellulose content in fish feed needs to be assessed as it differs among various fish species which are discussed here.

Misgurnus anguillicaudatus, commonly known as pond loach, is a freshwater fish that belongs to the family Cobitidae of the order Cypriniformes. The richness of nutrients and pleasant taste makes the fish popular among the Chinese, with its market demand expanding every year. Prior to the experiment, the juvenile Taiwanese loaches were fed initially with commercial feed to acclimatize the fish to the experimental environment. The wheat bran was modulated to attain different fiber levels. The six groups of feed contained crude fiber content of 4.70%, 4.92%, 5.15%, 5.44%, 5.79% and 6.06% respectively. The results indicated that the feed containing crude fiber had significant effects on loaches, in reference to feeding rate, protein efficiency ratio, and feed conversion rate. However, there was no difference in specific growth rate. Also research indicated high fiber content will have a negative effect in utilization of other nutrients. This was due to the excessive amount of cellulose that makes the nutrients pass at a faster rate through the digestive tract. This eventually leads to a shorter digestion time and therefore lower digestibility rate (Krogdahl et al. 2005; Sun et al. 2019). Hence, appropriate fiber content is always recommended to improve the digestive efficiency as well as to yield enough nutrients and energy in loach. Therefore the optimal level of cellulose fiber applicable for Taiwanese loach ranges from 5.52% to 5.65%. In addition, the fiber content could also improve the antioxidant ability of Taiwanese loach. This was due to the presence of beta glucan in the fiber, which is also known to enhance the immunity level in loaches (Guzmán-Villanueva et al. 2014; Zhao et al. 2012; Sun et al. 2019). While in juvenile Tilapia (*Oreochromis mossambicus*) the optimal level of cellulose fiber was much lower than the loaches. Initially the juvenile tilapia was acclimatized to experimental conditions for about a week. The diet formulation either contained a minimum amount of fiber or without any fiber at all. The results indicated, the tilapia fed with 2.5% or 5% cellulose showed a better growth rate than those fed with a higher fiber or those without cellulose. Also, the survival rate declined as the cellulose content increased above the optimal level. Similarly the protein efficiency ratio increased with cellulose fiber up to 2.5%, beyond which it decreased (Dioundick and Stom 1990). Another study on tilapia however contradicts with the optimal level of fiber, as the study confirms a 10% dietary fiber is applicable (Anderson et al. 1984). This higher amount of cellulose fiber did not show reduction in growth rate which therefore indicates the optimal level could be beyond 5%. Research on tilapia even suggested that the long digestive tract allows them to utilize cellulose and other carbohydrates at high efficiency rates. Also, the extreme low pH in the stomach of tilapia becomes an additional factor for cellulose hydrolysis in the digestive tract. Generally the pH value for most fishes ranges between 2 and 2.2. While in tilapia it can reach up to 1.25 or 1. This extreme low pH facilitates the cellulolytic enzymes from the gut microflora to the fiber (Dioundick and Stom 1990). Therefore, these factors allow tilapia to take up the fiber content up to 10%. Moreover, the optimal fiber content in Nile tilapia (*Oreochromis niloticus*) partially supports the results of both (Dioundick and Stom 1990; Anderson et al. 1984). The Nile tilapia was fed with three diets, each containing α-cellulose from barley husk at 5%, 10%, and 15% respectively. The fish fed with all three diets had a survival ratio of 100%. Also, the average value of weight gain of the fish fed with 5% cellulose was similar to those fed with 10%. While those fed with 15% cellulose showed a lower value than the other two. Although the weight gain of fish was similar in the feed containing 5% and 10% cellulose, the best results for specific growth rate and feed conversion ratio were obtained from the fish feed containing 5% cellulose. In addition, the results also confirmed, the diet formulations should not exceed 10% cellulose in Nile tilapia (Ighwela et al. 2015). Certain other fishes like red sea breams and yellowtails also exhibit a better growth at 10% cellulose (Kono et al. 1987). Similar results were observed in sea bass juveniles, which can uptake up to 10–20% cellulose without affecting the growth parameters (Bromley and Adkins 1984).

Another freshwater fish, Rohu (*Labeo rohita*) is known to be one of the most important fish. The significance is due to consumer preference and it fetches the fish farmers a higher price when marketed. Therefore, the fish farmers are likely to stock this species among their major aquaculture species. On an average it contributes 35% of total stocking and produces 23% of total aquaculture production. In terms of growth among the Indian major carps, rohu comes after catla. Rohu gains maturity in 2 years from spawning and breeds by hypophysation from June to July. In regard to cellulose content, the fish is able to tolerate up to 16% α-cellulose. However, 12% α-cellulose is optimal in terms of growth rate and digestibility of nutrients; beyond this level the growth rate gradually decreases (Ashraf et al. 2014).

While the Atlantic cod (*Gadus morhua* L.) juveniles were able to retain up to 18% cellulose fiber. Generally, the natural diet of cod contains high levels of chitin (Link et al. 2000) which allows the cod to retain high levels of cellulose as well, since both share a β-1,4 glycosidic bonds between the monomers (Lekva et al. 2010). Two dietary mixtures were prepared for the experiment. One diet contained protein from both plant and fish meal while the other contained fish meal alone. Both the diets were supplemented with increasing amounts of cellulose (0%, 6%, 12%, and 18%). Minerals and vitamin sources were also added to the diets. The results confirmed the α-cellulose has not induced a negative effect on protein utilization at any concentration level. The protein efficiency ratio, protein productive value, and fillet yield were found to be equal in both the diets. Indeed, the growth was improved by the addition of cellulose. The addition of cellulose had a positive impact on the fish except the digestibility of fat and dry matter, which decreased upon increase in cellulose. Similarly the liver and muscle size were not influenced by the addition of cellulose. Therefore, as per the results the cod could retain a greater amount of cellulose up to 18% without affecting the growth parameters (Lekva et al. 2010).

A much higher fiber was found to be applicable in rainbow trout with a significant influence on its growth rate. Six experimental diets were prepared containing cellulose ranging from 0% to 50%. Each of these diets was tested for 51 days and the food intake was recorded on a daily basis. Also, the initial and final weight of the fish were analyzed. Prior to weighing, the fish were narcotized in 2-phenoxyethanol and the excess of water was removed. The total weight of food uptake increased due to the addition of dietary cellulose content. Also, there was an occurrence of rapid growth in fish having 0–30% dietary cellulose. The results also confirmed, the fish that took 40–50% dietary cellulose were comparatively smaller in size and had half growth in reference to those having up to 30% cellulose. However, there was a significant increase in stomach size of the fish fed with 40–50% cellulose. Slight variation in weight of hindgut was present due to the influence of dietary cellulose. Yet the liver did not show any size difference, rather it was relatively stable irrespective of cellulose in the diet. Lipid level, protein, and energy gains did have an impact by a low amount of cellulose that is 0–30%. Also, the nutrient energy conversion efficiency and protein conversion efficiency were found to be stable upon a diet fed with 0–30% cellulose; however had a decline when the cellulose content was beyond 30%. Thus the results confirmed, the trout could retain 30% cellulose providing a greater impact on its growth than those fed with 40–50% cellulose in the diet. The diets fed with 40–50% cellulose, though influenced the size of stomach, however could not increase the nutrient intake (Bromley and Adkins 1984). Although, there were discrepancies regarding the same species, as various other research suggested the trout can incorporate cellulose up to 8%, beyond which the growth rate decreased (Hilton et al. 1983; Poston 1986). There was still variation on this aspect, as certain other studies indicated that up to 15% cellulose inclusion in trout does not influence the digestibility of main nutrients (Hansen and Storebakken 2007). Data regarding the contradictory results are inadequate; however the discrepancies may probably be due to quality of feed and variation in the type of cellulose used.

2.11 Enzymes in Aqua Feed: Factors to Consider

Using enzymes in feeds, to improve feed utilization is a concept that has been well researched in both terrestrial and aquatic animal nutrition. The preliminary objective of application of enzymes in feeds is to enhance digestibility. It is suggested that providing an extra supply of enzymes in the feeds would boost the digestive processes, resulting in increased efficiency of the feeds. The aquatic animal population lacks some digestive enzymes during their early developmental stage or throughout their lives. The nutrient fractions digested by the application of these enzymes can be utilized by the aquatic animal that lacks the digestive enzymes (Ghosh and Mukhopadhyay 2006). Phytase, carbohydrases such as amylase, mannanase, galactosidase, xylanase, cellulase, and pectinase, protease, and lipase are the most widely used enzyme additives (Abishag and Betsy 2018).

2.11.1 Anti-Nutritional Factors

Based on existing feed formulations, the amount of the wild fish that are caught is inaccessible, and confusion about the potential access to the resort is a key problem. In 24 years, the supply of pelagic products for fish oil and meals has not increased in a sustainable way (Shepherd and Jackson 2013). As an alternative the plant- and animal-based by-products as sources of proteins in aquafeed are being used. With significant drawbacks and multi anti-nutritional factors, the plant-based proteins are still an important ingredient in the aqua feed (Malcorps et al. 2019). The dry matter indigestion of plant-based nutrients is higher than fish meals. This leads to a large contribution of waste product due to undigested nutrient excretion. The indigestion is mostly due to the non-starch polysaccharides, fibers, and other anti-nutritional factors. Anti-nutritional factors are categorized as organic materials that affect the feed consumption, utilization, growth, and normal functioning of internal organs (Altan and Korkut 2011; Kokou and Fountoulaki 2018). The antinutritional factors are phytates, saponins, non- starch polysaccharides, lectins, tannins, cyanogenic glycosides, and gossypols (Krogdahl et al. 2010).

2.11.2 Phytates

Phytates are the major storage form of phosphorus in plant grains and seeds that monogastric animals as well as humans cannot degrade. Since phytate is a potent chelator of mineral ions, it is linked with a variety of health problems (Kumar and Sinha 2018). The phytates interact with minerals and other nutrients, insoluble complexes are formed in the small intestines and it does not accord with any absorbable requisite elements. The digestibility and utilization of proteins and amino acids are disrupted by phytates in fishes and higher organisms. For example, in acidic Nile tilapia stomach the negatively charged phosphate moiety binds at the lysine amino group, imidazole groups on histidine and guanidyl groups on arginine in soluble proteins. In the alkaline intestines of Nile tilapia, ternary complexes are formed. These complexes are resistant to proteolysis (Kumar et al. 2012).

2.11.3 Non-starch Polysaccharides

Non-starch polysaccharides (NSPs) present in the aqua feed as part of 90% of the plant cell wall. These consist of cellulose, hemicelluloses, and pectin polysaccharides, non-cellulosic polymers such as arabinoxylans, mannans, xyloglucan and mixed-linked b-glucans. The interaction of the non-starch polysaccharides depends upon the structural linkages between the sugar residues and the sugar residues itself. The NSPs are also present in the aqua feeds as soluble purified form, for example, guar gums used for the stability of the feed pellets (Sinha et al. 2011). The most utilized ingredient in aqua feeds are soybean meals. Approximately 200 g/kg of NSPs are there in raw soybeans and cereals contain about 100-200 g/kg of soluble and insoluble forms of NSPs (Felix et al. 2018). The increase in NSPs causes the higher level of digested viscosity which will affect the emulsification reducing lipolysis. The entrapment of bile salts by NSPs also impairs solubilization efficiency of fats. This will all lead to reduced lipid absorption and utilization. The NSP induced digesta viscosity also hinders the absorption of minerals (Sinha et al. 2011).

2.11.4 Protease Inhibitors

The relatively low quality protein content of plant-based feedstuffs is one of the key drawbacks of using high inclusions of plant-based feedstuffs. Protease inhibitors (PIs) are prevalent in storage organs such as seeds and tubers, accounting for 1–10% of total protein in almost all plants. PIs account for 6% of the protein in soybeans, and residual levels can persist despite the feed processing. Protease inhibitors reduce the proteolytic digestive enzyme activity. Proteases are enzymes that catalyze the hydrolysis of peptide bonds in proteins. The protease inhibitors bind to the protein and reduce the activity of protease enzymes, such as trypsin and chymotrypsin, along the digestive tract. This leads to the hindered protein utilization (Felix et al. 2018).

2.11.5 Phytase Enzymes

Most commonly used plant grains and seeds in aqua feed contain phytates stored as indigestible phosphorus. Phytases are enzymes which can cleave the phytates and convert it to inorganic phosphorus inositol. The feed ingredients undergo a pretreatment with phytase enzymes to improve digestibility. The studies have proved the enhanced phosphorus digestibility and increased absorption of minerals. Some studies report the increase and utilization of protein and lipids (Lemos and Tacon 2017). In Nile tilapia, 50% phosphorus added with 500 or 1000 units of phytase per kg increases the body weight, protein content, and lipid content. The phytase supplementation also increases the feed utilization efficiency than that of the fish population diet without the addition of phytases (Abo Norag et al. 2018).

2.11.6 Protease Enzyme

The most essential ingredient of feedstuffs is protein. Increasing the protein content of feeds can boost fish productivity, but too much protein is metabolized as an energy source, resulting in further nitrogen discharge into the water (Xue et al. 2012). Proteases (EC 3.4) can catalyze the reaction of hydrolysis which degrade protein molecules to peptides and ultimately to free amino acids (Ramos and Malcata 2017). Exogenous proteases introduced to feed may compensate for endogenous protease deficiency, allowing macromolecular protein to be hydrolyzed into smaller molecular peptides, peptones, and a variety of amino acids which can be easily digested and absorbed, thus decreasing stimulation, barriers to nutrition, improved feed utilization and promotion of growth (Shi et al. 2016). Neutral and acid proteases are widely used feed industry proteases. In a study by (Liu et al. 2018) upon addition of protease in a lower protein diet, the dietary protein requirement of juvenile gibel carp (*Carassius auratus gibelio*), was decreased. The feed conversion ratio, digestibility of protein and lipids, and the protein efficiency ratio were also enhanced considerably.

2.11.7 Carbohydrase Enzymes

Carbohydrates are one of the primary constituents of the aquafeed and are used by the fish as an energy source. Two main components of carbohydrates are monosaccharides which are easily digestible (disaccharides and oligosaccharides) and the indigestible, insoluble polysaccharides (cellulose and hemicellulose). The carbohydrate supplement in many species is required as it promotes utilization of proteins and lipids and stimulates growth. Therefore the substitution of plant-based proteins has gained interest for the expansion of the global aquaculture industry. The antinutritional factors like phytates, non-starch polysaccharides, and protein inhibitors in plant-based nutrition negatively affect absorption of nutrients and fish health (Francis et al. 2001; Malcorps et al. 2019). Amylase, β glucanases and β xylanases, cellulase and pectinases are the carbohydrases widely used in food industry. The functions of carbohydrases differ upon its types, yet two main enzymes, xylanase and glucanase, account for more than 80% of the global carbohydrate enzyme market. All feed-relevant carbohydrases are the members of the family hydrolase/glycosidase which converts polymeric carbohydrates into low molecular oligosaccharides or polysaccharides (Zheng et al. 2020). The linear polysaccharides are digested by the xylanase enzymes, breaking down the hemicellulose which is a main component of the plant cell wall. The use of xylanases in maize-soy-based diet

Table 2.3 Beneficiary effects of distinct enzymes on aquatic animals

Enzymes	Species	Effects	References
Bovine trypsin	*Cyprinus carpio*	Enhanced proteolytic activity	Dabrowski and Glogowski (1977)
Cellulase	*Ctenopharyngodon idella*	Increased growth performance Enhanced endogenous enzyme activity Intestinal microbiota changes	Zhou et al. (2013)
	Carassius auratus	Gain in weight Increase in feed intake and trypsin activity	Shi et al. (2017)
Lipase	*Sparus aurata*	Significant absorption of glycerol trioleate in 45 day old juvenile	Koven et al. (1993)
Phytase	*Ictalurus punctatus*	Bone ash increment Increase in bone phosphorus Gain in weight Increase in bioavailability of phytate phosphorus	Jackson et al. (1996)
	Carassius carassius	Weight gain by 25%	Yu (2000)
	Oreochromis sp.	Dry matter energy digestibility coefficients of palm kernel meal diet (40%)	Ng and Chong (2002)
	Channa micropeltes	40% substitution of fish meal with soya bean meal with corresponding reduction of feed cost.	Hien et al. (2015)
	Macrobrachium rosenbergii	Improved protein efficiency ratio Enhanced growth	Biradar et al. (2017)
Protease	*Oncorhynchus mykiss*	Feed efficiency in canola pea diet	Drew et al. (2005)
	Salmonids	Improved carbohydrate and protein digestibility	Chowdhury et al. (2014)
	Litopenaeus vannamei	Weight gain Less feed conversion ratio	Li et al. (2016)
	Carassius auratus gibelio	Increased protease activity	Liu et al. (2018)
Amylase	*Labeo rohita*	Increased growth rate Improved dry matter digestibility	Kumar et al. (2006a, b)
Mannanase	*Oreochromis niloticus*	Weight gain WBC count increment	Chen et al. (2016)

helps to impair the plant cell walls to degrade to allow hydration for the endogenous enzymes to perform its activity for a better breakdown of starch and proteins. Xylanases also help in releasing the proteins from the aleurone layer which is rich in xylan in wheat. Supplementing xylanase enhances bile acid conjugate activity in intestines and increases size of the small intestinal villi. It also leads to improved weight gain, feed intake, and feed efficiency. Liver vitamin E also increases by the addition of xylanases (Ganguly et al. 2013) (Table 2.3).

2.12 Conclusion

Sustainable development with sound socioeconomic benefits in aquaculture could be achieved only when there is a decrease in the dependency on fish meal and fish oil in aqua feeds. A comprehensive research on finding effective alternatives for fish meal has been well recognized by the aqua feed industries, which in turn depends upon the economics and environmental effects. Another threat to the growth of aquaculture is the recurrent disease outbreaks among fishes due to unhealthy practices and antibiotic resistance. Development of functional aqua feeds; crafted aqua feeds; and the emergence of aquaponics have paved the way to replace the roles of conventional feeds. Fish farming has now become an efficient practice to produce animal proteins. Single-cell protein, fish processing waste, novel industrial by-products, and probiotics have transformed the aquaculture sector into a fish meal- and antibiotics-independent platform, supporting wide-scale fish farming. Continued research will promise to uplift the economic and environmental status of aqua feed industry, enabling its healthy expansion.

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Chapter 3
Perspectives and Implications of Probiotics as Beneficial Mediators in Aquaculture Industry

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Abstract The diseases and nutrient deficiency in fish and other cultured aquatic species are of crucial concern as they cause severe economic loss in aquaculture sectors. The use of probiotics has proven to be an effective option to reduce the infections, improve the overall health and enhance the productivity. This review comprehensively brought out significance of probiotics, their mode of administration via encapsulation methods and their mechanism of action reported by several research works. We have also highlighted the other beneficial aspects of probiotics as potent functional agents influencing gut microbiota, competence with pathogenic bacteria, release of antimicrobial secretions and maintain the water quality in environmental friendly manner which ensures sustainable aquaculture and fish farming. This review has also covered up the role of probiotics in wide spectrum of functions such as antiviral and antifungal properties, reducing stress and diseases, modifying the gut microbiota, improving the fecundity, survival and modulating the immune system of the host. We have discussed every aspect of probiotics and correlated with the existing research and review articles till date. However, most

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of the studies are reported based on the laboratory trials and experiments; commercial and large-scale demonstration has to be scaled up and utilization of them in natural environments like ponds and lakes will be effective way to broaden aquaculture industry.

Keywords Aquaculture · Probiotics · Encapsulation · Strain selection · Properties · Mechanism of action

3.1 Probiotics and Aquaculture

Probiotics are identified as components that augment the aquatic animal growth, endurance and health. In aquaculture, probiotics are generally considered "Janus-faced", where a particular group of bacterium is a pathogen to a fish, while it may be a promoter in other marine organism. The sources of the probiotics are chiefly the bacteria including the gram- positive and gram-negative, while the non-bacterial group includes the bacteriophages, micro algae and yeasts. These sources can be anywhere from intestine, gills, mucus of the aquatic organisms or even their habitats as well. However, the administration routes of these differ from oral or feed additives depending on the pre-requisites of the host. Similarly, the choice of probiotics can be a single or in combination with immune stimulants with symbiotic concepts. Among wide options, encapsulating the probiotics is seemingly a suitable approach. The public concern to minimize the utilization of synthetic chemicals has probed the sound eco-friendly alternatives in the recent years. Aquaculture industry has shaped-up as the chief and essential source of animal protein and promoter of essential food across the world. Unlike animal farming, aquaculture has encouraged lower greenhouse gas emissions and followed periodic ban of fishing to promote breeding of aquatic species. It also keeps a check limit on overfishing and non-selective fishing as well. Ultimately the aquaculture industry has been a major contributor for the economic development of a nation. However the aim of the aquaculture industry is increase in productivity and sustainability (Fernandes and Kerkar 2019; Sattanathan et al. 2020a, b).

Despite the growth, the brackish water aquaculture is associated with several challenges like disease control, health and survival management, and reproductive strategies. These are resolved by the application of antibiotics, medicines, vaccines and essential supplements. The utilization of the above synthetic chemicals for improvement has caused cascade of changes like biomagnification, bioaccumulation and development of multidrug resistant microorganisms. In other words, the antibiotics are not target specific and tend to destroy the gut microflora along with the pathogenic bacterial strains resulting in less immunity to the organism resulting in unfavourable effect on the food safety and ecosystem as well (Chauhan and Singh 2019; Liu et al. 2020). Certain disadvantages like failure of uptake of vaccines by fry due to underdeveloped immune system (Xia et al. 2018), requirement of large commodities of drugs for treatment purposes, water pollution, etc. seem to search an alternative option. In the face of the above, adoption of probiotics can be the best option as it is largely beneficial to the host. It is considered to be the best suitable alternative and replacement for the synthetic chemicals like pesticides and antibiotics. Moreover, probiotics are usually harmless and they survive through symbiosis with the host organisms. For instance, probiotics enhance the digestive capacity of the fish larvae and fingerlings and allow them to counteract the digestive complications. The mode of delivery can be through direct addition as feed or through pellets, encapsulated forms and even bioencapsulations which uses live feeds like planktons, diatoms, etc. (Krishnaveni et al. 2021). Encapsulated foods are preferred over the conventional feeding because of the more efficient targeted delivery without wastage, prevention of water pollution due to turbidity and effective carriage into the gut for absorption (Jantarathin et al. 2017).

3.2 Selection of the Probiotic Strain

Selection of probiotic bacteria is usually an experimental process based on scientific evidence and reports. Inappropriate choices on the selection of strains lead to failure in probiotic feed formulations. In general the choice is mainly dependent on biosafety considerations and is dependent on the following criteria: (1) common availability and ease of culture and production, (2) reliable method of administration, and (3) knowledge on the location of proliferation in the host's body. The mode of action of probiotics in host organism includes the acceleration of gut microbiota, immunomodulation and strengthening the defence system to counteract various infections. In addition, the probiotic strain can influence the surplus proliferation of beneficial bacteria and eliminate the pathogenic/harmful bacteria from the gut and play a vital role in the detoxification and inactivation of toxins in the fish. Therefore it is apparent that the probiotic microorganisms have direct influence over the gut which in parallel acts as another hub of influential gut microbiome (Pandiyan et al. 2013). The choice of probiotics plays a crucial role in aquaculture industry. The probiotic strain may play a positive role in the host but may be detrimental to another host surviving in the same habitat. Hence, the choice of the probiotic isolated from the host such as gut, primary/secondary hosts, water, soil, fermented food, etc. (Tang et al. 2018; Balasubramanian et al. 2018; Makridis et al. 2021) has produced effective results in the growth and immunity of the fishes (Sharifuzzaman and Austin 2017). Several studies have suggested that the probiotic identified and characterized from the natural habitat of the fish provided better growth and enhanced immunity. Recent approaches conveyed that probiotics isolated from the fish gut itself were very popular and effective. They are effective candidates in limiting the metabolism of aquatic organisms. The chiefly used strains are lactic acid bacteria (LAB), *Bifidobacterium, Streptococcus, Aeromonas media, Bacillus subtilis, Lactobacillus helveticus, Enterococcus faecium*, and *Carnobacterium inhibens* (Giri et al. 2013). However the other groups of gram-negative bacteria that include the *Vibrio*, *Pseudomonas*, *Plesiomonas* and *Aeromonas* were also reported to be effective probiotics in gut of fish (Lakshmi et al. 2013). In addition, commercially available probiotics

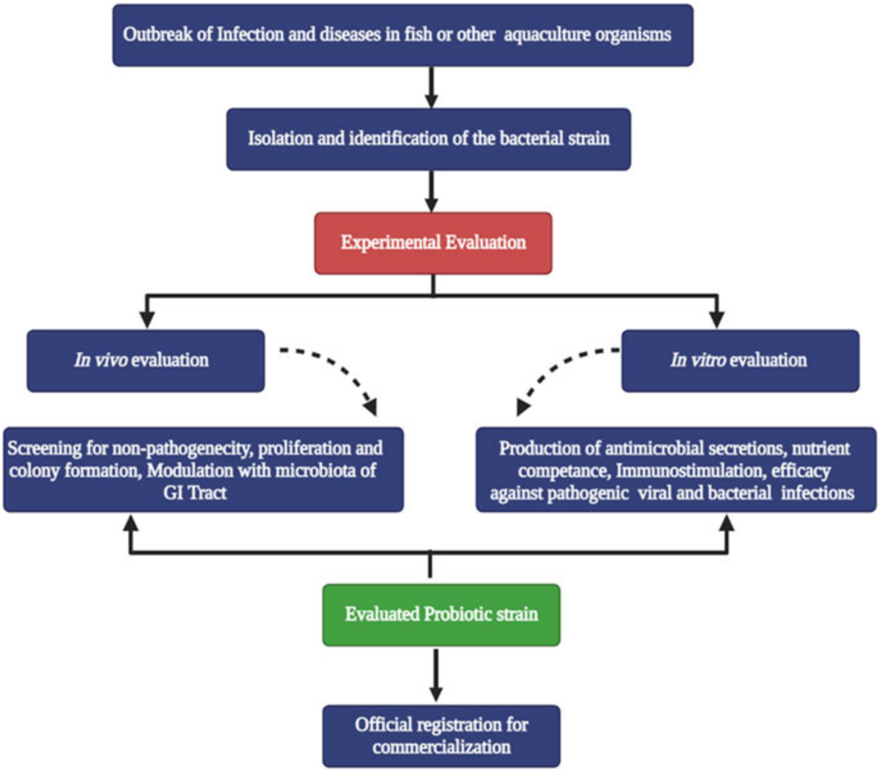

Fig. 3.1 Step-by-step screening and evaluation of probiotics through experimental protocols

were also proven to be potential in aquaculture. Figure 3.1 illustrates the steps involved in the selection and evaluation of a probiotic strain.

Certain studies have reported that the introduced strains failed to adapt and progress growth inside the host (Hosain and Liangyi 2020) Above all, the probiotic should possess integrated key features like resistance to in vivo abiotic stress like alkaline pH inside the host gut, bile and other digestive juices, etc.; also the binding potential to the mucosal surface is considered a significant trait for the candidate probiotic. Expression of low pathogenicity to the host and functioning as an immune modulator is also mandatory requirement for nourishment and growth of the fishes. However, prior identification and characterization through microbial cultures and 16sRNA sequencing can aid in the commercialization and preservation purposes. Further, their antibacterial activity with other pathogens should be evaluated (Vílchez et al. 2015) and the storage properties should be checked for the preservation purposes (Kumaree et al. 2015).

Based on the routes of action, the probiotics were broadly classified into gut and water probiotics. Each of them has a particular mode of function; gut probiotics are augmented with food and administrated orally and they improve the gut-associated microflora, whereas the water probiotics proliferate in water medium consuming the dissolved nutrients in medium and particularly eliminates the pathogenic bacteria from the medium. Table 3.1 lists the several bacterial species used as probiotics.

To put in a nutshell, the selected pathogenic strain must possess the following characters:

1. Resistance to the acidic environment due to secretion of HCl in stomach, also to other secretions such as bile and pancreatic enzymes in accessory digestive glands.
2. Transport, modulation and compliance with the intestinal mucosal cells.
3. Extended viability, resistance to transport and handling stress, efficiency to rapidly establish colonies inside the host.
4. The species should be non-pathogenic, such as lactic-acid bacteria and yeasts.
5. Production of antimicrobial secretions to eliminate pathogenic bacteria and devoid of translocation.

3.3 Bioencapsulation

Encapsulation is the method of immobilizing probiotics in a biodegradable material or capsule. The encapsulation may be a live prey or a polymer enables controlled release and effective delivery of the nutrients to the target. The common live food organisms include the nauplii of the brine shrimp (*Artemia* sp.) and rotifers (*Brachionus* sp.) which are supplied as bioencapsulated foods to different larval stages of aquaculture organisms. The common polymer material includes the alginate, chitosan and agar which are used in microencapsulation of the live feeds which ensures long shelf-life and viability for long time. The probiotics serve the dual purpose of satisfying the nutritive value and also improving the life span of fishes. In order to ensure the durability and avoid contamination, the live food organisms which are encapsulated were sterilized by using antibiotics. The live organisms have potency to absorb bacteria by filter feeding mechanism and adhere to their digestive track and exoskeleton in short time approximately between 10 and 48 h. This phenomena is likely beneficial for encapsulating the common probiotics like *Bifidobacterium* sp. and *Lactobacillus* sp. which showed increased growth rate in *Chirostoma jordani*; however decline in feed uptake was noted after first 30 days and fishes that were exclusively fed with Lactobacillus sp. showed an appreciable growth rate compared to other encapsulated live feeds (Vázquez-Silva et al. 2016). In a parallel study, fortified sea bream larvae with bioencapsulated *Planococcus* fed to rotifers and *Artemia* subsequently increased the growth, survivability, digestibility and biochemical indices of the larvae (Ghoname et al. 2020). In the case of ornamental fishes like *Poecilia latipinna*, the bioencapsulated feed enriched *Artemia* and *Saccharomyces cerevisiae* reported remarkable growth parameters, improved survival, increased fecundity and immunity (Rezaei Aminlooi et al. 2019).

Alginate encapsulated *Saccharomyces cerevisiae* JCM 7255 fed to *Oreochromis niloticus* imparted maximum absorption of nutrients due to increased villi height,

Table 3.1 List of probiotics and their optimizing effects on aquatic organisms

S. No.	Name of the probiotics	Potential benefits	Mode of action/ place	Reference(s)
1.	*Lactobacillus rhamnosus*	Enhanced immunity Improved blood quality	Gut probiotic	Nikoskelainen et al. (2003), Panigrahi et al. (2010)
2.	*Lactobacillus* sp.	Reduce pathogenicity against *Aeromonas hydrophila*	Gut probiotic	He et al. (2017)
3.	*Bacillus cereu*	Protection against *Aeromonas hydrophila* infections	Gut probiotic	Dey et al. (2018)
4.	*Alcaligenes* sp. AFG22	Enhancement of the lipid profile	Gut probiotic	Asaduzzaman et al. (2018)
5.	*Bacillus amyloliquefaciens*	Enhanced antibody production and stress reduction	Gut probiotic	Nandi et al. (2018)
6.	*Streptococcus* sp.	Improve feeding efficiency and growth rate	Gut probiotic	Lara Flores and Olvera-Novoa (2013)
7.	*Bacillus* sp., *Arthrobacter* sp., *Paracoccus* sp., *Acidovorax* sp.	Reduce pathogen load and provide nutrients	Gut probiotic	Nandi et al. (2018)
8.	Integrated probiotics (*Bacillus subtilis* + *Lactococcus lactis* + *Saccharomyces cerevisiae*)	Improved survival rate and metabolism, increased body weight	Gut probiotic	Abareethan and Amsath (2015)
9.	Bacillus NL110, Vibrio NE1	Reduces nitrogenous concentration in medium	Water probiotic	Mujeeb Rahiman et al. (2010)
10.	Pseudomonas sp.	Enhanced transcription rate of antimicrobial peptides	Water probiotic	Ruangsri et al. (2014)
11.	Nitrosomonas sp., Nitrobacters sp.	Reduces nitrogenous wastes in the medium	Water probiotic	Padmavathi et al. (2012)
12.	*Bacillus* spp.	Promotes the beneficial algal growth	Water probiotic	Lukwambe et al. (2015)
13.	*Rhodopseudomonas palustris, Lactobacillus plantarum, Lactobacillus casei, Saccharomyces cerevisiae*	Maintain water pH and improves dissolve oxygen level	Water probiotic	Melgar Valdes et al. (2013)
14.	*Paenibacillus polymyxa*	Enhanced immunity and limited the pathogenic stress in host	Water probiotic	Giri et al. (2013)

improved immunity and resistance also showed resistance against *Streptococcus agalactiae* (Motlagh et al. 2012). The probiotic strain *L. delbrueckii delbrueckii* (AS13B) impregnated in *Dicentrarchus labrax* was bioencapsulated in rotifer and artemia which showed overall gain in growth parameters and increased IGF1 mRNA (Giorgia et al. 2018). Probiotic Alken Clear-Flo® 1006 (ACF-1006) infused in host *Rachycentron canadum* bioencapsulated in rotifer provided notable results in the elimination of pathogenic and undesired bacteria and accounted for the enhancement in health (Chen et al. 2019). Similarly, *Lactobacillus* spp. grown in *Sparus aurata*, L. bioencapsulated in rotifera and artemia markedly increased the specific growth rate, increased the secretion of digestive enzymes and metabolism and improved the survival of the fishes (Shefat 2018).

3.4 Microencapsulation

In cultured fish systems, the nutrition with live foods is coupled with challenges like limitation in delivering feed, expensive rate for rearing and culturing for commercial purposes, non-targeted approach and wastage of nutrients, etc. In order to cope-up the existing drawbacks, various techniques like pelletization and microencapsulation have been applied. These are techniques which have the basic idea of incorporating the biological nutrients like enzymes, anti-oxidants, hormones, carbohydrates, vitamins and minerals and even the probiotic strains in immobilized form enclosed in biopolymers. Microencapsulation is the method of encapsulating the live probiotics using naturally occurring biopolymers derived from sea weeds. This technique ensures the extended storage and targeted delivery of nutrients while the biopolymer capsule may act as immunostimulant (De et al. 2014). Novel advances in microencapsulation technology promise improved feeding systems in aquaculture. The microencapsulated diet comprised formulated active nutrients and probiotics and agents infused in digestible capsules (Willer and Aldridge 2017). Advancement in microencapsulation technology such as “BioBullets” are vectors designed to transport high levels of nutrients such as EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) extracted from marine algae to support the juvenile growth which dramatically improved the production rate and success. The rationale of biobullets includes customisable physical characteristics and nutritional contents, extended shelf-life and ease of production in large quantities (Costa et al. 2011). Recent investigations by Willer et al. (2020) demonstrated that Schizochytrium-based microencapsulated diets remarkably improved sexual maturation in oyster broodstock than the conventional live algal diets. Histo-pathological studies showed that the gonads of treated oysters fed with microencapsulated feed had greater weight than controls and contained high levels of omega-3 fatty acids which are key factors for sexual maturation and spermatogenesis. Consumption of probiotic-enriched aqua foods highly benefits the humankind. Bivalves are one among the commonly consumed aquatic food which house unique micronutrients which are presumably digested by humans. Novel microencapsulated carriers are developed for delivering ample amount of additional nutrients to bivalves to meet human nutrient deficiencies. Microcapsules sealed with vitamin A and D fed to oysters increased the vitamin content in the tissue and serving them for dietary intake will accomplish to meet the human dietary need. In parallel, the application of such microencapsulation techniques and scale-up of this technology involving other bivalve species like clams and mussels would offer a low-priced and sustainable mechanism towards tackling the nutrient deficiencies globally (Balasubramanian et al. 2016; Willer and Aldridge 2020). Effects of dietary supplements in the striped catfish *Pangasianodon hypophthalmus* were investigated by feeding the fish with freeze-dried microencapsulated probiotic *S. cerevisiae* in guar gum (Boonanuntanasarn et al. 2019). The results showed notable increase in humoral immune parameters, growth and improved efficacy in intestinal villus and other metabolic indicators. Significant and beneficial effects were observed on rates of growth and feed conversion ratio.

3.5 Microencapsulation of Biological Compounds for Cultured Fish Feeds

Apart from the immobilization of probiotics, other essential dietary components like proteins, lipids, carbohydrates and active plant metabolites are also used as necessary dietary components for carnivorous and omnivorous fishes. The sources of protein included both animal and plant sources, such as egg albumin, casein and fish protein hydrolysates from animals, while the proteins extracted from plant sources included soy proteins, cotton seed, lecithin and barley protein (Aghbashlo et al. 2013). However, limitations are laid on the usage of plant proteins due to low digestibility, palatability and high fibre content which negatively affect the physiology of fish. Dietary supplement of lipids has become relatively important for fish growth and exploration of varied alternative sources for lipids other than fish oil provides new avenues for sustainable aquaculture (Jin et al. 2017). Common vegetable oils like sunflower, soybean, palm and olive oils contain low levels of fatty acids; however, linseed and canola are exceptions as they are rich sources of alpha linoleic acid. In addition, certain studies have reported the significance of using oils extracted from microbes (Chatterjee and Judeh 2016) and silkworm pupae (Stoica et al. 2016) as fatty acids source. The oxidative stability of the omega-3 fatty acids is improved in microencapsulation techniques and targeted delivery to the host improves the fecundity and growth rate of fishes. On the other hand, carbohydrates impart energy metabolism and non-nutritional content to the fishes. Pectins, chitosan, cellulose and maltodextrin are commonly used in microencapsulations (Jung et al. 2013). Their amicable properties make them ideal candidates as sealing or plastering agents, increased transit time, site specific delivery of bioactive materials and targeted controlled release (Wang et al. 2011). On the other hand, bioactive molecules from plant extracts of garlic and pepper are actively involved to enhance food uptake by triggering appetite and weight gain, while mistletoe functions as immunostimulant, anti-pathogenic and parasitic effects are rendered by various algae and cinnamon and magnolia bark, respectively (Hai 2015)

3.6 Mechanisms of Action of Probiotics

Several modes of action are established by the probiotics like antagonistic action against virulent pathogens, potential to secrete active metabolites like vitamins, enzymes, improve adhesion properties towards the gut lining, development of immunity and establishment of competitive exclusion of pathogenic bacteria. Figure 3.2 illustrates the various roles of probiotics in fish culture.

3.6.1 Production of Antibacterial Substances and Blocking Adhesion Sites

Out of all known common probiotic bacteria such as *Bacillus* sp. only a few are used as probiotics in human, animal an aquatic feeds these include *B. coagulans*, *B. clausii*, *B. cereus*, *B. subtilis* and *B. licheniformis*. They produce secretions like various organic acids, bacteriocin and hydrogen peroxide which are bactericidal in action. They promote acidic environment in the gut to facilitate the elimination of pathogens. Their strong hydrophobicity due to aminopeptidase (the surface-associated proteins), flagellin and metelloprotease specifically bind to gastro-intestinal mucin and fibronectin which play prominent roles in adhesion to GI tract. Moreover, the resistance of the spores against low pH of the gastric-intestinal barrier represents great advantage over other probiotic microorganisms. In addition, several other extracellular enzymes like amylase, protease, lipase, phytase, cellulase and xylanase aid in the digestion and improve the absorption of nutrients. In addition, certain strains produce hydrolase activity, reduction of cholesterol and antioxidant activity which exerts strain on the reduction of oxidative stress (Mingmongkolchai and Panbangred 2018).

For instance, the probiotic bacterium *Lactobacillus reuteri* discharges an antimicrobial agent, reuterin, that has efficient activity on variety of pathogens, including bacteria, fungi, protozoa, and viruses. Through the release of antimicrobial substances, e.g., exopolysaccharides, the probiotic bacteria function to limit the biofilm development by pathogens and shield against infection (Spinler et al. 2008). A notable compound called indole (2,3-benzopyrrole) has proven inhibitory potential against various virulent pathogens including *Vibrio anguillarum*, *Aeromonas salmonicida*, *Edwardsiella tarda* and *Yersinia ruckeri* which cause severe infections in aquatic organisms (Abbass et al. 2010).

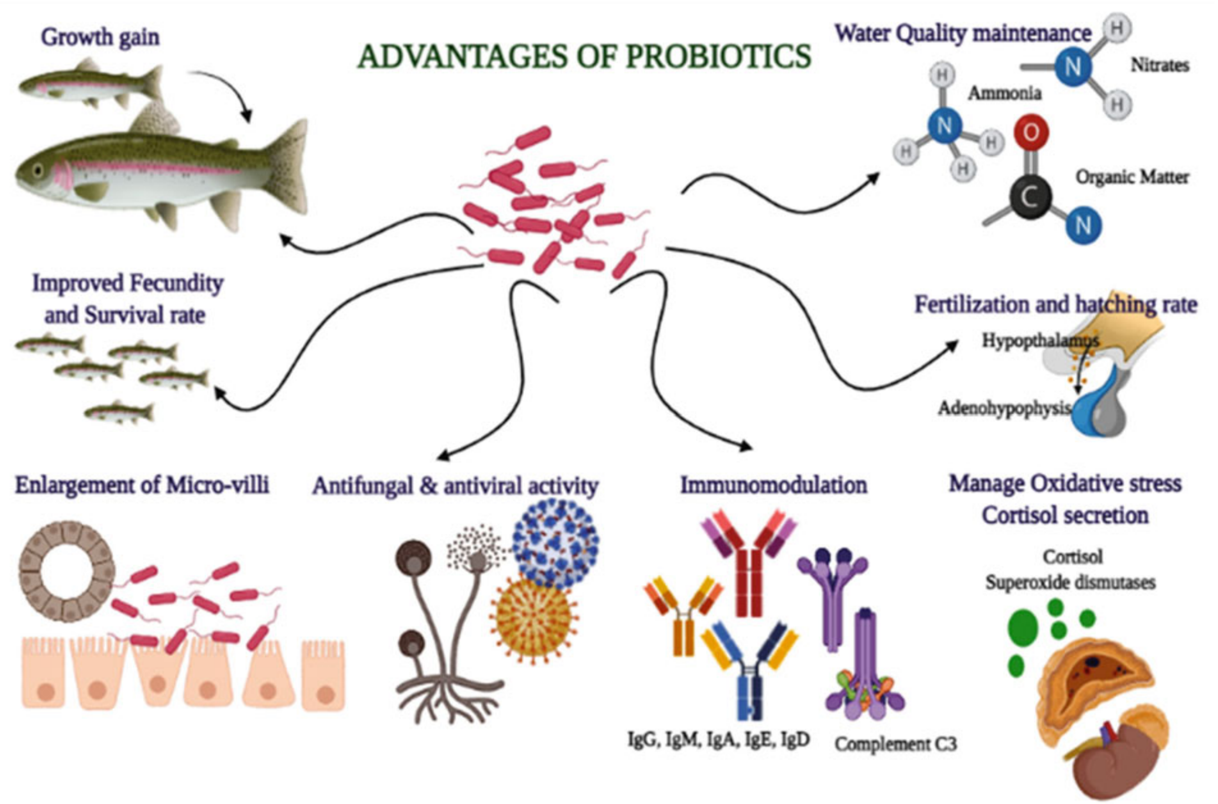

Fig. 3.2 Diagrammatic representation of the role of probiotics in aquaculture. Improvisation on various parameters like body weight, increased digestion rate due to the enlargement of surface area of microvilli, stress tolerance through secretion of antioxidative enzymes, active immune response through increased immunoglobulin production, better fecundity rates and improvement of water quality of the aquatic environment. Downregulation on the colonization of pathogenic bacteria in GI tract, antiviral and antifungal are highlighted

3.6.2 Antiviral and Antifungal Activity of Probiotics

Certain species of *Aeromonas*, *Corynebacterium*, *Pseudomonas* and *Vibrio* reported antiviral activity against infectious haematopoietic necrosis virus (Zorriehzahra et al. 2016). Supplementation of probiotic strain *Bacillus megaterium* and *Vibrio* sp. has aggravated resistance against white-spot syndrome virus in the shrimp, *Litopenaeus vannamei* (Li et al. 2009). Combination of *lactobacillus* with sporlac provided improved resistance against lymphocystis viral disease *Paralichthys olivaceus* (olive flounder) (Harikrishnan et al. 2010). Scanty literature is available on the antifungal effects of probiotics. *Pseudomonas* species (M162, M174) and *Lactobacillus plantarum* FNCC 226 showed inhibitory activity against *Saprolegnia parasitica* in catfish (Nurhajati et al. 2012). In another study, the probiotics such as *Pseudomonas* sp., *Vibrio* sp., *Aeromonas* sp., and groups of *Coryneforms* isolated from salmonid hatcheries exhibited antiviral activity against infectious IHNV. In addition, Vibrio sp. (NICA 1030 and NICA 1031) isolated from black tiger shrimp hatchery exhibited antiviral activity against IHNV and *Oncorhynchus masou* virus (Loh 2017). Even though probiotic bacteria prevent the viral transmission in fish population, their mechanism is still unclear

3.6.3 Competence for Nutrients

The deficiency of nutrient availability for pathogenic bacteria is a limiting factor for their proliferation. The availability of micronutrients proportionally enhances the presence of the microbiota in the GI tract. The survival and the competence exist between the microbes existing in the same environment. Probiotics have the capacity to outrage the pathogenic bacteria by utilizing all the available nutrients that is supposed to be accounted for the growth of pathogenic strains. Presence of siderophores producing probiotic strain can effectively accomplish nutrient competence by sequestering the free iron inside them, thus making them unavailable for the growth of pathogens. Apart from the above, probiotics play crucial roles in maintaining physiological activities of treated fishes, it is widely accepted that probiotics effectively reduce the levels of stress hormone called cortisol and activate the secretion of superoxide dismutase, catalase, and glutathione peroxidase which are potent antioxidative enzymes to manage the stress (Zolotukhin et al. 2018). It is also reported to increase the body weight by inducing the mRNA transcription of insulin-like growth factor (IGF)-I (Hoseinifar et al. 2016). The probiotic culture of *Pseudomonas fluorescens* sequesters the free iron which limited the growth and proliferation of *Vibrio anguillarum* and *Aeromonas salmonicida* by restricting the free iron availability (Maltz et al. 2015).

3.6.4 Stimulation of Immune System

Certain probiotic strains of bacteria possess potency of stimulating the immune response. They trigger the cascade of immune response such as producing antibodies, activating macrophages, initiating the T cell proliferation and accelerating the production of interferon. Probiotics are advantageous over other groups of microorganisms as they play critical role in the immune-stimulation and aid in protection of the cultured aquatic species through curtailing diseases and infestation of pathogens. The probiotics induce the cellular and humoral mediated immune response by accelerating the production of IL-1b, TNFα and lysozymes in carp species when fed with the formulated probiotics *Aeromonas veronii*, *Vibrio lentus* and *Flavobacterium* (Dawood et al. 2018). In a similar study, Allameh et al. (2016), observed the improved activity of albumin, IgG, IgA, C3 complement, myeloperoxidase, lysozyme and phagocytic activity in RBCs of selected fishes. They have experimentally evidenced as a potent immunomodulator which hind to specific MAMPs (microbial associated molecular patterns) depending on the specific pathogens and its respective pathogen pattern recognition receptors (PRRs). They provoke cascade of immune response leading to intracellular signalling events resulting in the discharge of specific cytokines and interleukins activated by T cells to counteract an array of antigenic responses (Akhter et al. 2015)

3.6.5 Disruption of Quorum Sensing and Activation of Quorum Quenching

Beneficial and non-pathogenic bacterial communities form the basis of aquacultural feeds. Studies on analysing the Quorum Sensing (QS) among the pathogenic and the probiotics provide insights for anti-infective models in aquaculture systems (Zorriehzahra et al. 2016). QS is a bacterial regulatory mechanism which is accountable for the expression of proteins based on cell density-dependent manner. The regulation of gene expression is controlled by AI (auto-inducers) which communicate among the bacterial communities to regulate the physiological activities. Disruption of the QS system among the pathogens provides insights for anti-infective strategy in aquaculture. Several research works have cited the significance of the QS, wherein Chu et al. (2014) reported Bacillus sp. QSI-1 served as a potential quorum quencher on virulent factors and biofilm producing factors in the *Aeromonas hydrophila*, a fish pathogen in Zebrafish (Danio rerio). The biochemical parameters indicated a significant inhibition of protease synthesis, haemolysis and reduction in biofilm formation; overall the survival percentages were escalated.

Other probiotic bacteria like *Lactobacillus*, *Bifidobacterium*, *Bacillus cereus*, *Lactobacillus acidophilus,* etc. disintegrate the signal molecules of harmful bacteria through targeted action of enzymes and auto-inducer antagonists (Mundi et al. 2013). It is well understood that effectiveness of virulence and biofilm formation

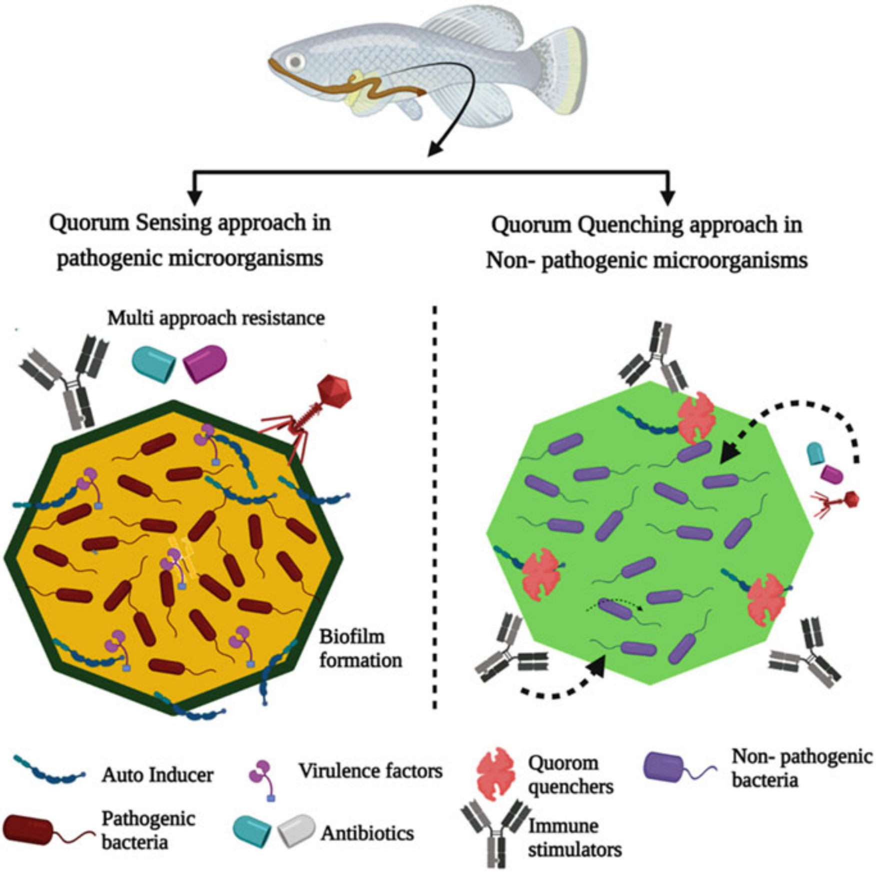

Fig. 3.3 Role of Quorum Sensing (QS) and Quorum Quenching (QQ) in disease prevention in fishes. The GI tract usually harbours a combination of natural and commensal flora which can be pathogenic and non-pathogenic. Pathogenic bacteria produce auto-inducers (AI) that act as signalling molecules and modulate between the bacterial colonies to from biofilm to acquire resistance from all drugs. In the case of non-pathogenic bacteria or probiotics, they secrete enzymes that are QQ which function to degrade the AI and disrupt the biofilm formation; thus it allows the action of drugs to target the harmful bacteria and prevent the fish from further infections

induces the diseases and infections in fishes. These are effectively deregulated Quorum quenching (QQ) which is often called as disruption of quorum sensing, which hare considered advantageous and preventive approaches for eco-friendly management of infections in aquatic organisms. Jayaprakashvel and Subramani (2019) demonstrated recent advances on the influence of QS and QQ with the virulence of bacterial pathogens both in vivo and in vitro conditions and provided insights for the disease management in aquaculture systems. Figure 3.3 illustrates the phenomena of QQ and QS in the pathogenic and non-pathogenic strains of bacteria which provided better understanding on the attributes of preventive steps to counteract infections.

3.6.6 Maintenance of Water Quality

Probiotics aid in improving the water quality by utilizing the organic nutrient which helps to improve water quality. They have the tendency to convert the organic matter into carbon dioxide. Probiotics individually cannot assure the water quality, but the combination of them along with photosynthetic bacteria, nitrifiers, and denitrifiers can have a multi-faceted approach in maintain the water quality in the culture fish systems (Hasan and Banerjee 2020). In contrast, Bacillus species has been proven experimentally to maintain the water quality in fish culture. It has ability to have strong influence on the physico-chemical properties of waters such as pH, conductivity, BOD, COD, DO, etc., and also in modulating the microbial balance. However the efficiency depends on the method of application, selection of strain and nutrient availability (Hlordzi et al. 2020). New and integrated approach of coupling biofloc and probiotics termed as exogenous addition of known probiotic bacteria to the biofloc are known to give promising results on the maintenance of water quality. Besides, it also promoted highest survival, growth rate and elimination of pathogenic microorganisms in fish culture systems (Daniel and Nageswari 2017). It is also reported that probiotic bacteria possess algicidal activity and limits the growth of several microalgae. The nitrifying probiotic bacteria are also used to eliminate ammonia, nitrates and nitrite toxicity which improves the physico-chemical standards of the water and provides healthier environment for shrimp and prawn larval culture (Aguirre-Guzmán et al. 2012).

3.6.7 Role of Probiotics in Aquaculture as Biocontrol Agents

Usage of antibiotic for controlling pathogenic infections and diseases in aquaculture resulted in multidrug resistance microorganisms and created negative impact on environmental health. As an effective alternative, probiotics have been successfully employed as biocontrol agents to limit the harmful microorganisms in culture systems (Román et al. 2013). The most commonly employed biocontrol probiotic strains are *Lactobacillus, Carnobacterium* and *Bacillus* species, although other optional candidates are *Aeromonas, Enterobacter*, *Pseudomonas*, *Shewanella*, *Lactococcus*, *Leuconostoc*, *Vibrio*, *Enterococcus* and *Saccharomyces* species. An experimental study reported that Lactobacillus acidophilus served as an effective probiotic agent against the fish pathogens *Staphylococcus xylosus*, *Aeromonas hydrophila* and *Streptococcus agalactiae* in the *Clarias gariepinus* (El-Ezabi et al. 2011). Similarly, the probiotic strain *Bacillus amyloliquefaciens* FPTB16 showed inhibition against *Aeromonas hydrophila*, *Vibrio* sp. and *Edwardsiella tarda* in vitro and conferred high resistance with a dietary dose of 109 CFU/g. The golden shrimp production is affected severely by the Acute hepatopancreatic necrosis disease (AHPND) caused by *Vibrio parahaemolyticus*. The probiotic bacteria CDM8 and CDA22 isolated from the hindgut of *Penaeus vannamei* demonstrated antagonist activity against VpAHPND toxin genes of *V. parahaemolyticus* through the secretion of catalase (Wang et al. 2018)

3.7 Conclusion and Future Perspectives

The current researches on probiotics have improvised and elevated the aquaculture industry. In particular, the future prospective appears optimizing as the need for probiotic are ever increasing for farming of fish and other aquatic organisms. Further, focussing and screening on specific probiotic strains from rearing systems will reveal information on the quality and functional properties. Moreover, in-depth understanding on the mechanism of action on the fecundity and survival rates will accelerate the growth of the industry in large scale. However, there are limitations associated with the use of probiotics because of non-specificity; this can be improved by the strain-improvement to enhance their efficiency. Multi-disciplinary approaches such as rDNA technology, molecular techniques, gene ontology and annotation studies would presumably aid in design and improvement of the genetic makeup of the probiotic strain. Finally, probiotics has emerged to be a sound alternative for antibiotic in controlling diseases, which is undoubtedly an environmentally friendly approach.

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Chapter 4
Different Animal Feeds and Their Role in Aquaculture

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Abstract Aquaculture, particularly protein-rich food for the world's increasing human population, is the most reliable food supply in the future. On the other hand, poor practices and feed issues lead to illness susceptibility and, as a result, infections and disease outbreaks. A diet specially designed for their nutritional needs. This chapter provides information on animal feeds used in the aquaculture sector, primarily for finfishes and crustaceans. It mainly included the general importance and requirements of the nutritional compounds and common diet supplements of fish feed. Overview of the most common feeds, their role in aquaculture, the animal feeds used in hatcheries, and the nutritional quality of feeds in aquaculture, with particular attention to the most suitable and common feeds used in freshwater and marine aquaculture, is provided.

4.1 Introduction

Aquaculture is the fastest-growing sector, with recent global growth and the capability to continue filling the gap in aquatic food sources. The aquaculture industry has been expanding rapidly over time, even though aquaculture contributes to the global economy. Aquaculture is viewed as the only natural substitute for developing and improving fisheries resources and ecosystem regeneration. The formulation of nutritionally balanced commercial feeds can impact the growth and production of aquatic species and support the growth of the aquaculture industry. Nutrition is defined as the science of a nutrient's interaction with a living organism's component, including feed composition, ingestion, energy release, disposal of waste, and synthesis for growth and reproduction. Nutrients play an essential position in intensive aquaculture because they influence aquaculture production costs, especially fish

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growth, health, and waste output. The nutritional requirements of these cultured organisms can be addressed by including nutritionally enriched foods. Diets should have all of the necessary protein, amino acids, lipids, minerals, and vitamins for aquatic animals to grow and reproduce correctly. These are significant elements essential for living organisms for their proper growth and maintenance (Dawson et al. 2018). Diets that are prepared or artificial can also be comprehensive or supplemental to aquatic animals.

The development in aquafeeds production supports the aquaculture industry's sustainability, economy, and upholds nutritious finfish and shellfish production. The majority of commercial diets provide the necessary nutrients that are compatible with the needs of fish. Aquatic species cultured in indoor systems or confined cages may not have access to natural foods. Aquaculture's future growth is entirely reliant on the success of sustainable feed formulations. Over time, the aquaculture business has grown faster than the other industries (chicken, hog, and beef) (Troell et al. 2014; Sattanathan et al. 2020a, b). Commercially available fish and shrimp diets usually comprise 25–50% crude protein, which is the most important and expensive component for fish. These diets are attributable to more than just their good amino acid profile and exhibit the quality of palatability and digestibility. Based on current aquaculture production rates and rising usage, it is expected that worldwide demand for fishmeal would soon outstrip worldwide supply.

4.2 Different Animal Feeds Used in Aquaculture

Numerous animal protein sources have been examined as viable alternatives for fishmeal, including processed byproducts with high crude protein content, such as meat and bone meal and chicken byproduct meal (45–65%) and a diverse amino acid profile (Allen Davis and Arnold 2000).

4.2.1 Fishery Byproducts

Currently, fishery byproducts account for about 20% of global fish meal output. The raw materials are derived from fisheries and aquaculture and they typically include trimmings (i.e., blood, viscera, skin, bones, and heads) which are then processed to make fish feeds. Fish meal and fish oil are extensively used in fish feeds, especially salmonids and marine fish derived from pelagic species. Fish oil is vital in long-chain omega-3 fatty acids, which are suitable for health, and fish meal is a vital source of protein with a good balance of essential amino acids. After processing, 50% and 70% of the byproducts are considered "inedible" (Stevens et al. 2018). Despite this, fish meal generated from byproducts of fisheries aquaculture has been successfully used in aquafeeds. Byproduct fish meal is a potential substitute for conventional fish meal and a more cost-effective and long-term protein source. Fish meals from a variety of fish, including tuna, Korean rockfish (*Sebastes schlegeli*) have been established as a byproduct meal in fish feeds without compromising growth and feed usage in fish culture.

4.2.2 Insects

The scarcity of fishmeal and fish oil and their increasing costs have prompted sustainable aquaculture feed alternatives. Insects play an essential role in the natural diet of fish and have a modest environmental impact and a minimal need for arable land. Thus they could be a viable alternative. Insects are a natural element of a fish's diet; studies of replacing fish meal with insects in the regime of fish have arisen in the last decade, and the encouraging results have prompted more research. Many fish species eat insects in the wild: omnivore species eat insects found on the water bodies, while carnivorous species eat insects as juveniles before transitioning to fish-based diets.

Regarding nutritional characteristics, ease of raising, and biomass output, insects are the best candidates for partial or complete substitutes for fish meal proteins in fish feed. Compared to other feeds, insect meal also has the potential to be a good aqua feed element. Seven bug species have been approved for use, and they must be grown on feed-grade substrates. Even though none of these species are pathogenic, vectors, or invasive (Riddick 2014).

4.2.2.1 Nutritional Composition of Insects

It is essential to determine the insect's specific composition before introducing any insect species as an aquafeed. Insects have the best nutritional properties, are accessible to the rear, and produce large biomass. Thus they might be used in fish feed as a partial or total replacement for fish meal proteins. Insects have gained much interest in recent years as a potential protein source (51.52%) for aquaculture (Makkar et al. 2014). Insects have short life cycles and can be raised on high-productivity bio-waste with high feed conversion coefficients. Insect nutritional composition changes according to life stage, growing conditions, and nutrition. A good quality fish diet can have up to 73% protein, whereas a soybean meal can have up to 50% protein. Depending on the insect species and processing method, insects' protein content ranges from 50 to 82% (dry matter).

In general, amino acid profiles in an insect are taxon-dependent. The amino acid content in Diptera is comparable to fish meal profiles, and amino acids in Orthoptera and Coleoptera resembling soybean meal are deficient in lysine and methionine (Barroso et al. 2014). The grasshopper (*Zonocerus variegatus*) and termite *Macrotermes bellicosus* have been found to have many amino acid deficiencies. Several insects, such as mosquitoes, honeybee (*Apis mellifera*), locusts, mealworms, and houseflies, as well as the common fruit fly (*Drosophila melanogaster*), Eri silkworm (*Attacus ricini*), and cockroaches, produce large amounts of taurine (up to 26 mol/g) (Sowa and Keeley 1996). Insects have crude lipid content ranging from 8.5% to 36%; variances in lipids and fatty acids composition are primarily due to food preference. Due to their involvement in various processes such as membrane formation and eicosanoid formation, HUFA deficiency in terrestrial insects could impair fish growth and development (Tocher 2015). Combining insect feeds with nutritional profiles suitable for or modifying the substrate utilized as a nutrient source can help enhance insect nutrition. Minerals such as calcium, potassium, zinc, selenium, iron, and magnesium are found in insects. Ca and P levels, on the other hand, are frequently lower than in fish meal. The vitamin and mineral profiles of insects, on the other hand, are heavily influenced by the insect diet (Makkar et al. 2014; Rumpold and Schlüter 2013).

4.2.2.2 Sources of Insect Feeds

Orthoptera, which includes locusts, grasshoppers, and crickets, is a major crop pest and is utilized in fish diets as a fish meal alternative. Isoptera has a high protein content (37–49%) and fat content (30%), although it has a low mineral content (Sogbesan and Ugwumba 2008).

Yellow mealworm larvae, Palm weevils (*Oryctes rhinoceros*) are currently being used in fish, poultry, and pig diets (Makkar et al. 2014; Veldkamp and Bosch 2015). The super worm (*Zophobas morio*) contains very rich quantities of protein and sufficient quantities of the essential amino acids needed for optimum fish development, but minerals such as phosphorus and calcium scarce in the super worm (Ghaly and Alkoaik 2009). Silkworm Puppets (Dried Silkworm Puppets) food has been utilized as a fish meal and fish oil replacement in fish diets, and Lepidoptera, domesticated silkworm (*Bombyx mori*) meal is a good source of lipids (30%) and protein (50–71%) (Rumpold and Schlüter 2013).

4.2.2.3 Feeding Trials

Grasshopper or Locust and Termite Meal

Adult Orthoptera (locusts or locust) was included in catfish diets and tilapia at 13.5% and 25%, respectively, with no effect on their digestibility or development. The addition of 13% variegated grasshopper meal to fish diets increased essential amino acid and high PUFA content in African catfish development, while complete substitution of fish meal with grasshopper meal resulted in growth reduction in African catfish and *Clarias batrachus* (Alegbeleye et al. 2012). Fish meal replaced by 15% termite meal reported best growth performance on vundu catfish, *Heterobranchus longifilis*. Higher (22.5–30%) dietary inclusion of termite meal resulted in slow growth in fish (Sogbesan and Ugwumba 2008). Fish meal-based diets supplemented with termites and soybean meal at varying ratios enhanced development and nutrient utilization in African catfish fingerlings (Solomon et al. 2015). Regarding the positive results of partial fish meal substitution, fish diets supplemented with a higher percentage of termite meal are significant methane generators (Martius et al. 1993).

Yellow Meal Worm

The incredibly edible yellow mealworm larvae (9% mealworm) resulted in optimal growth of African catfish when sun-dried or oven-dried. *Rainbow trout* have also been shown to benefit from yellow meal worm larvae, which can be fed at up to 50% of their diet (Gasco et al. 2014). Yellow meal worm larvae (26% dietary inclusion) have been demonstrated not to affect African catfish growth or feed utilization, while greater inclusion rates (35–43% equivalent to 80–100% fish meal replacement) have been reported to impair fish development, feed, and protein production (Ng et al. 1992). Although few studies reported dietary inclusion of yellow mealworms reported a negative impact in test organisms. The use of dried yellow mealworm in fish meal reduced the growth of common catfish, *Ameiurus niger*.

Silkworm Meal and Silkworm Protein Oil

Unlike other insect feeds, silkworm meal appears to generate beneficial effects, whether defatted or not, and silkworm fat may also be regarded as a benefit. Domesticated silkworm (*Bombyx mori*) meal is an abundant source of protein and lipids, and dried Silkworm Puppets food has been used as a replacement for fish meat in fish feeds (Rumpold and Schlüter 2013). The common carp, *Cyprinus carpio*, is attracted to and stimulated by silkworm oil (Begum et al. 1994). The use of silkworm protein oil instead of sardine oil in the feed of common carp resulted in enhanced growth and body composition of fish, showing that silkworm protein and lipids are suitable for cyprinids. Increases in dietary non-defatted silkworm protein flour or silkworm protein oil in the feed of common carp, *Cyprinus carpio* significantly increased growth, higher digestibility of protein and lipid and body composition (Nandeesha et al. 1990).

Furthermore, adding defatted or non-defatted silkworm protein meal to cyprinids' diet and Nile tilapia (Oreochromis niloticus) resulted in excellent digestion (Boscolo et al. 2001; Jayaram and Shetty 1980). Silkworm protein supplemented food resulted in good development in olive flounder (*Paralichthys olivaceus*), chum salmon *Oncorhynchus keta* and *Trichopodus pectoralis*, where a low inclusion level (5%) severely affected the growth of tilapia (Boscolo et al. 2001). Several studies showed that 30–50% (upto100% fish meal substitution) of defatted or non-defatted silkworm protein included in fish meal diets resulted in increased growth and proximate composition on rohu (*Labeo rohita*), common carp, and cat fish (*Heteropneustes fossilis*), (Nandeesha et al. 2000).

Maggot Meal

Maggots or common housefly larvae have been widely studied in fish diets, either whole or maggot meal, sometimes known as mag meal, due to its ubiquity. According to studies, maggot meal added to a fish's diet increased their growth, immune health, and disease resistance. Studies have shown that maggot meal-fed Nile tilapia, hybrid catfish (fed 7.5% maggot diet), and African catfish (fed 12.5% or 25% maggot diet) reported significant growth improvement (Ajani et al. 2004; Ogunji et al. 2007). When amino acids were added to the maggot meal diet (81%), it was superior to a soybean meal diet (Ossey et al. 2014). Furthermore, fish given the maggot meal diet showed increased cannibalism of fish larvae, which could have dramatically improved the diet's balance. Maggot meal was also tried in rainbow trout; however, it was ineffective because a 9.2% dietary inclusion had a substantial impact on fish development, and the fish fillets were lacking in omega-3 fatty acids (St-Hilaire et al. 2007).

Black Soldier Fly

Black soldier fly diets (5–25%) treated with AAs (Lys and Met) fed with Atlantic salmon, *Salmo salar*, resulted in better growth and development grew well (Belghit et al. 2019). An increased dietary concentration of (12–30%) black soldier flies decreased growth in turbot (*Psetta maxima*), channel catfish, and rainbow trout. Black soldier flies feed content reduced by 33%, reducing the diet's palatability, protein digestibility, and turbot growth performance. The protein and fat digestibility of Atlantic salmon, on the other hand, was excellent (Kroeckel et al. 2012; Newton et al. 2005).

Super Worm Meal

Nile tilapia have gained significant growth, development, and feed utilization by incorporating 7.5 and 15% super worm meal into the basal diet (Omoyinmi and Olaoye 2012). Super worm meals improved Nile tilapia growth, while in the diet, yellow mealworm larvae demonstrated the beneficial result of both marine and freshwater fish. The introduction of (14 and 16%) *Oryctes rhinoceros* in both African and vundu catfish diets showed optimal growth but reduced growth in Nile tilapia (Fakayode and Ugwumba 2010).

4.2.3 Microbial Biomass (Single-Cell Proteins)

4.2.3.1 Importance and Nutritional Quality

Aquaculture, the fastest-growing animal protein industry, is well-positioned to meet the world's demand for high-quality, protein-rich foods. However, establishing a sustainable, renewable protein source is a significant problem. The microbial biomass, also known as "microbial protein" or "single-cell protein," provided by various microorganisms, is a possible substitute for aquatic animal diets. Microalgae, yeasts, and bacteria are typically recognized as having a tremendous potential for aquafeeds among the immensely diverse group of microorganisms. Bacteria and yeasts have a higher nutritional quality than bacteria and yeasts, with acceptable protein content and a better amino acid profile. Single-cell proteins, which are equal to fish meal, have been shown to have the potential to be employed as feed supplements in experiments. Changing growth conditions, altering the culture media, and post-harvest treatments can increase single-cell proteins' nutritional quality (Øverland and Skrede 2017). All are effectively being researched and marketed and show attractive benefits and challenges.

4.2.3.2 Sources of Single-Cell Protein in Aquaculture

Fungi

Animal feed has historically included yeasts and fungus, mainly for cattle and direct human consumption. *Saccharomyces cerevisiae*, *Fusarium venenatum*, Aspergillus sp., *Penicillium* sp., *Rhizopus* sp., *Scytalidium* sp., and *Trichoderma* sp. are the most well-known fungal species. They offer aquaculture potential. Methionine and lysine are the abundant amino acids vitamin B complex present in fungal proteins (Turnbull et al. 1992). The fungus *Kluyveromyces fragilis* grows on whey; it can create sulfur-containing amino acids. Compared to algae, fungi have a higher nucleic acid concentration, ranging from 7% to 10%. In larval feeding and brood stock diets, essential fatty acids such as ARA, DHA, and EPA are required, and fungal oil extract offers a superior alternative supply of these fatty acids (Nasseri et al. 2011; Ugalde and Castrillo 2002).

Bacteria

Bacillus megaterium, *Bacillus sustilis*, *Streptococcus faecium,* Streptomyces sp., *Thermomonospora* sp., and *Lactobacillus* sp. are all helpful bacteria in aquaculture. Bacteria have a short generation time since their cell masses multiply quickly within a short period (within 20–120 mint). They may also grow on a variety of raw materials and edible substrates such as starches and sugars. Bacterial single-cell proteins contain an 80% of protein and contain certain essential amino acids. Bacteria can quickly multiply over organic material waste and petrochemical products, including methanol, ethanol, and nitrogen. They may also reproduce in mineral- and nutrient-supplemented natural water, which helps them overcome vitamin deficiency during growth (Sharif et al. 2021).

Microalgae

Demand for protein and high fishmeal prices has made it necessary to find new alternatives to plant and animal protein sources to address the aquaculture needs in recent years. The main use of microalgae in aquaculture is related to their use as animal feed. Currently, 30% of world algae production is used for animal feed. Macroalgae or seaweed is an easily available and relatively inexpensive food ingredient that can successfully address the challenges posed by aquaculture. The use of seaweed as an aquaculture additive has gained considerable attention because of its advantages, including increased weight gain, improved triglyceride level and protein content in the body, improved disease resistance, reduced nitrogen release into the environment, and improved fish digestibility. The combination of different types of algae provides a more balanced diet and increases fish growth than a single algae feed. Various microalgae such as Chaetoceros, Tarashioshira, Tetracelmis, Isocrisis, and Nannochloropsis are used to feed larval stages of bivalves, shrimp, and some fish species in intensive monoculture systems. Algae are ingested either directly by the target animals (at the larval stage or beyond) or indirectly by other organisms such as Artemia, rotifers, and Daphnia, which feed the target larval species. Zooplankton consumes nutrients such as fatty acids and vitamins, which are then transmitted to higher trophic levels (the larvae and other stages of fish, shrimp, and mollusks) (Becker 2013; Liu et al. 2020). Microalgae protein synthesis is affected by both growth conditions and media used for culture. The protein content of the dry biomass of microalgae ranges from 26.5% to 53.3%.

In particular, microalgae protein has a much higher yield than soybean protein and is a suitable protein source for aquaculture. According to previous studies employing microalgae for aquaculture, the feed conversion ratio of fish raised with microalgae is higher than the feed conversion ratio of fish raised with conventional feed. Microalgae biomass contains substantially higher concentrations of PUFAs than standard aquaculture diets, under unusual circumstances, such as freezing temperatures. Microalgae with high PUFA content offer a far better potential for aquaculture than soybeans and peanuts, which are widely employed as raw materials for traditional fishmeal manufacturing. As a result, microalgae can be considered a low-cost and effective source of PUFA for aquaculture. Natural pigments in microalgae, such as astaxanthin, chlorophyll, and carotene, are essential for the growth of several fish species. Microalgae-based diets, on the other hand, may not function well in aquaculture in some circumstances due to palatability difficulties. Because of the increased microalgal concentration in fishmeal, most growth parameters such as ultimate body weight, absolute feed consumption, and specific growth rates are reduced in fishes. (0% to 30%) (Li et al. 2014; Walker and Berlinsky 2011). Therefore, in practical applications, the palatability of microalgae-based foods should be comprehensively evaluated to ensure the sustainable functioning of microalgae-supported aquaculture.

4.2.3.3 Single-Cell Protein Feeding Trial Results

Fungi and Bacteria

The majority of the trials were designed to evaluate how single-cell protein substituted fishmeal in terms of growth and feed conversion in fishes although several also looked at broader nutritional implications. The most common yeasts utilized in feeding studies are *Saccharomyces cerevisiae*, *Candida utilis*, and *Kluyveromyces marxianus*, and the majority of feeding trials have been done on shrimp and salmon. In fishmeal diets, alternatives such as *Candida utilis* and *Kluyveromyces marxianus* resulted in growth and nutrient retention in test organisms (up to 40%). It was observed that *S. cerevisiae* is a poor supplier of protein (Hansen et al. 2019; Øverland et al. 2013). Salmon fed single-cell protein (bacterial protein meal 36%) showed a higher feed conversion ratio and growth rate than those fed a control diet while having lower nutritional digestibility. Increased growth and development have been recorded in shrimp fed a mixture of two purple non-sulfur bacteria (1%). *Corynebacterium ammoniagenes* (10–20%) and *Methylobacterium extorquens* single-cell protein were found to be capable of totally replacing fishmeal in shrimp diets (Chumpol et al. 2018). Salmon did not develop soybean meal-induced enteritis when *Methylococcus capsulatus* single-cell protein was included in their diet with soybean meal (Romarheim et al. 2011). Salmon (52%) and trout (52%) grew faster when *M. capsulatus* single-cell protein was given to their diet (38 percent). A microbial biomass combination of bacteria and microalgae (10%) diet increased growth in black tiger shrimp (*Penaeus monodon*) (Arnold et al. 2016; Glencross et al. 2014; Øverland et al. 2010). Diet constituent of *Methylobacterium extorquens* (up to 55%) in salmon diets and up to 10% in trout rations resulted in no significant growth of fishes (Hardy et al. 2018; Romarheim et al. 2011). Decreased growth reported with *S. cerevisiae* (up to 24%) added fishmeal or soybean meal in basal diet of Pacific white shrimp *Litopenaeus vannamei* (Guo et al. 2019). Compared to shrimp on a complete fishmeal diet, *Candida utilis* (50%) diet-fed shrimp had no adverse side effects and developed faster (Gamboa-Delgado et al. 2016). Fungi *Yarrowia lipolytica* fed with salmon and shrimp reported significantly decreased growth (Hatlen et al. 2012).

Microalgae

A variety of algal species (*Chondrus* sp., *Chlorella* sp., *Scenedesmus* sp., *Spirulina* sp.) that can be utilized as feed supplements for traditional protein sources in aquaculture. Protein content in microalgae (60–70%) farmed for animal or human consumption is typically high. There are also fats (especially omega-3 fatty acids and carotenoids), vitamins A, B, C, and E, mineral salts, chlorophyll, and a small nucleic acid quantity. The crude protein values range from 45 to 73%, with fat and mineral content varying from 2 to 20% and 5 to 10%, respectively. Algal single-cell protein features a significant amino acid profile, including the sulfur-containing amino acids methionine and cystine. They are also rich in vitamins, particularly water-soluble ones. *Euglena gracilis* is one of the most commonly utilized algae for single-cell production due to its high nutritional content and ease of digestion. As the cell wall is rigid, digestion of algal meals can be a challenge for aquatic animals. Various methods adopted (chemical, physical, and mechanical) have been used to damage the algal cell wall; it makes these meals more easily digestible. Both the whole-cell meal and the cell-ruptured meal had equivalent nutritional and protein quality. The cell-ruptured meal was more digestible and had a superior essential amino acid, lipid, and carbohydrate composition than the whole-cell meal (Teuling et al. 2019). Salmon feed containing 20% *Desmodesmus* sp. single-cell protein resulted in satisfactory growth and final body composition (Kiron et al. 2016). Biomasses of *Scenedesmus almeriensis* (40%) in the fishmeal diet reported moderate growth in trout. *Nannochloropsis oculata* and Spirulina (*Arthrospira platensis*) were used as fishmeal substitutes in shrimp diets, with *N. oculata* demonstrating a poor alternative (Gamboa-Delgado et al. 2019; Sirakov et al. 2012; Tomás-Almenar et al. 2018).

4.2.4 Poultry by-Products

Feather meal and poultry byproducts have long been promoted, and their production methods have been discussed elsewhere. Poultry meal is primarily used in the pet food sector, especially in aquatic diets. Compared to a fish diet, a poultry meal has a higher production volume, higher nutritional value, and a lower price, making it a viable protein supplement for many aquaculture species. With the growing need for aquafeeds, poultry as a product can be used as a feed ingredient in fish farming systems. They appear to be strong sources of protein and lipids, with crude protein (69%), 10–21% fat, and around 10% ash, and they have calcium hydroxide and an essential amino acid profile comparable to fish meal in fish nutrition, poultry byproduct is a pleasant and high-quality feed element (Fasakin et al. 2005). Feather meal contains 80–85% protein and has been examined in both freshwater and marine species. It is abundant in sulfur-containing amino acids. In comparison to fishmeal, the amount of lysine in poultry byproducts is less. Poultry byproducts (15%) supplemented diets for catfish showed improved growth and body composition. Even though trout diets comprising more than 75% chicken byproducts are insufficient in the amino acid, trout performed far better on a mix of feather meal and yeast single-cell protein than when one of these commodities was utilized as the only source of protein (Rawles et al. 2006).

4.2.4.1 Feeding Trial

Poultry byproducts can substitute up to 75% in the diet of juvenile gilthead seabream (*Sparus aurata*) and up to 100% replaced feed of red seabream (*Pagrus major*). On a diet containing 66.7% poultry byproduct, they fed juvenile red drum, *Sciaenops ocellatus* showed significant growth (Kureshy et al. 2000). Poultry byproducts can also substitute half of the fish meal in the diet of juvenile cobia (*Rachycentron canadum)*, gilthead seabream (*Sparus aurata*), and *Cuneate drum* (Nengas et al. 1999; Wang et al. 2006; Zhou et al. 2011). Poultry byproducts have also been found to be effective in the diets of freshwater fish. Sunshine bass (*Morone chrysops x M. saxatilis)* showed improved growth and development when of the basal diet (100%) protein was replaced with turkey meal (Muzinic et al. 2006). Gibel carp (*Carassius auratus gibelio*) showed higher growth by replacing 50% of fish meal with poultry byproduct protein (Yang et al. 2006). 25% poultry byproduct could replace fish meal protein in the diet of juvenile tench (Tincatinca), resulting in good growth performance (González-Rodríguez et al. 2016). Poultry meal is considered a suitable replacement protein in fish diets. The activity of digestive enzymes is being used as a proxy for the suitability of protein sources in fish diets. Fillet quality and proximate composition have long been used as a fish feed element to study the effects of dietary changes on fish growth performance. Feed conversion, growth and survival were observed in Coho and Chinook salmon fed a poultry byproduct diet (Fowler 1991).

4.2.5 Tubifex

Tubifex (Annelida: Tubifex tubifex) is a living bait widely used in ornamental fish. It is best suited for production due to its adequate size and availability. It is high in n-3 (C18:3n3 and C20:5n-3 C18:2n-6 and C20:4n-6) fatty acids and has a stable nutritional profile. Studies proved that tubifex has high nutritional content (high protein, lipid, and certain fatty acids) and palatability. Feeding of tubifex improved survival percentage and growth of *Pseudoplatystoma fasciatum* (Arslan et al. 2009*).* Tubificidae, particularly *Tubifex tubifex* (Muller), have been utilized to produce and rear shrimp *Macrobrachium lanchesteri* (De Man) (Paniker et al. 2010). Catfish (*Clarias macrocephalus*) supplemented with live Tubifex increased feed utilization and growth performance (Roshada hashim et al. 1993). Tubifex fed with guppy (*Poecilia reticulata*) increased growth substantially (Görelşahin et al. 2018). Tubifex has been demonstrated to increase the growth of ornamental fish such as *Chitala chitala* and *Betta splendens* (Mandal et al. 2010; Sarkar et al. 2006).

4.2.6 Shrimp and Crab Meal

Commercially available shrimp feeds have a crude protein level of 30 to 50%, while fish meal has a much higher protein level, and various factors influence its nutrition composition. This makes it a desirable element in animal feeds. Shrimp waste meal is an animal protein that comes from shrimp. It exhibits rich lysine and chitin; this source contains a lot of nutritional compounds. Shrimp waste meal has protein and ash contents ranging from 35% to 55% and 13% to 38%, respectively, and could benefit fish diets.

Red crab was previously used as a food source for aquatic species. It was used in diets as a source of pigments. Crabs are a good source of -carotene, with astaxanthin being the most common carotene found in red crabs (Spinelli and Mahnken 1978). Salmonids were fed a diet enriched with red crab meal as a source of pigment. In the diets of American lobsters and shrimp, red crab meal was added to pelletized meals. (Table 4.1).

4.2.7 Krill Meal

The interest in evaluating and using the Antarctic krill *Euphausia superba* in the diet as a supplement arose from its great biological value and great abundance. Antarctic krill (*Euphausia superba*) is a giant marine crustacean that dwells in the Antarctic Ocean and has sparked worldwide interest. Because most large marine species feed on shrimp-like krill, it is vital to the Antarctic food system (Nicol and Endo 1999). The krill meal is made from a boiled and dried aqueous extract of powdered whole Antarctic krill, producing a brownish-orange powder with a nutritionally stable amino acid profile (Storebakken 1988). Krill is a high-protein, somewhat fatty, food, and it has a high nutritional value. Lipid content varies from 5% to 14%, and it is a good source of unsaturated fatty acids like eicosapentaenoic acid. Whether the

Table 4.1 Dietary inclusion of crab meals and their applications in fish culture

Fish	% of crab meal in diet	Remarks	Reference
Litopenaeus vannamei	–	Growth, feed conversion,	Goytortúa-Bores et al. (2006)
Farfantepenaeus californiensis	5.4, 100	Growth rate, feed conversion	Villarreal et al. (2004)
Pagrus pagrus	10, 20	Fatty acids profile	García et al. (2010)
Pagrus pagrus	10, 20	Growth performance, proximate composition, skin coloration	García-Romero et al. (2014)
Paralichthys lethostigma	5	Proximate analysis, fatty acid composition	González et al. (2006)

krill specimen uses glycerols or waxes as energy storage determines the nutritional value of the lipids. Krill includes carotenoids and is a vital source of essential fatty acids. It is said to be a palatable feed element. The chitin-containing carapace of krill makes it heavy and carries much ash. In fish feeding studies, krill has proven to be a good source of protein, energy, and flesh-pigmenting carotenoids. Furthermore, despite krill's high fluoride level, the mineral does not concentrate in the edible sections of the fish. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), two marine omega-3 polyunsaturated fatty acids, are predominantly bound in phospholipids in krill. Fatty acids in krill meals are linked to phospholipids. Because omega-3 fatty acids attach to phospholipids, the building blocks of all cells, are more effectively taken into tissues and cells than omega-3 fatty acids that attach to triglycerides, the structural variation in delivering omega-3 fatty acids may be relevant to fish health. Krill oil and krill meal are two common krill products. Krill oil exhibited higher levels of EPA and DHA than krill meal or fish oil. Krill meal contains protein, omega-3 phospholipids, feed attractants, and astaxanthin. It is also high in chitin, which comes from the exoskeleton and soluble compounds, including trimethylamine oxide, free amino acids, and nucleotides, which may work as feeding stimulants (Burri and Nunes 2016). Fish development is aided by partially substituting krill meal for fishmeal in diets, which also happens in cases of feeding depression (e.g., high plant-based diets) or stress (e.g., change in salinity or temperature). Krill meal is also used to boost growth and fillet quality in functional fish meals. The antioxidant astaxanthin produces pigmentation and is thought to have anti-inflammatory qualities by scavenging free radicals and reducing oxidative stress and damage to proteins, lipids, and DNA (Graf et al. 2010). In the form of omega-3 lecithins, krill meal provides protein, feeding stimulants, phospholipids, omega-3 fatty acids, and choline in an all-in-one package. This combination of features makes krill meal an especially appealing shrimp feed ingredient for improving larval, post-larvae, juvenile growth, stress resistance, and survival (Table 4.2).

4.2.7.1 Krill Meal as a Feeding Stimulant

Crustacean and krill meal is the most efficient feed intake drivers in tiger shrimp among diverse feeding effectors commercially utilized in shrimp feeds (betaine, hydrolysates, squid, and crustaceans). This can be achieved through a shorter culture period and higher crop turnover. The growth-enhancing activity is partly explained by the supply of inorganic elements and nutrient balance positively influencing feed intake. The insoluble protein part of crustacean-derived components contains a growth factor. Juvenile tiger shrimp grow 20% faster on meals containing crab meal or krill meal. At the same time, the growth of shrimp increased from 0.58 g/wk. with a basal diet to 1.22 g/wk. with diets containing krill meal (Smith et al. 2005; Williams et al. 2005). As a growth promoter, krill meal in shrimp farming, rapid growth is one of the most desirable cultural criteria. Production efficiency, survival rates, and yield can all benefit from high growth rates (Derby et al. 2016). Furthermore, krill meal increases the number of pellets consumed by white leg shrimp in a

Table 4.2 Dietary inclusion of krill meals and their applications in fish culture

Fish	% of crab meal in diet	Remarks	Reference
Gadus chalcogrammus,	–	Growth performance Feed utilization Plasma chemistry	Choi et al. (2020)
Red seabream	5.10, 20	Growth, amino acid profile	Shimizu et al. (1990)
Cynoglossus semilaevis	100 and 200 g kg–1	Growth, development	Xu et al. (2017)
Paralichthys olivaceus	3%, 6%, 9%,12%	Growth performance, feed utilization, innate immunity, disease resistance	Tharaka et al. (2020)
Mink kits	0%, 8%, 17%, 33%	Growth, amino acid profile, blood characteristics	Krogdahl et al. (2015)
Pagrus major	0.05	Growth performance, feed utilization	Tharaka et al. (2020)
Atlantic salmon	0.074	Gene expression	Jalili et al. (2020)
Acipenser gueldenstaedtii	0%, 10%, 20%, 30%	Growth performance, feed utilization, body composition	Gong et al. (2016)
Penaeus monodon	0–15%	Growth	Williams et al. (2005)

concentration-dependent way by encouraging a slower feed intake. Krill meal enhances juvenile white leg shrimp culture performance fed a soy protein concentrate-based diet containing only 5% fishmeal (Sá et al. 2013).

4.2.7.2 Krill Meal Used in Plant-Based Diets

The limited supplies and high fish meal prices increased the interest in plant-based protein diets, mainly using soybean meal. These diets often lack palatability and contain anti-nutritional properties that can disparage shrimp performance. The amount of krill meal that must be used in plant-based meals to boost growth is minimal. Although a dietary inclusion of 1% krill meal is sufficient to boost feed intake, the inclusion of 2% krill meal in a diet results in faster growth, increased production, and a lower food conversion ratio in white shrimp, *Litopenaeus vannamei* (Sabry-Neto et al. 2017).

4.2.7.3 Krill Meal in Animal Byproduct Diets

When krill meal was utilized, however, feed palatability and shrimp growth were only improved. As a result, formulators should be aware of the necessity to add krill meals to feeds containing a high percentage of animal byproducts. Feeds comprising animal byproduct meals as a protein source have weak feeding stimulation. Hence krill meal has been added to compensate.

4.2.8 Zooplankton

4.2.8.1 Importance in Aquaculture

Zooplankton plays a crucial role in the transport of energy from producers to higher trophic levels. They are the starter feed for most fish larvae and many plankton-eating adult fishes and the abundance and distribution of zooplankton impact pelagic fisheries and ecosystem. Zooplankton is a rich source of food for farmed fish in the aquaculture industry, particularly for fry and finfishes. Most aquatic animals, especially in their larval stages, depend on zooplankton for survival. Its significance relates to the fact that most fishes rely on it as a source of nutrition after the yolk sac has been digested. Furthermore, certain large aquatic animals, such as whales, are filter feeders, which means that planktonic organisms make up most of their food, with zooplankton. The high metabolic rate of young fish in the tropics makes zooplankton a significant dietary source.

4.2.8.2 Nutritional Quality and Role of Live Feeds in Aquaculture

Many aquaculture species can benefit from live feedings, such as phytoplankton and zooplankton. It is widely referred to as “living capsules of nutrition” since it includes high nutritional components of macro- and micronutrients. These organisms contain a vital resource of lipid and fatty acid, vitamins, protein, and amino acids (Evjemo et al. 2001). There are several studies on nutrient levels of various zooplankton groups from different geographical areas that reported that zooplankton contains vital growth factors such as fatty acids and amino acids (Boëchat and Giani 2008; Hamre 2016). They provide essential antioxidants, and micronutrients such as pigments, sterols, vitamins, protein, and fatty acids passed down the food chain.

Artemia

Artemia is also known as brine shrimp or sea monkeys (Minami et al. 2010). The more widely used live feed Artemia species in hatcheries are *Artemia salina* and *Artemia franciscana* (Aragão et al. 2004; Kadhar et al. 2014). Artemia nauplii have some advantages as a food source, although it is nutritionally deficient since it lacks essential fatty acids almost entirely (Navarro et al. 1992). Enriching Artemia with essential fatty acids is indicated to increase the growth and survival of fish larvae, which contains HUFAs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Copeman et al. 2002; Smith et al. 2002). Various enrichment procedures have been devised to improve the nutritional quality of *Artemia nauplii*, (Han et al. 2001). Enrichment diets, such as microalgae, lipid emulsions, and microcapsules, have also increased the necessary fatty acid content for Artemia. Dried single-cell algae and fungus belonging to *Schizochytrium* sp., *Mortierella alpina, Nannochloropsis* sp., or *Haematococcus pluvialis* are examples of enrichment products; these contain an abundant of DHA, EPA, ARA, or astaxanthin, as well as a higher amount of lipids. Artemia, which contains high levels of essential fatty acids EPA and DHA levels, is critical to consider to estimate the dietary value of Artemia for fish from the sea (Watanabe et al. 1978). Artemia strains' lipid and energy content decrease as they grow, with the maximum lipid and energy content at the beginning. The energy content in a decapsulated cyst: nauplii, is found to be high, as well as the proximate composition of protein (37–71%), lipid (12–13%), carbohydrate (11–23%), and ash (4–21%21) % (Watanabe et al. 1983). Because Artemia is naturally low in critical HUFAs, it is frequently supplemented with various natural algal concentrates, oil emulsions, and commercial diets. Artemia has been supplemented with a wide range of commercially available supplements, including various vegetable and animal oils, DHA, and spirulina (Palma et al. 2011; Sorgeloos et al. 2001) (Table 4.3).

Rotifer

Rotifers, even better than copepods and other live feeds, show a wide variety of nutritional quality and a source of nutrition for newly hatched fish larvae and plankton-eating fishes (Howell 1973). The rotifer is regarded as a living food capsule that delivers necessary amounts of macro- and antibiotics, vitamins, and micronutrients to fish larvae (Gatesoupe 1982). Rotifers are ideal for the first stages of fish and shrimp larvae due to their small size. Rotifers are the most preferred live feed because they can be cultured at great densities, are temperature and salinity tolerant, and are the right size for most marine fishes, and have a high reproductive rate. In freshwater aquaculture, *Brachionus calyciflorus* and *Brachionus rubens* have been presented as great feed for fish larvae. However, their minimal use is mainly due to the supply of appropriate inert food for freshwater fish (Schlüter and Groeneweg 1981). Because inert food is scarce in brackish water aquaculture, the marine rotifer species *Brachionus plicatilis* is mass cultured and a live food source for various finfish and shrimp. Commercialization of rotifers is completely limited to marine species of the genus Brachionus (Mills et al. 2017). Different culture methods are used; batch culture is the most widely used culture method (Dhert et al. 2001).

Since the 1960s, Japanese researchers reported that rotifers had been recognized as valuable starter feeders for fish larvae. After algal feeding, rotifers were the primary feeding source for most of the fish larvae. Rotifers are usually utilized based on the mouth size of the fish larvae (50–110 m length or100–200 m length). They were an ideal feed for most farmed finfish and shellfish. Due to their nominal

Table 4.3 Dietary inclusion of Artemia and their applications in fish culture

Fish	Remarks	Reference
Goldfish	Growth, survival	Abi-Ayad and Kestemont (1994)
Danio rerio	Growth, survival	Carvalho et al. (2006)
Barbus barbus	Growth, survival	Fiala and Spurny (2001)
Oreochromis niloticus	Growth, survival	Drossou et al. (2006)
Leuciscus idus	Growth, survival	Hamáčková et al. (2007)
Solea senegalensis	Development, gene expression, antioxidants	Jiménez-Fernández et al. (2018)
Lates calcarifer	Survival, larval growth	Fehér et al. (2013)
Octopus vulgaris	Growth, survival	Fuentes et al. (2011)
Hippoglossus hippoglossus	Growth, survival	Hamre and Harboe (2008)
Scophthalmus maximus	Survival, growth rate, weaning success	Gatesoupe (1982)
Stizostedion	Growth, survival, stress resistance	Kolkovski et al. (2000)
Hippocampus abdominalis	Growth, survival	Woods (2003)
Catla catla	Growth, digestive enzyme activity	Kandathil Radhakrishnan et al. (2020)
Poecilia reticulata	Growth, survival, stress resistance	Lim et al. (2002)
Xiphophorus maculatus		
X. helleri		
P. sphenops		
Tetra		
Corydoras aeneus	Growth, survival	Lipscomb et al. (2020)
Synodontis eupterus		
Synodontis nigriventris		
Epalzeorhynchos bicolor		
Pterophyllum scalare		
Trichogaster lalius		
Poecilia latipinna	Reproductive indices, lysozyme activity, disease resistance	Rezaei Aminlooi et al. (2019)
Leuciscus cephalus	Growth, survival	Shiri Harzevili et al. (2003)
Pseudapocryptes elongatus	Growth, feed utilization	Anh et al. (2011)
Gadus morhua	Growth, survival, lipid composition	Garcia et al. (2008)

size, ability to persist in the water column, rapid reproductive rate, and ability to be cultivated in huge quantities, they became popular and most efficient prey for the aquaculture industry (Lubzens et al. 1989). Rotifers constitute protein content ranging from 28 to 63% (calculated the dry weight), of which nearly half is a soluble protein (Srivastava et al. 2006). The rotifers' nutritional content (protein and lipid) changed with feeding and development rate, particularly the DHA/EPA ratio. Rotifers have a lipid composition of 9–28% (dry weight), with triacylglycerols (20–55%) and phospholipids (34–43%), and the rotifers have significant levels of micronutrients Vitamin B1, B2, E, and C (Lubzens et al. 2003). Rotifers can transmit micro, macro, and even antibodies to their prey (Gatesoupe 1982). Different enrichment diets, such as algae and commercial diets rich in vitamins, fatty acids, amino acids, and antioxidants, have been utilized to enhance the nutritional content of live feeds, such as copepods, Artemia, and rotifers. Feeds such as various microalgae, baker's yeast and oil emulsions have been used to enhance the nutritional quality of rotifers (Cavalin and Weirich 2009; Lubzens et al. 1987; Morizane 1991). One of the most significant uses of microalgae in aquaculture is as rotifer feed, which is widely recognized because of its higher nutritional content and the ease with which numerous species may be grown. Numerous marine oil emulsions (n-3 HUFA) mixed with or without baker's yeast are currently being used as enrichment diets for rotifers. Some microalgae, such as *Isochrysis galbana* and *Nannochloropsis oculata,* exhibit high concentrations of essential fatty acids like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), making them good sources of indispensable fatty acids for rotifers. To increase fish nutrition, rotifers can be enhanced with specific substances such as selenium, vitamins, and iodine during the enrichment process (Table 4.4).

Copepod

Copepods are a type of zooplankton that can be found in both fresh and marine habitats. Copepoda is the largest Crustacea division, with approximately 6000 species. They are insignificant; typically, they are less than 1 mm long, free-swimming, and plentiful in freshwater and marine habits. Copepods are an essential part of the food chain, providing nutrients to a wide range of aquatic species. Copepods are typically thought to be preferable to rotifers and Artemia for hatcheries and the aquaculture industry. Copepods have high nutritional profiles as well as the right amount of digestive enzymes to meet the needs of larval fish (Conceição et al. 2010). Copepods have a high concentration of essential fatty acids (EPA, DHA, and ARA), which enhances the growth and survival of fish larvae. Copepods typically constitute significant carotenoid levels, proteins, minerals, and vitamins, such as manganese and copper.

On the other hand, copepods are only used in hatcheries due to their limited adaptability and difficulty scaling up in laboratory conditions. Less appropriate

Table 4.4 Dietary inclusion of rotifers and their applications in fish culture

Rotifer	Fish	Remarks	Reference
Brachionus plicatilis	*Trachinotus carolinus* L.	Growth, survival, fatty acid content	Cavalin and Weirich (2009)
Brachionus calyciflorus	*Lota lota* L	Growth, survival,	Harzevili et al. (2003)
–	*Orthopristis chrysoptera*	Growth, survival,	Broach et al. (2015)
Brachionus calyciflorus	Gudgeon and perch	Fatty acid profiles	Awaïss et al. (1996)
–	Bass Morone chrysops x Saxatilis	Growth, survival, fatty acid	Ludwig et al. (2008)
–	*Gadus morhua*	Growth, mineral status	Penglase et al. (2015)
Brachionus angularis	*Pangasianodon hypophthalmus*	Growth, survival	Vu et al. (2021)
–	*Pagrus major*	Growth, survival, stress resistance	Takaoka et al. (2011)
–	*Seriola lalandi*	Growth, survival	Woolley and Partridge (2016)
Brachionus plicatilis	*Gadus morhua*	Survival	Hamre and Harboe (2008)
Brachionus plicatilis	*Dicentrarchus labrax*	Growth, survival, biochemical	El-Sayed et al. (2021)
Brachionus plicatilis	*Scophthalmus maximus*	Growth	Gatesoupe (1991)
–	*Limanda ferruginea*	Growth, survival, lipid composition, pigmentation	Copeman et al. (2002)

species were mass cultured and exploited as a live food source. Few studies have explored copepod enrichment using artificial diets or oil emulsions, probably due to its restricted use and inherently high nutritional value. Copepods are unable to profit from routine enrichment procedures, which are commonly used for rotifers and Artemia. Copepods can be enriched during the culture stage by supplementing with various vital nutrients. Copepods high in docosahexaenoic acid and EPA can help larval fish generally develop by reducing the occurrence of morphological defects (Matsumoto et al. 2009). Copepods are rich in vitamins C and E and antioxidants such as astaxanthin (Drillet et al. 2011). Copepods contain antioxidants that can protect HUFAs from peroxidation and are crucial for the proper growth of fish larvae (McKinnon et al. 2003) (Table 4.5).

Cladocerans

Aquaculture hatchery procedures rely heavily on mass production and supplementing of live feed for larval nutrition. The newly hatched larvae depend

Table 4.5 Dietary inclusion of copepods and their applications in fish culture

Copepod	Fish	Remarks	Reference
Acartia clausi	Lates calcarifer Bloch	Nutritional profile, growth, survival	Rajkumar and Kumaraguru vasagam (2006)
Temora longicornis and *Eurytemora* sp.	*Hippoglossus hippoglossus*	Nutritional value	Evjemo et al. (2003)
Parvocalanus crassirostris	*Plectropomus leopardus*	Survival, growth	Burgess et al. (2020)
–	*Scophthalmus maximus*	Faster growth	Abate et al. (2016)
Cyclopina kasignete and	*Lates calcarifer*	Growth, survival	Rasdi and Qin (2018)
Moina			
Daphnia magna	*Rutilus frisii kutum*	Growth, survival, stress resistance	Fereidouni et al. (2013)
Moina micrura and Artemia	*Litopenaeus schmitti*	Growth rate, soluble protein content, osmotic stress and digestive enzyme activity	Martín et al. (2006)
Moina micrura	*Macrobrachium rosenbergii*	Growth, survival, nutritional composition	Alam et al. (1993)
Moina macrocopa	*Lates calcarifer* (Bloch)	Growth	Fermin (1991)

on live feed, which supplies nutrients for growth and physiological functions. Cladocerans (Phylum Arthropoda, Class Crustacea, subclass Branchiopoda), also known as "water fleas," have high reproductive rates, and extensive temperature tolerance, and the capacity to adapt to a variety of environments. Cladocerans such as Moina and Daphnia sp. provide a good supply of nutritional food supply for several cultivable fishes. In freshwater aquaculture, daphnia is an essential live feed, and it is utilized as a whole live feed in many freshwater fish hatcheries due to the ineffectiveness of commercial feed during early larval culture. Daphnia and Moina, in particular, are commonly utilized as live food, particularly in a freshwater culture (Qin and Culver 1996). In large-scale cultivation, freshwater species such as *Diaphanosoma birgei* and *Moina micrura* may be better species for feeding fish larval stages and fingerlings of different finfishes (Sipaúba-tavares and Bachion 2002). They have high intrinsic rates, a short life span, a small size, rapid embryonic development, and much energy used on reproduction.

Fish larvae feed Moina as their principal source of live food, and *Moina macrocopa* and *Moina salina* have been found abundantly in freshwater sources. Adult Moina has a substantial lipid content than juveniles and significant protein content (50% dry weight) (Rottmann et al. 1992). Because of their rapid development ability, Moina is increasingly being used as a live feed for larval stages of finfish fingerlings worldwide. In the enrichment and development of Moina, the availability of food sources in natural conditions is crucial, and it influences their nutritional quality. The fats in the meal also impact population growth and reproduction rates (Patil et al. 2010). Daphnia can be found in a variety of freshwater ecosystems around the world. Rapid jerks of the two big antennules are used to swim. Daphnia contains 20–27 percent lipid, Proteinases, peptidases, amylases, lipases, and cellulases, and they can all be used as digestive enzymes for fish larvae (Pangkey 2009). Several enrichment diets for Daphnia have been tried, including canola oil and potassium (Fereidouni et al. 2013) (Table 4.5).

Fairy Shrimps

Fairy shrimps are a cost-effective freshwater microcrustacean that is often used as live aquaculture feed. They live in pools and artificial water bodies all over the world in the natural system. Due to their vast individual biomass, ability to reproduce, and fast growth, fairy shrimps are potentially excellent live-feed organisms. Fairy shrimps are preferred in both freshwater and marine hatcheries, and it is economically feasible. Also, it is easily consumed, easily digestible nature, contains vital development factors, and does not change water quality. Fairy shrimp nutritional compounds are most comparable to Artemia, which may include significant levels of nutritious compounds. Besides, they contain essential amino acids, fatty acids, substantial amounts of protein, antioxidants from the carotenoid complex, such as astaxanthin and canthaxanthin, and antheraxanthin (Dararat et al. 2012) (Table 4.6).

Table 4.6 Dietary inclusion of fairy shrimps and their applications in fish culture

Fairy shrimp	Fish	Remarks	Reference
Streptocephalus proboscideus	*Acipenser persicus*	Growth	Namin et al. (2007)
Branchinecta orientalis	*Oncorhynchus mykiss*	Growth, survival	Pormehr et al. (2018)
Streptocephalus proboscideus	*Persian sturgeon, Acipenser persicus*	Growth	Namin et al. (2007)
Streptocephalus dichotomus	*Pterophylum scalare*	Growth	Velu and Munuswamy (2003)
Streptocephalus sirindhornae	*Macrobrachium rosenbergii*	Growth rate	Sornsupharp et al. (2013)
Streptocephalus sirindhornae	*Amphilophus citrinellus* (Günther, 1864) × *Cichlasoma trimaculatum* (Günther, 1867)	Skin pigmentation, carotenoid deposition	Sornsupharp et al. (2015)
Branchinecta orientalis	*Oncorhynchus mykiss*	Growth, survival, nutritional quality	Pormehr Yabandeh et al. (2017)

4.3 Conclusion

Animal feeds are the primary source of nutrition for both freshwater and marine fish culture, even though their nutritional composition is altered either naturally or nutritionally dense supplements to meet the needs of the target species. In the future, further improvements in feed formulation and different diets are likely to lead to a gradual substitution of traditional basal diets for other sources. Live feeds encapsulated with micro diets are the most often used animal meals in aquaculture, and they have been found to boost larval performance significantly. In comparison to inert diets, enriched live feeds improve feeding behavior and nutritional composition. Other animal feeds, such as single-cell proteins and animal byproduct feeds, play an essential part in hatchery operations. The nutritional quality of live feed organisms reflects the culture conditions and feed quality when cultured. Fish meal-based diets are now regarded as one of the most significant animal feeds, but given the high cost and scarcity of other economically viable animal feeds, other options should be explored. On the other hand, the availability of various types of animal feeds may help save a significant number of fish and, as a result, the aquaculture industry.

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Chapter 5
Novel Feed Ingredients for Approaching Aquatic Sustainability

Waleewan Changpasert, Utthapon Issara, Aarranee Chotiko, and Alexander Chouljenko

5.1 Introduction

Heading to the mid-twenty-first century, one of the greatest challenges will be to supply the approximately 9.7 billion human population in 2050 with high-quality food (Hua et al. 2019). Based on information in 2018, approximately 88% of fishery products were consumed by humans and this statistic tends to increase from year to year (FAO 2020). Fishery production is a main global food source that is related to the aquafeed market, both of which are experiencing continued growth (Shah et al. 2018). Fish meal is an essentially digestible feed ingredient that is used by aquatic farms because of its high content of proteins, lipids, minerals, vitamins, and small amounts of carbohydrates. Approximately 30 million tonnes of wild fish have been caught for producing fish meal (Olsen and Hasan 2012). The requirements and the restrictions related to obtaining wild-caught fish have created market uncertainty for producing fish meal, with prices increasing by nearly 300% in the last 10 years (Shah et al. 2018). Utilization of fish feeds for aquaculture is estimated to trend upward through 2025 while prospects of procuring wild-caught fish may be limited. Meanwhile, the prices of fish meal have been continuously rising (Tacon and Metian 2015). Therefore, in this chapter, we brief what is known about the various alternative protein feed sources together with antibiotic replacement and growth promoters which can potentially be used in order to approach aquatic sustainability.

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5.1.1 Nutrition of Microalgae Used in Aquaculture

One of the most costly ingredients present in the manufacturing of fish food is protein. This nutrient accounts for over 60% of the costs related to the production of fish food. Fish meal is utilized as a major protein for inclusion in aquatic feeds and comprises over 55% of feed protein content (Han et al. 2016; Alex et al. 2020). However, the available supply of wild-caught fish for fish meal production has recently decreased because of overfishing, also resulting in fish meal becoming more expensive (Han et al. 2016). Therefore, substituting fish meal with an alternative protein source for use in aquaculture is very important.

Microalgae are a primary food source for many kinds of aquatic animals (Norambuena et al. 2015). They are a group of small-celled algae that can conduct photosynthesis. The major biochemical composition of microalgae consists of protein, carbohydrate, and lipid, along with other constituents such as vitamins, pigments, and antioxidants being significantly present in some algal species (Niccolai et al. 2019). Microalgae are able to survive in a large range of habitats, have fast cell division under optimum environmental factors, and produce valuable metabolites. Some varieties of algae have several-fold greater biomass yield than land plants (Hemaiswarya et al. 2011). Utilizing microalgae as a food source and in food supplements has been done for centuries (Wells et al. 2017). Historically, the earlier studies working on the possibility of incorporating algae in aquafeed have been published since the 1970s and a rising number of commercial applications has been realized in many countries for several decades (Vigani et al. 2015; Stanley and Jones 1976). Algal biomass is becoming increasingly well established in a variety of food applications for human consumption in Asia and Western diets; moreover, varying algal species based on their therapeutic and nutritional properties has recently drawn interest in European gastronomy (Peteiro 2018; Wells et al. 2017; Rioux et al. 2017; Mouritsen et al. 2019). Presently, the current global microalgal yield is sold commercially to feed industries at approximately 30% (Hamed 2016; Spolaore et al. 2006).

Microalgae play a vital function in the aquaculture sector as a dietary source for the growth and development of many kinds of aquatic animals based on the availability and proportion of biochemical constituents such as protein, lipid, carbohydrate, and vitamins of microalgae strains (Chu and Dupuy 1981; Wikfors et al. 1992). In general, microalgae obtain 30–40% of protein, 10–20% of lipid, and 5–15% of carbohydrate of total biomass at their late logarithmic growth phase (Brown et al. 1997). Some species of microalgae are excellently rich in protein, as high as 60% depending on culture conditions. In Table 5.1, referent studies demonstrated the various biochemical compositions of microalgae frequently used in aquaculture. Proteins are the essential component for enzyme production and biosynthesis of tissue. Additionally, they are vital to fast-growing juveniles and adults, being essential for undergoing gametogenesis, metabolic processes, and tissue production in all animals (Langton et al. 1977; Wikfors et al. 1992; Kreeger 1993). Protein content differs widely across groups of microalgae. For instance,

Table 5.1 Proximate composition of microalgae

Feed ingredient/algae	Protein (%)	Carbohydrate (%)	Lipid (%)	Ash (%)	Fiber (%)	Moisture (%)	Reference
Fish meal	57.71	–	5.51	9.25	5.15	6.07	Alex et al. (2020)
Spirulina platensis	59.00–60.00	19.10–23.85	6.77–7.80	8.88–10.30	–	–	Yilmaz (2012)
Spirulina fusiformis	65.00	13.62	6.84	9.93	11.37	9.92	Michael et al. (2019)
Chlorella sp.	52.00	7.50	24.30	8.20	–	–	Shields and Lupatsch (2012)
Chlorella vulgaris	56.80	5.90	16.90	9.30	–	4.90	Niccolai et al. (2019)
Chlorella sorokiniana	39.90–51.30	10.70–15.50	22.70–17.90	5.40–9.40	–	7.50–8.50	Niccolai et al. (2019)
Chlorella pyrenoidosa	35.67	22.05	19.23	5.63	3.80	13.60	Changpasert et al. (2019)
Scenedesmus obliquus	50.00–56.00	–	–	–	–	–	Becker (2007)
Dunaliella salina	11.30	38.30	28.60	–	–	–	Colusse et al. (2020)
Nannochloropsis sp.	32.60	17.60	11.10	–	–	–	Arkronrat et al. (2016)
Nannochloropsis oculata	34.11	12.51	19.81	–	–	–	Banerjee et al. (2011)
Nannochloropsis granulata	33.50	23.60	36.20	6.70	–	–	Tibbetts et al. (2015)
Tetraselmis sp.	21.70	14.50	9.40	–	–	–	Arkronrat et al. (2016)
Tetraselmis chuii	56.40	20.00	44.00	–	–	–	Helena et al. (2018)
Tetraselmis suecica	18.30–40.20	10.20–36.80	22.40–28.50	14.80–15.70	–	6.10–7.20	Niccolai et al. (2019)
Chaetoceros calcitrans	41.60–43.10	6.62–8.70	11.71–26.80	–	–	–	Banerjee et al. (2011)
Chaetoceros muelleri	10.82–16.05	5.31–8.86	6.65–8.95	45.21–48.41	–	–	Jesús-Campos et al. (2020)
Cyclotella cryptica	20.31	38.48	18.60	6.00	3.48	11.44	Changpasert et al. (2019)
Isochrysis galbana	60.08–62.20	–	17.89–20.72	11.68–13.77	–	3.94–6.46	Shekarabi et al. (2019)
Tisochrysis lutea	36.70–41.70	7.50–9.40	19.30–22.00	–	–	–	Arkronrat et al. (2016)

– = not available.

the excellent cyanobacterium *Spirulina* (commercially known as the name *Arthrospira*) boasts up to 60% of its dry weight as protein. It also contains all the essential amino acids required for animals, especially rich in lysine and threonine which plants lack (Li et al. 2015). Among other commercial species, the unicellular green algae *Chlorella* holds a high protein content of approximately 50–55% of dry weight. This alga is abundant in an essential amino acid profile that cannot be synthesized in humans and must be supplied by foods. Nowadays, *Spirulina* and *Chlorella* are growing on a large scale throughout the world and are added in many kinds of foods to increase valuable nutrition, especially protein content (Wells et al. 2017).

Scenedesmus is a freshwater green microalga, frequently added as an ingredient for feeding many kinds of fish. *S. obliquus* contains a maximum protein content of around 50–56% of dry weight with abundant essential amino acids (Becker 2007). *Dunaliella salina* has been widely used as natural food for fish because it contains a lot of diverse nutrients. The report of Colusse et al. (2020) demonstrated 11.3% of protein and 28.6% of lipids were found in *D. salina*. However, the growth media may have an effect on the biochemical composition of *D. salina* (Pratiwi 2020). *Nannochloropsis* is exploited in aquaculture and contains 32–34% of protein content (Arkronrat et al. 2016; Banerjee et al. 2011; Tibbetts et al. 2015). The flagellated green algae *Tetraselmis* is an outstanding diet addition in the marine fishery and can accumulate 18–56% of protein content (Arkronrat et al. 2016; Helena et al. 2018; Niccolai et al. 2019). Sharawy et al. (2020) stated that Pacific white shrimp (*Litopenaeus vannamei*) obtained the maximum protein and lipid levels when fed with 7.5 g/kg of *T. suecica* which was added in shrimp feed. However, each consumer demands different amounts of protein for their growth application. In the case of juvenile Eastern oyster (*Crassostrea virginica*), it can consume protein content in the range of 15.6–57.4% of dry weight without limiting the oyster's growth (Wikfors et al. 1984). However, if protein content dropped below 40%, the growth of juvenile mussels (*Mytilus trossulus*) was limited (Kreeger and Langdon 1993).

In general, carbohydrate is the main part of the compounds obtained from microalgae's photosynthesis system and can be found abundantly at 50% dry weight which varies in different algal species (Yen et al. 2013). Normally, there are no requirements for providing carbohydrate content in the fish diet. Therefore, the optimum concentration of carbohydrates in the fish feed should be realized. For instance, feed utilization of Cod (*Gadus morhua*) reduced when adding starch more than 10% in feed (Hemre et al. 1993). Starch (12%) in feed was an appropriate concentration for carbohydrate energy utilization in rainbow trout (Phillips Jr. et al. 1948). Although, growth rates of some fish species decreased when reared with carbohydrate-free diets. If no carbohydrates are added to the diet, proteins and lipids would be broken down for energy and used to synthesize other biological compounds by supplying metabolic intermediates (Wilson 1994).

Dietary lipids play an essential role in providing the main energy source, fat-soluble nutrients, and essential fatty acids that in adequate quantities are critical for normal growth and development of fish larvae (Sargent et al. 2002; Kim et al.

2012). On the other hand, decrease in utilization of feed and growth performance of multiple fish species can be found when excessive dietary lipids are supplied (Duan et al. 2001; Segato et al. 2005; Wang et al. 2006). According to Huang et al. (2016), reported excess dietary lipid at 15% of lipid content results in decreased growth performance and enhanced oxidation rates of chu's croaker (*Nibea coibor*) while low dietary lipids (6 and 9%) are inadequate for the basic needs of this fish. One of the microalgae that have the potential to produce a high level of lipid in their cell is diatoms (Bacillariophyceae), a very important class of unicellular algae that can be found in marine and freshwater environments (Hildebrand et al. 2012). Marine diatoms such as *Chaetoceros calcitrans*, *Isochrysis galbana,* and *Tisochrysis lutea* contain protein up to 20–26% dry weight (Banerjee et al. 2011; Shekarabi et al. 2019).

5.1.2 The Growth Factors Involved in Microalgae Cultivation

Microalgae have a simple cellular structure and desire varied environmental conditions for their growth. Importantly, microalgae are able to produce biochemical constituents as a result of changes in different growth factors (Levasseur et al. 2020). Consequently, the optimality and selection of the cultivation factors are required to induce biomass production and promote the valuable composition of microalgae for aquafeed.

The brief overview scheme of the process for culturing microalgae to producing animal feed is shown in Fig. 5.1, including upstream and downstream processes. Focusing on environmental factors, there are many variables that have an influence on cell physiology and biochemical compositions of microalgae, namely pH, light,

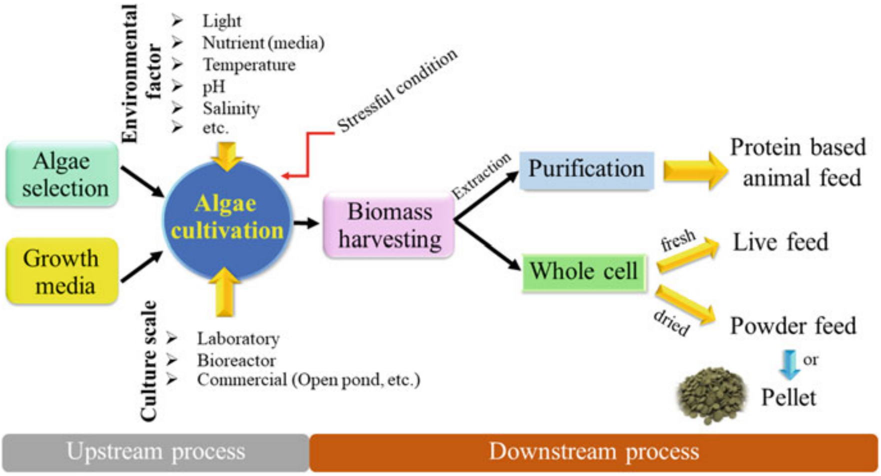

Fig. 5.1 Overview scheme of algae process for producing animal feed

temperature, available nutrients, and salinity. The appropriate temperature of algal culture leads to maximum algal productivity and effectively enhances metabolic processes in different algal species (Borowitzka 2016; Sibi et al. 2016). Singh and Singh (2015) demonstrated that 15–30 °C of water temperature was optimally required for microalgae cultivation. In Table 5.2, it is shown that all reported microalgae can grow in the range of 25 to 35 °C. Yilmaz (2012) studied the effect of different temperatures on biochemical composition of *S. platensis*. The proximate analysis demonstrated an increase in carbohydrate and lipid levels of *S. platensis* when the temperature was higher while protein decreased, and the best growth rate for *S. platensis* was found at 35 °C which resulted in protein and lipid contents of 60.59% and 7.22%, respectively. Similar to the report of Colyer et al. (2005), *S. platensis* accumulated high protein contents around 57–70% and 7–10% of lipid under optimum growth temperatures between 30 and 35 °C. Moreover, Kessler (1985) reported that biomass of *C. saccharophila*, *C. protothecoides*, and *C. vulgaris* to be plentiful when grown at 26–32 °C.

The growth and biochemical compositions of microalgae could be seriously affected by medium pH which essentially factors into enhancing algal metabolism and has an effect on biosynthesis of secondary metabolites. Generally, the appropriate pH differs according to different species of algae. Hence, shifting pH in media could be limiting algal growth through metabolic inhibition. Khalil et al. (2010) stated that moving the pH value to acidic or alkaline levels leads to significantly reduced protein content of *D. bardawil,* which was 61.9, 82.8, 74.6, and 64.5%, when grown at pH of 4, 6, 9, and 10, respectively. It can be explained that carbon is inaccessible for algae growth under high alkalinity because carbonate ion is the notable form of inorganic carbon for algae growth (Falkowski and Raven 1997). Touloupakis et al. (2016) studied the effect of different pH levels (7.5–11.0) on biochemical compositions of *Synechocystis* sp., which is frequently used as a rotifer feed. It was found that protein content was consistent at all pH values (65.1–66.4%) while carbohydrate content increased at pH 11.0. Therefore, it was suggested that setting pH to around 11.0 can serve to harvest high protein, meanwhile growth under this alkaline condition can prevent contamination by other organisms such as protozoa.

Three non-mineral nutrients are needed for microalgae growth: hydrogen, oxygen, and carbon. Macronutrients are required for algal media, for instance, nitrogen, sulfur, phosphorus, potassium, magnesium, etc. Micronutrients are desired in small concentrations (2.5–30 ppm), namely manganese and iron, but other elements, namely copper, cobalt, boron, molybdenum, and zinc, are necessary as essential trace elements (2.5–4.5 ppm). BG-11 medium has been widely used in freshwater cultivation for blue-green algae or other green algae such as *Chlorella* and *Scenedesmus* species (Changpasert and Wong 2020; Gris et al. 2014). However, suitable culture media selection is important for isolation and robust growth of various algae species. Colusse et al. (2020) evaluated the effects of different culture media on green microalgae *D. salina* cultivation. The result showed Conway medium highly affected lipid content at 43%, whereas protein content and total carbohydrate were greatly improved in Johnson medium. The research of Khalil

Table 5.2 Effect of environmental conditions on proximate composition of microalgae

Algae	Environmental condition	Culture system	Protein	Carbohydrate	Lipid	Reference
Spirulina platensis	Schlösser medium Temperature; 35 °C	Batch culture system (2 L)	60.59%	22.88%	7.22%	Yilmaz (2012)
Spirulina platensis	Temperature; 30 and 35 °C Light intensity; 31.35 μmol photons m^{-2} s^{-1}	–	57.00–70.00%	–	7.00–10.00%	Colyer et al. (2005)
Chlorella vulgaris	Basal medium (with nitrogen-rich) Basal medium (with nitrogen-deficient) Temperature; 28 °C pH; 6.20 Light intensity; 60 μmol m^{-2} s^{-1} 2.0% CO_2 with an aeration rate of 0.2 vvm	Photobioreactor	47.78% 22.84%	18.16% 48.59%	12.74% 15.28%	Ho et al. (2013)
Chlorella pyrenoidosa	BG-11 medium Temperature; 24.70 °C pH; 8.74 Light intensity; 178 μmol photons m^{-2} s^{-1}	Closed sterile bottle (2 L)	–	–	27.12%	Changpasert and Wong (2020)
Dunaliella salina	Conway medium Temperature; 23 ± 0.1 °C pH; 8.48 Light intensity; 100 mmol m^{-2} s^{-1}	Flasks (2 L)	23.40%	6.90%	43.30%	Colusse et al. (2020)

(continued)

Table 5.2 (continued)

Algae	Environmental condition	Culture system	Protein	Carbohydrate	Lipid	Reference
Dunaliella bardawil	MH medium Temperature; 28 ± 2 °C pH; 6.00 Light intensity; 78 μE m^{-2} s^{-1}	Erlenmeyer flasks (250 ml)	82.80%	–	–	Khalil et al. (2010)
Synechocystis sp.	BG-11 medium Temperature; 28 °C pH; 7.50–11.00 Light intensity; 50 μmol photons m^{-2} s^{-1}	Pyrex roux-type photobioreactor (1 L)	65.10–66.40%	12.10–18.80%	9.00–12.50%	Touloupakis et al. (2016)
Scenedesmus obliquus	BG-11 medium Temperature; 23 ± 1 °C pH; 8.00 Light intensity; 150 μmol photons m^{-2} s^{-1}	Flat plate photobioreactor	350 μg/mg	300 μg/mg	430 μg/mg	Gris et al. (2014)
Isochrysis galbana	F/2 medium Temperature; 25 ± 1 °C pH; 7.00 Light intensity; 100 μmol m^{-2} s^{-1} Salinity; 30 ± 1‰	Erlenmeyer flasks	27.98%	25.26%	9.78%	Thu et al. (2015)

Nannochloropsis oculata	F/2 medium (Fe deficiency at 0.15 mg/l) Temperature; 26 ± 1 °C pH; 8.00 Light intensity; 80 μmol photons m^{-2} s^{-1} Salinity; 25‰	Erlenmeyer flasks	–	–	32.47%	Sabzi et al. (2018)
Nannochloropsis oculata	Conway medium Temperatures; 21 ± 1 °C Light intensity; 350 μmol photons m^{-2} s^{-1} Salinity; 33‰	Erlenmeyer flasks	26.10%	29.30%	26.30%	Paes et al. (2016)
Tetraselmis sp.	F/2 medium Temperature; 25 ± 1 °C pH; 8.30 Light intensity; 80 μmol m^{-2} s^{-1} Salinity; 25‰	Conical flasks (3 L)	26.40%	9.40%	13.80%	Renaud et al. (1999)

– = not available.

et al. (2010) found that the optimum medium for *D. bardawil* growth was MH medium. F/2 medium is an enriched seawater medium designed for culturing algae, mainly marine diatom. For example, marine diatom *I. galbana* was grown under optimum conditions in F/2 medium at pH 7.0, salinity 30‰, temperature 25 °C, and 100 μmol m^{-2} s^{-1} of light intensity which resulted in 27.98% and 9.78% of protein and lipid content, respectively (Thu et al. 2015). Moreover, culturing under stressful conditions (namely, extreme temperature or pH, nutrient depletion, high or low light intensity, salinity or metal level) induce microalgae to greatly increase their levels of carbohydrate or lipid because this forces them to transform peptides or protein to carbohydrates or lipids (Levasseur et al. 2020; Ho et al. 2013; Siaut et al. 2011). As shown in Table 5.2, stressful nitrogen-starvation dramatically influenced the biochemical composition of *C. vulgaris*, where protein content was shown to decrease from 47.78 to 22.84%. In contrast, the carbohydrate and lipid contents increased from 18.16 to 48.59% and 12.74 to 15.28%, respectively (Ho et al. 2013). Fe is required to synthesize DNA and iron-sulfur proteins (Fillat 2014). Sabzi et al. (2018) stated that low Fe concentration in the growth medium can stimulate lipid production in *N. oculata*.

In general, higher photosynthetic efficiency is found in algae compared to land plants due to enhanced abilities to capture light followed by conversion into available chemical energy (Melis 2009). Gris et al. (2014) demonstrated that microalgae metabolisms are supplied with the energy provided by the light. Therefore, the appropriate level of light intensity and temperature provides a crucial role in stimulating the development of high biomass. Generally, light intensity in the range of 150–200 μmol photons m^{-2} s^{-1} influenced abundant algal growth. *S. obliquus* had the highest growth rate at 150 μmol photons m^{-2} s^{-1} which accumulated ~350 and ~ 430 μg/mg of protein and lipid content, respectively, while when lowering this light intensity level, *S. obliquus* growth was limited (Gris et al. 2014). According to Csavina et al. (2011), the growth of certain algae can be limited under low light and temperature. In contrast, *N. oculata* prefers high light intensity of 350 μmol photons m^{-2} s^{-1} and can produce high protein and lipid at 26% dry weight (Paes et al. 2016). Salinity is very essential for marine organisms. Different levels of salinity can influence growth, physiological mechanisms, and biochemical production of algae (Fava and Martini 1988). *N. oculata* and *Tetraselmis* sp. can grow well at a salinity of 33% and 25%, respectively, and stimulated content of protein at 26 and 14% while lipid content was 26 and 30%, respectively (Paes et al. 2016). Interestingly, employing the response surface methodology program (Box-Behnken design) to investigate the functional relationship between various factors (pH 7.5–9.5, 20 to 30 °C, 100 to 200 μmol photons m^{-2} s^{-1} of light intensity) and lipid content that efficiently calculates appropriate conditions for optimizing *C. pyrenoidosa*'s lipids. It was found that the agreeability between predicated data and trial data could be successfully validated. Per model conditions, at designed levels of pH 8.74, 24.70 °C, and 178 μmol photons m^{-2} s^{-1} of light, the maximum lipid content at 27.12% was produced by *C. pyrenoidosa* in 11 days (Changpasert and Wong 2020).

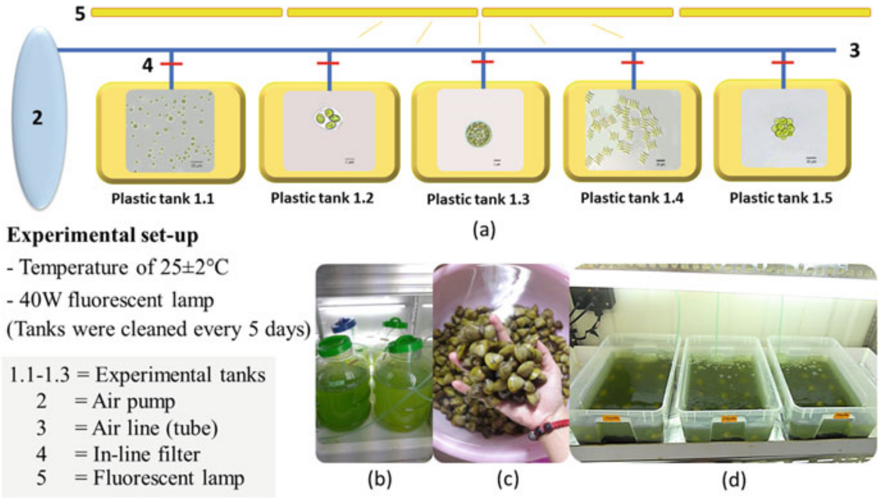

Fig. 5.2 Experimental setup of 100% live microalgal diets fed to Asian clam (*C. fluminea*); experimental setup (*C. pyrenoidosa*; tank 1.1, *O. multisporus*; tank 1.2, *C. cryptica*; tank 1.3, *S. acutus*; tank 1.4, *C. microporum*; tank 1.5) (**a**), microalgal plastic carboys (**b**), Asian clam (**b**), and Asian clam culturing for 8 weeks (**d**) (Changpasert et al. 2019)

In Fig. 5.2, around 80% of the algal cultivation cost is provided for the organic substrates (Mitra et al. 2012; Pal et al. 2019). Generally, many studies cultured microalgae in a laboratory to find out the optimum growth conditions to be included in a database for commercial scale-up. Open pond culture systems are comparatively inexpensive; however, they become easily contaminated. Besides, biomass recovery accounts for around 20–30% of total operation costs for biomass harvesting. Industrially useful compounds such as proteins, carbohydrates, pigments, and vitamins would keep their functionality after downstream processing. Different forms (fresh or dried) of microalgal production can be produced depending on the objective for utilization. Indeed, ease of use, low energy costs, and scalability need to be taken into account for commercial production as well (Perez-Garcia and Bashan 2015).

5.1.3 The Use of Microalgae in Formulated Aquafeeds

Currently, microalgae have been receiving strong interest due to their high protein content and usefulness when added directly into feed of animals to enhance growth performance in the aquaculture sector. In this section, current information/knowledge on general attributes of microalgal species used as dietary inclusion ingredients in aquafeed for replacement of fish meal are described. There are several advantages in using microalgae to substitute for or reduce the inclusion of traditional fish meal due to microalgae's valuable nutrition and beneficial impact on the growth rate of aquatic animals such as high growth performance, enhanced muscle protein, increased carcass quality, disease resistance, and improved immune response (Becker 2004). Some strains of microalgae selected for fish rearing appear based on convenience and low cost commercially. For instance, filamentous blue-green *Spirulina* sp. can be grown by commercial open pond systems for producing a dried powder, which has considerable popularity in the food industry and aquaculture (Soni et al. 2017).

The availability of information regarding possible ingredients for feeding aquatic animals has been rising exponentially. There are several reports on the utilization of biomass of blue-green algae, green algae, and diatom species (*Spirulina* sp., *Chlorella* sp., *Dunaliella* sp., *Nanochloropsis* sp., *Scenedesmus* sp., *Tetraselmis* sp., etc.) as an outstanding protein source for partial replacement of fish meal in the diet of many aquatic species and indicated as high potential food stock for aquafeeds (Badwy et al. 2008). Many studies demonstrated that microalgae could be used as a feed additive/replacement in a fish meal or feed to promote the growth of aquatic animals or improve their immunity effect in aquaculture (Table 5.3). The previous research replaced fish meal by using *Spirulina* species in the range of 7.5–32%, stimulating positive growth. Many reports showed that feed replacement with more than 10% of *Spirulina* has been successful for supporting aquaculture growth. For example, using 20% of *S. maxima* in formulated feed led to significantly better growth when substituted for animal protein in the diet for tilapia (*Oreochromis mossambicus* Peters fry) (Olvera-Novoa et al. 1998). Substituting 20% of *S. platensis* in feed for hybrid red tilapia (*O. mossambicus* × *O. niloticus*) as well as 20% replaced fish meal for Nile tilapia fingerlings (*O. niloticus*) supported specific growth rate and survival rate of these fish (Ungsethaphand et al. 2010; El-Ward et al. 2016). Similarly, when fish meal was replaced with 32% of *S. platensis*, there was no negative impact on the growth of red tilapia (*O. aureus* × *O. mossambicus*) fry (Sarr et al. 2019). Likewise, Rainbow trout (*Oncorhynchus mykiss*) had a higher weight gain and increased carotenoid concentration of skin and fillet when a fish meal was substituted with a low concentration of *S. platensis* at 7.5% (Teimouri et al. 2013).

One of the species known to be beneficial is *Chlorella* spp., a famous alga that is used for producing valuable health foods and supplements for human. However, high production costs for producing *Chlorella* for animal feed still prevents many crossover applications (Patterson et al. 2013). The useful *Chlorella* is rich in protein content, which stimulates growth performance, boosts immunity, and increases animal coloration depending on the level of algae in the aquafeed. For example, the common carp is a famous ornamental fish in Asia and Europe (Parkos et al. 2003). *Chlorella* inclusion in the diet did not negatively affect feed intake and survival of common Carp juveniles (*Cyprinus carpio* var. Koi) at 15% of replacement (Yadav et al. 2020). Giant freshwater prawn postlarvae (*Macrobrachium rosenbergii*) and Nile tilapia (*O. niloticus*) displayed significantly increased growth rates and enhanced immune response when fish meal was substituted by *C. vulgaris* at 4% and 15%, respectively (Maliwat et al. 2017; El-Habashi et al. 2019).

Microalgal cultures play a pivotal role as the principal feed for the early stages of larval development in shrimp hatcheries (Chepurnov et al. 2018). *Dunaliella* has

Table 5.3 Reported use of algae as an additive/replacement in fish feed for aquatic animals

Microalgae species	Aquatic species	Inclusion level	Type	Effect on aquatic animal	Trial duration	Weight gain (%)	SGR (%/ day)	Survival rate (%)	FCR	Reference
Spirulina maxima	Tilapia (*Oreochromis mossambicus* Peters fry)	20% (replacement)	Dried	Beneficial effects on growth of tilapia and substitute for the animal protein in the diet	9 weeks	1915.34	4.76	68.89	1.07	Olvera-Novoa et al. (1998)
Spirulina platensis	Hybrid red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*)	20% (replacement)	Dried	The final weight gain, specific growth rate, survival rate of fish was not affected by *Spirulina platensis* supplementation	120 days	1344.43	1.78	90.00	1.82	Ungsethaphand et al. (2010)
Spirulina platensis	Nile tilapia fingerlings (*Oreochromis niloticus*)	20% (replacement)	Dried	*Spirulina* were most effective in stimulating fish growth	56 days	184.56	1.87	98.00	1.83	El-Ward et al. (2016)
Spirulina platensis	Red tilapia (*Oreochromis aureus* × *Oreochromis mossambicus*) fry	32% (replacement)	Dried	Better growth and survival of red tilapia fry	8 weeks	448.33	2.84	72.00	1.95	Sarr et al. (2019)
Spirulina platensis	Rainbow trout (*Oncorhynchus mykiss*)	7.5% (replacement)	Dried	Carotenoid concentrations of skin and fillet increased significantly	10 weeks	131.40	1.39	–	1.00	Teimouri et al. (2013)

(continued)

Table 5.3 (continued)

Microalgae species	Aquatic species	Inclusion level	Type	Effect on aquatic animal	Trial duration	Weight gain (%)	SGR (%/ day)	Survival rate (%)	FCR	Reference
Chlorella sp.	Common carp juvenile (*Cyprinus carpio* var. koi)	15% (replacement)	Dried	*Chlorella* sp. did not affect the feed intake and survival, which showed the quality of developed feed to be at par with commercial feeds	60 days	55.70	0.74	100.00	2.17	Yadav et al. (2020)
Chlorella vulgaris	Giant freshwater prawn postlarvae (*Macrobrachium rosenbergii*)	4% (replacement)	Dried	Increased specific growth rate, enhanced immune response	50 days	442.11	1.47	65.56	–	Maliwat et al. (2017)
Chlorella vulgaris	Nile tilapia (*Oreochromis niloticus*)	15% (additive)	Dried	Increased the fish body protein and immune response	9 weeks	–	–	100.00	–	El-Habashi et al. (2019)
Dunaliella salina	Juveniles of white shrimp (*Fenneropenaeus indicus*)	2% (replacement)	Dried	Enhanced growth and body color in white shrimp *Fenneropenaeus indicus*	180 days	300.00	0.89	60.00	–	Ahmad et al. (2015)

Dunaliella salina	Giant tiger prawn (*Penaeus monodon*)	5–10% (additive)	Dried	Strongly enhanced the immunological and antioxidants factors (superoxide dismutase and catalase) and increased the survival rate	30 days	–	–	–	–	Madhumathi and Rengasamy (2011)
Haematococcus pluvialis	Pacific white shrimp juvenile (*Litopenaeus vannamei*)	12.5% (replacement)	Dried	High growth rate, appeared redder and contained higher free and esterified astaxanthins	8 weeks	943.40	4.11	95.80	2.13	Ju et al. (2012)
Tetraselmis chuii	Pacific whiteleg shrimp postlarvae (*Litopenaeus vannamei*)	50% (additive)	Dried	High survival and lower oxidative stress of shrimp	12 days	–	26.60	97.60	–	Rahman et al. (2017)
Nannochloropsis sp.	Kuruma shrimp postlarvae (*Marsupenaeus japonicus*)	4% (replacement)	Dried	Increased growth and survival of the shrimp	37 days	333.33	3.78	75.00	–	Adissin et al. (2019)
Nanofrustulum sp. + *Tetraselmis* sp.	Whiteleg shrimp postlarvae (*Litopenaeus vannamei* Boone)	5% (replacement)	Dried	Growth and feed performance of shrimp fed diets with included algae was not different from shrimp fed control diets	9 weeks	398.00	2.55	90.67	1.72	Kiron et al. (2012)

(continued)

Table 5.3 (continued)

Microalgae species	Aquatic species	Inclusion level	Type	Effect on aquatic animal	Trial duration	Weight gain (%)	SGR (%/day)	Survival rate (%)	FCR	Reference
Nannochloropsis gaditana	Gilthead seabream juvenile (*Sparus aurata*)	2.5% (additive)	Dried	High muscle growth	90 days	–	1.54	–	1.01	Ayala et al. (2020)
Tetraselmis suecica	European sea bass juveniles (*Dicentrarchus labrax*)	20% (replacement)	Dried	Growth performance and major quality traits of sea bass were not diminished	63 days	68.80	0.82	–	–	Tulli et al. (2012)
Scenedesmus almeriensis	Gilthead Sea bream juveniles (*Sparus aurata*)	20% (replacement)	Dried	Positive effects observed on gut functionality	45 days	220.39	2.58	–	1.70	Vizcaíno et al. (2014)
Scenedesmus obliquus	Spotted wolffish juveniles (*Anarhichas minor*)	4% (replacement)	Dried	Fast muscle cellularity of spotted wolffish	12 weeks	88.20	0.73	100.00	–	Knutsena et al. (2019)
Isochrysis galbana	Carnivorous marine fish (*Trachinotus ovatus*)	4.8% (replacement)	Dried	Improved growth performance, enhanced total n-3 fatty acids, DHA, and EPA in neutral and polar lipids of muscle and liver of fish	80 days	364.06	1.92	83.33	1.83	He et al. (2018)
Phaeodactylum tricornutum	Atlantic salmon (*Salmo salar*)	6% (replacement)	Dried	No negative effect on growth, feed conversion of protein, lipid, energy, ash, and growth performances	82 days	69.34	0.64	–	0.82	Sørensen et al. (2016)

Spirulina platensis	Bighead carp (*Hypophthalmichthys nobilis*)	100% Only algae	Live	A positive impact on improved growth performance and better feed efficiency of fish	90 days	625.00	2.20	96.60	2.10	Arumugam et al. (2020)
Chlorella vulgaris	Bighead carp (*Hypophthalmichthys nobilis*)	100% Only algae	Live	Good growth and improved fish appetite, which leads to increased feed intake and thereby improved growth	90 days	543.39	1.90	95.60	1.86	Arumugam et al. (2020)
Chlorella pyrenoidosa	Asian clam (*Corbicula fluminea*)	100% Only algae	Live	Increased survival rate, shell length, and wet weight gain of clam	8 weeks	8.43	1.01	97.78	–	Changpasert et al. (2019)
Chlorella cryptica	Asian clam (*Corbicula fluminea*)	100% Only algae	Live	Increased survival rate, shell length, and wet weight gain of clam	8 weeks	7.97	0.95	93.33	–	Changpasert et al. (2019)
Ourococcus multisporus	Asian clam (*Corbicula fluminea*)	100% Only algae	Live	Increased survival rate, shell length, and wet weight gain of clam	8 weeks	8.19	0.98	95.56	–	Changpasert et al. (2019)
Isochrysis galbana		100% Only algae	Live	Fresh weight, length, width, and	15 days	63.80	–	20–30	–	Thu et al. (2015)

(continued)

Table 5.3 (continued)

Microalgae species	Aquatic species	Inclusion level	Type	Effect on aquatic animal	Trial duration	Weight gain (%)	SGR (%/day)	Survival rate (%)	FCR	Reference
	Snout otter clam (*Lutraria rhyncheana* Jonas 1844)			thickness of the clam body increased						
Isochrysis galbana + *Chlorella* sp.	European flat oyster (*Ostrea edulis* Linnaeus 1758)	50:50% Only algae	Live	Growth and survival rates significantly higher than only *Chlorella* sp. and unfed groups	16 days	–	0.02	24%	–	Acarli and Lok (2011)

– = not available, *SGR* = specific growth rate, and *FCR* = feed conversion ratio.

been used as natural food. Some researchers have also proven that *D. salina* can increase the growth of several types of aquatic animal because of its nutritional contents such as beta-carotene which augments the immune system (Alishahi et al. 2014). For instance, juveniles of white shrimp (*Fenneropenaeus indicus*) and giant tiger prawn (*Penaeus monodon*) have been fed with *D. salina* at the level of 2 and 5–10% substitution in a fish meal without adverse impact on animal growth, increasing the survival rate and enhancing body color (Ahmad et al. 2015; Madhumathi and Rengasamy 2011). Whiteleg shrimp (*Litopenaeus vannamei*), also known as king prawn or Pacific white shrimp, is largely cultivated in Southeast Asia. However, the loss of shrimp production may be caused by environmental problems, pathogenic infection, low survival rate, and weak larvae (Lightner 2011). The green microalgae, *Haematococcus pluvialis* contains useful pigment (carotene and astaxanthin) that has been added in aquafeed which enhances color pigments for many kinds of fish and shrimp (Chien and Shiau 2005). According to the study of Ju et al. (2012) that fed 12.5% of defatted *H. pluvialis* in diet, a high growth rate was supported and astaxanthin accumulation was stimulated in Pacific white shrimp juveniles. Moreover, Pacific whiteleg shrimp postlarvae had high survival and oxidative stress decreased by feeding 50% of microalgae *T. chuii* replacing a fish meal (Rahman et al. 2017). Increased growth and survival of the kuruma shrimp postlarvae (*Marsupenaeus japonicus*) was observed by replacing a small amount of *Nannochloropsis* sp. at 4% in the diet (Adissin et al. 2019). In addition, 5% of the algal combination of *Nanofrustulum* and *Tetraselmis* substituted in the fish meal had potential to improve the growth of white leg shrimp postlarvae (*L. vannamei* Boone) (Kiron et al. 2012).

Adding 2.5% *N. gaditana* inclusion in the diet had a positive effect on muscle growth of gilthead seabream juvenile (*Sparus aurata*) (Ayala et al. 2020). Dried algae *T. suecica* did not hamper the growth of European sea bass when substituted at 20% in the fish diet (Tulli et al. 2012). The closely related *S. almeriensis* exhibited significantly positive effects on gut functionality in gilthead seabream by replacing 20% of the fish meal in the diet without negatively affecting fish growth (Vizcaíno et al. 2014). Knutsena et al. (2019) stated that the muscle cellularity of spotted wolffish juveniles (*Anarhichas minor*) was growing rapidly when substituting 4% of *S. obliquus* in fish meal.

Marine diatoms have been extensively used in the aquaculture industry such as the species of *I. galbana* which is applied most often to strengthen initial feeding, as these algae are rich in valuable oil (Bruce et al. 1940; Li et al. 2015). For example, He et al. (2018) added 4.8% for replacing *I. galbana* in a fish meal for feeding carnivorous marine fish (*Trachinotus ovatus*) that has been frequently found in Southern China due to its delicious meat. *I. galbana* stimulated better quality fish muscle and liver with high DHA and EPA contents and enhanced fish growth performance. In addition, replacing 6% of the diatom *Phaeodactylum tricornutum* in the fish meal resulted in normal function of feed utilization, growth performance, and nutrient digestibility in Atlantic salmon (Sørensen et al. 2016).

Live microalgae are considered as primary food sources for growing many life stages of mollusks and other aquatic animals that need highly valuable nutrients for larval and early juvenile stages. Moreover, they have an appropriate cell size, fast growth rate, and are easily digested for rearing abalone, clam, crustaceans, and some fish species (Thu et al. 2015). Various reports use microalgae as an effective live feed for culturing aquatic animals. Better feed efficiency and growth performance were found in bighead carp (*Hypophthalmichthys nobilis*) when separately fed with 100% live *S. platensis* and *C. vulgaris*, rather than the conventional pellet (Arumugam et al. 2020). Changpasert et al. (2019) studied five live microalgae, *C. pyrenoidosa*, *C. cryptica*, *O. multisporus*, *S. acutus,* and *C. microporum*, that were isolated for separately rearing the Asiatic clam (*Corbicula fluminea*), which is one of the popular Taiwanese clams for producing food ingredients and nutritional supplements. The results showed that *C. pyrenoidosa*, *C. cryptica*, and *O. multisporus* had appropriately small cell sizes and their biochemical compositions were suitable for stimulating the growth and survival rate of the clam (Fig. 5.2). Moreover, snout otter clam (*Lutraria rhyncheana*) reared 100% on live feed of *I. galbana* experienced a positive effect on its width, length, weight, thickness, and survival rate (Thu et al. 2015). In addition, the mixed live algae (50:50%) of *I. galbana* + *Chlorella* sp. enhanced high daily mean length increment of European flat oyster larvae (*Ostrea edulis*) (Acarli and Lok 2011). However, various types of microalgae were used to culture different aquatic animals at different developmental stages based on the nutrient requirements of the aquatic animals (Li et al. 2015).

Notwithstanding, replacing higher inclusion levels of microalgae in aquafeed may have a negative effect on feed efficiency and fish growth performance because of the inhibitory substances in microalgae such as protease, amylase, tannins inhibitors, etc. (Norambuena et al. 2015). Thus, adding or replacing the microalgae at a low to medium level of inclusion would be appropriate for aquafeed (Yadav et al. 2020). Nevertheless, recent knowledge and information on the utilization of microalgae for fish meal replacement and sustainable aquaculture industry development needs to be updated.

5.1.4 Application of Yeast and Alternative Protein Sources as Marine Feed Ingredients

Yeast are single-celled microorganisms that are classified in the fungi kingdom. This fungus ranges in size from 3 to 4 micrometers and it contains a cell wall like plant cells, but without chloroplast. Yeast is classified as a facultative anaerobic microorganism that can grow with or without oxygen (Øverland and Skrede 2016). With their activity, the yeast can produce alcohol and carbon dioxide via the conversion of sugar and oxygen with oxidative metabolism, contributing to yeast cell proliferation.

Some reports have illustrated that yeast and its derivatives have been fed to animals for a long time (more than 100 years) (Shurson 2017). In present, several countries with livestock, as well as aquaculture, are concerned about the use of antibiotics for feeding animals. Elimination of this process is desirable and consideration of methods to accomplish this is trending. As a consequence, many researchers are attempting to find alternative ingredient sources that can impart animal health and enhance growth performance (Shurson 2017; Shurson et al. 2015). Moreover, Shurson (2017) also noted that feed ingredients obtained from the fermentation process of yeast (for instance distillers dried grains with solubles) in animal feed have dramatically increased nowadays. Thus, there are several types of animal or aquatic feeds containing yeast ingredients that have been produced for stimulating the animal's growth rate or activity. However, only few studies of potentiality, efficiency, or different effects of those feeds containing yeast on the animals' health have been noted. There is no report on the adverse effects of utilization of yeast in animal feeds on animal metabolisms, as well as aquaculture environments such as fish farming.

To produce potential feeds with high nutritional value and reduce cost in the feed industry, strategies to increase the utilization of yeast in fish feeds are of great interest nowadays in the field of aquatic feed development. Yeasts have been highlighted as a potent ingredient source in fish feed because of their nutritional value (protein, lipids, B-vitamins, etc.). Furthermore, yeast can transform low-value by-products from agricultural biomass manufacture into a high-value product without the extravagant utilization of land, water, as well as changing climatic conditions. Thus, it is one of the reasons why yeast is esteemed as a good ingredient and has high potentiality for aquaculture feed production (Agboola et al. 2021). *Saccharomyces cerevisiae, Kluyveromyces marxianus, Wickerhamomyces anomalus, Blastobotrys adeninivorans,* and *Cyberlindnera jadinii* are commercial yeasts that provide a nutritional composition that is promising for aquaculture. The crude protein of these yeasts ranges from 382 to 528 g/kg of dry matter (Agboola et al. 2021). Besides, yeast is composed of high ash content, moderate levels of carbohydrates, and a low lipid content which is mostly unsaturated fatty acids (Øverland et al. 2013). Aside from macronutrients, yeasts also present small sources of micronutrients such as vitamins (vitamin B groups), minerals, together with some enzymes (Lapeña et al. 2020). Focusing on the protein source for aquaculture feeding, yeast can enhance amino acids, small molecules from protein structure digestion that can be absorbed and used for the health benefits of animals through the optimization of feed formulation. The imbalance of amino acids supplementation for marine feeds is caused by the wrong augment of amino acids presented in yeasts. Nevertheless, the difference between of two intrinsic or extrinsic amino acids produced by yeast has demonstrated to the aquatic animal metabolisms which were the extrinsic amino acids (crystalline amino acids) tend to be easier than intrinsic when process into the intestine lumen (Larsen et al. 2012). Hence, controlling diet optimization in fish feeds through the strategy of interaction between intrinsic and crystalline amino acids is interesting in the development or improvement of dietary utilization of yeasts. Some studies have reported the synchronization of both amino acid types with long-term frequency feeding for the fish which was well documented for the aquaculture farming, especially in rainbow trout, common carp (Nwanna et al. 2012), Nile tilapia, and channel catfish (Lanna et al. 2016).

Up to now, the application of dietary enzymes for utilizing fish feed supplementation has been conducted to stimulate or enhance the efficiency of nutritional value digestibility and yeast in fish. Indeed, the cell wall of yeast generally composes the polysaccharides complex structure which is unsusceptible for the endogenous enzymes produced by aquatic animal species (Shurson 2017; Agboola et al. 2021). Based on the reason above, it is a great challenge for supplementation of exogenous enzymes in aquatic diets leading to promote the degradation of the yeast cell wall and stimulate nutrient utilization. Mannanase, glucanase, chitinase, and glucosidase are examples of commercial dietary enzymes that are specific for the yeast cell wall (Agboola et al. 2021). The synchronization between bio-substance and single-cell microorganisms like yeast may be a possible technique for unlocking potential nutrients of several yeast species and enzymes and contributing to the success of novel aquatic feed ingredient formulations. *Phaffia* yeast is the one species of yeast that is of addressed interest to the aquaculture industry. According to its nutritional factors associated with the yeast's products, *P. rhodozyma* contains astaxanthin (most abundant in carotenoid for the marine environment) which is the major pigment in the salmonid fish (Weber et al. 2003; Zhuang and Zhu 2021). One kind of yeast like *Saccharomyces cerevisiae* has been classified and indexed as a macro-ingredient in aquaculture feeds. There were many studies reported about the effect of *S. cerevisiae* supplementation in fish feeds. For instance, in African catfish (*Clarias gariepinus*) Essa et al. (2011) reported that *S. cerevisiae* could be used to improve the profitability of African catfish within 186 days. Around 40% of replacement of fish meal protein had utilized an intact and extracted *S. cerevisiae* without compromising feed conversion ratio (FCR) in Artic charr for 99 days (Vidakovic et al. 2016). Feeding trials of 84 days found that *S. cerevisiae* up to 45% in the fish feed formulation improved performance of goldfish (Gumus et al. 2016). Furthermore, the study of Nguyen et al. (2019) reported that it is possible for a partial replacement of 60% fish meal in giant freshwater prawn reared diets without adverse effects in the aquatic animal by way of recirculating aquaculture system (RAS) or a biofloc system for 3 months.

Even though many studies illustrate the beneficial effect of yeast cell wall derivatives in fish, only a few studies have considered yeast as a macro protein ingredient in fish feeds (Agboola et al. 2021). However, some yeast such as *Saccharomyces cerevisiae* that are useful for the brewing industry (Ferreira et al. 2010) or baked products has been emphasized for the replacement of soy protein in aquaculture feeds which was found to not have any adverse effects on the growth performance rate of marine fish species such as Atlantic salmon (Øverland et al. 2013). On the other hand, Øverland and Skrede (2016) noted that the yeast strain, fermentation media, and post-fermentation step, as well as aquaculture species and diet optimization and formulation, are vital factors leading to the decreased growth rate of some fish species caused by the increasing levels of *S. cerevisiae* utilization. Øverland et al. (2013) noted that use of *S. cerevisiae* at 40% in Atlantic salmon for 89 days of feeding depressed salmon growth and nutrient utilization. Nevertheless, there is mounting scientific evidence regarding the utilization of other species of yeast supplemented in aquatic feeds. Babu et al. (2013) studied and reported fish feed that contained 10% of *Candida aquaetxtris* (CA) on black tiger shrimp (*Penaeus monodon*) activity. The white spot syndrome virus was inhibited after CA intake every 7 days. For Nile tilapia (*Oreochromis niloticus*), up to 1% of *Rhodotorula mucilaginosa* inclusion with a fish diet for almost 2 months could enhance beneficial attributes including growth performance, nutrient composition, and immune response, as well as the antioxidative properties of Nile tilapia species (Chen et al. 2019). In addition, the combination of two yeast species (about 40–60%), *Wickerhamomyces anomalus* mixed with *S. cerevisiae* ratios 70:30, substituted for fish meal into feeds of rainbow trout (*Oncorhynchus mykiss*) showed that both yeasts stimulated and modulated the gut microbiota in rainbow trout intestines (Huy et al. 2020). Vidakovic et al. (2020) also agreed with the finding of the previous study which the result showed consistency for maximum acceptable replacement of fish meal (at 60%) in the diet of rainbow trout fish species. Therefore, the replacement of fish meal protein with one or a combination of yeast caused no negative effects on the aquatic animal growth rate, nutrient utilization, and digestibility along with gut microbiomes activity which promoted good intestinal health of rainbow trout. Consequently, the responsible alternative ingredient sources of the aquaculture environment and/or other marine species are concerned with the overall sustainability index of several fish feed manufacturers together with traditional fish farms. In this part, the use of yeast or yeast derivative products as the alternative protein source and perhaps conversion to the main protein ingredient in aquaculture feeds compared to traditional feed ingredients and formulations depends on the contribution of the feed industry. With the above, to best understand the impact of attributable yeast on fish protein ingredients, a holistic life cycle investigation of the process related to aquatic feed production should be realized.

Generally, fish meal is used as a major source of protein in fish feeds or marine species because of its contained high nutritional value and palatability. However, in the aquaculture process, the fish feed has constituted more than 60% of the total cost of operation, contributing to several researchers trying to develop more cost-effective new ingredients with equivalent efficiency to be used as the main protein source (Daniel 2018). According to the plant-based protein trend in recent years, various plants that contain high levels of protein such as soy, seaweed, or other plant tubers are recommended regarding the cost efficiency. They seem to be cheaper than fish meal while providing adequate animal nutrition as well as aquaculture performance. Researchers have reported that there is no difference in the growth rate of aquatic animals with replacement of fish meal at suitable ratios by plant-based protein supplemented into the fish feeds when compared with original fish meal-fed animals (Yu et al. 2014; Suprayudi et al. 2014; Bonaldo et al. 2015; Minjarez-Osorio et al. 2016; Valente et al. 2016). The response and ability of each aquatic animal to utilize alternative plant proteins is different. Moreover, significant concerns related to growth reduction and feed efficiency are anti-nutritional factors (ANFs), toxicity, and low digestibility of amino acids, minerals, non-soluble fiber and starch (Daniel 2018). In the last decade, Sicuro et al. (2012) studied the replacement of fish meal by corn gluten at more than 50%, but not to exceed 60% and observed no negative effects for hybrid sturgeons “AL” (*Acipenser naccarii* ×

A. baeri), while substitution with pea meal showed a reduction in utilization efficiency (Table 5.4). This phenomenon may be caused by ANFs. In the same way, the substitution of defatted rubber seed at 50% into marine feed that contained fish meal and was fed to common carp juvenile (*Cyprinus carpio* L.) had also presented positive effects on the growth performance. Although other protein sources are beneficial and have a potentiality for alternative fish feed ingredients, the cyanide content in the diet is an important limitation as well (Suprayudi et al. 2014). Consequently, the study of this substance's limitation needs to find out and clarify its mode of action on the health functionality of aquatic species and the marine environment.

The esteemed protein source of animal feeds is soybean or its related extracted product. Soybean is inexpensive and can be utilized in livestock and aquaculture on a large scale. It is composed of beneficial amino acids and some essential amino acids that play a vital role in animal cell activity and growth (Kader et al. 2012). The substitution of soybean protein into fish feeds has been recognized at 50–75% of total fish meal. Some aquatic species such as red drum (*Sciaenops ocellatus*) and shortfin corvina (*Cynoscion parvipinnis*) have been reported to acceptably utilize soybean protein at 25 and 75% replacement for fish meal, respectively, as described by Minjarez-Osorio et al. (2016). Bonaldo et al. (2015) supported that a mixture of fish meal and soybean meal, wheat gluten, or soy protein isolate/concentrate up to 50% levels in aquaculture feeds did not influence the nutrient utilization and digestion in turbot (*Psetta maxima*), meanwhile only 5% of the whole plant or plant extracted substitute caused a worsening of growth effectiveness and fish welfare, probably due to insufficient feeding and nutrient intake. The study of Yu et al. (2014) evaluated the effects of feeding cottonseed meal with added lysine to juvenile Chinese sucker (*Myxocyprinus asiaticus*). This combination did not adversely affect growth, though Chinese sucker did not improve in growth performance when ferrous sulfate was added into cottonseed meal.

From this point of view, therefore, reducing fish meal inclusion levels and replacing fish meal with cost-effective, widely available, and environmentally friendly sources of nutrients would be good for aquatic health and considered essential for the future development of the aquaculture industry.

5.1.5 Probiotics as Antibiotic Alternatives and Immune Stimulants

Based on input utilization and stocking density, aquaculture production can be divided into three systems, including extensive, semi-intensive, and intensive methods. Extensive fish farming usually refers to spacious ponds with low number of fish per unit area. Supplied inputs and control for this system are less significant factors than labor. On the other hand, intensive fish farming consists of systems that intend to use the minimum quantity of water to obtain maximum production of fish,

Table 5.4 Reported use of yeast or plant protein sources as an additive/replacement in fish feed

Yeast or plant protein source	Inclusion level	Aquatic species	Effect on aquatic animal	Trial duration	Weight gain (%)	SGR (%/day)	Survival rate (%)	FCR	Reference
Corn gluten	55% (replacement)	Hybrid sturgeons "AL" (*Acipenser naccarii* × *Acipenser baeri*)	Corn gluten was acceptable as a partial substitute for fish meal	60 days	–	0.54	–	1.30	Sicuro et al. (2012)
Defatted rubber seed meal	50% (replacement)	Common carp (*Cyprinus carpio* L.) juvenile	Resulted in a comparable growth and feeding performance without any adverse effect	40 days	260.00 (relative growth)	–	86.70	–	Suprayudi et al. (2014)
Non-genetically modified soybean meal	25% (replacement)	Red drum (*Sciaenops ocellatus*)	Highest relative weight gain	7 weeks	755.04	–	85.00	–	Minjarez-Osorio et al. (2016)
Soybean protein concentrate	75% (replacement)	Shortfin corvina (*Cynoscion parvipinnis*)	High survival rate and weight gain	8 weeks	455.81	–	92.50	–	Minjarez-Osorio et al. (2016)
Mixture of wheat gluten, soybean meal and soy protein concentrate	50% (replacement)	Turbot juveniles (*Psetta maxima*)	Results highlighted the consistency between growth performance and welfare status	9 weeks	612.37	3.11	–	–	Bonaldo et al. (2015)
Blend of soybean meal, peas, corn gluten, and wheat	75% (replacement)	Senegalese sole (*Solea senegalensis*)	Significantly affected muscle growth dynamics	140 days	103.59	–	–	–	Valente et al. (2016)
Groundnut cake	10% (replacement)	Fingerling of vundu (*Heterobranchus longifilis*)	Resulted in best achieved weight gain and specific growth rate of *Heterobranchus longifilis*	84 days	–	1.67	66.67	6.59	Ovie and Ovie (2007)

(continued)

Table 5.4 (continued)

Yeast or plant protein source	Inclusion level	Aquatic species	Effect on aquatic animal	Trial duration	Weight gain (%)	SGR (%/ day)	Survival rate (%)	FCR	Reference
Cottonseed meal with lysine	26.65% (replacement)	Chinese sucker (*Myxocyprinus asiaticus*)	No adverse effects on growth performance, body composition and digestive enzyme activities	8 weeks	336.74	2.63	97.00	–	Yu et al. (2014)
Plant protein diet mixed with 10% hydrolysed fish protein	80% (replacement)	Atlantic salmon (*Salmo salar*)	Resulted in high levels of amino acids in blood	12 weeks	338.74	–	–	0.88	Egerton et al. (2020)
Torula yeast (*Candida utilis*)	30% (replacement)	Tilapia (*Oreochromis mossambicus* Peters) fry	Best growth performance and diet composition, did not affect feed or protein utilization	63 days	–	–	–	–	Olvera-Novoa et al. (2002)
Brewer's yeast (*Saccharomyces cerevisiae*)	2% (replacement)	Hybrid striped bass juvenile (*Morone chrysops* × *Morone saxatilis*)	Enhanced growth performance	7 weeks	404.00	–	93.80	–	Li and Gatlin (2004)
Brewer's yeast (*Saccharomyces cerevisiae*)	15% (replacement)	Nile tilapia (*Oreochromis niloticus*)	Promoted growth and efficient diet utilization without affecting body composition	51 days	–	–	–	1.50	Ozório et al. (2012)
Baker's yeast (*Saccharomyces cerevisiae*)	3% (additive)	Nile tilapia (*Oreochromis niloticus*)	A pro-health ingredient, effected growth performance, immune and stress tolerance status	84 days	–	0.77	95.00	1.36	Abass et al. (2018)

– = not available, *SGR* = specific growth rate, *FCR* = feed conversion ratio.

while great farm management and use of inputs are required (Pillay and Kutty 2005). Due to the high demands of aquatic animals in global markets, intensive aquaculture methods have been generally applied to earn more profits. However, there are several challenges that could occur, especially a high risk of infectious disease devastation (Naylor et al. 2000). This could threaten the sustainability of the fish production industry (Dawood et al. 2019). Obviously, antibiotics are generally used in order to not only prevent the infection, but also to promote the fish growth. However, drug resistant microorganisms could result and antibiotic residues may contaminate the fish or remain in the environment (Douet et al. 2009), causing the increase of safety concerns. Therefore, the quest for alternative methods to prevent fish from infections have been launched. Different substances, both chemical and biological agents, extracts from animals or plants, as well as living microorganisms and their components have also been applied to enhance fish health. Various kinds of probiotics and their components like bacteriocins, such as lactoferrin, nisin and recombinant transferrin have been reported for their antimicrobial activities and inhibitory effects, growth promoting effects, and immune stimulating functions (Chauhan and Singh 2019). Those substances could be supplemented into fish diets or administrated via immersion or injection.

Probiotics are defined as “live microbes, which when administered in sufficient amount, confer a health benefit to the host” (FAO/WHO 2002). They could prevent several infectious diseases, enhance immune responses, and increase fish growth performance, as well as improving water quality (Dawood et al. 2019). Several probiotic species have been introduced to aquatic animals, including not limited to bacteria, but also microalgae and yeasts. Table 5.5 demonstrates potential probiotic species used in aquaculture (Camacho et al. 2019; Chauhan and Singh 2019; Wang et al. 2019a).

Probiotics exhibit several modes of action against pathogens and harmful intestinal bacteria. Competitive exclusion for adhesion and secretion of a variety of inhibitory substances of probiotics such as acetic acid, lactic acid, peroxide, bacteriocins, siderophore, and lysozyme have been well documented as the direct action of probiotics against pathogenic inhibition (Van Doan et al. 2020). Probiotics could enhance digestive capability of the fish, improving feed utilization and weight gain by producing extracellular enzymes to hydrolyze both diet nutrients and indigestible components. They produced some vitamins and degraded some toxins in diets as well as that produced by intestinal microbial populations (Hasan and Banerjee 2020). Probiotics also played an important role in modulating the immunology by interacting with epithelial and dendritic cells and with monocytes/macrophages and lymphocytes, resulting in increase of immunoglobulins production, macrophage and lymphocyte activities, as well as γ-interferon production (Hasan and Banerjee 2020). Due to their antimicrobial activities, probiotics also could function as a bioremediation tool. They helped improve rearing water quality by modulating the microorganism community, inhibiting pathogenic bacteria in the aquaculture environment, and removing some organic wastes in the water (Dawood et al. 2019). In this chapter, the effects of probiotics supplemented into the fish feed will mainly focus on the health of tilapia, rainbow trout, and shrimp.

Table 5.5 Probiotic species used aquaculture

Bacteria	Microalgae	Yeast
Bacillus aerophilus *Bacillus aerophilus (KM277363)* *Bacillus amyloliquefaciens* *Bacillus cereus* *Bacillus coagulans* *Bacillus firmus* *Bacillus licheniformis* *Bacillus mesentericus* *Bacillus mojavensis* *Enterococcus faecium* *Lactobacillus acidophilus* *Lactobacillus farraginis* *Lactobacillus fermentum Lactobacillus plantarum* *Lactococcus lactis* *Nitrosomonas* species *Pediococcus acidilactici* *Solibacillus silvestris* *Streptococcus faecium*	*Artemia* *Chaetoceros* sp. *Hippocampus reidi* *Isochrysis* sp. *Nannochloropsis oculata* *Pavlova* sp. *Spirulina* sp. *Tetraselmis suecica*	*Hanseniaspora opuntiae* *Metschnikowia* sp. *Rhodotorula benthica* *Rhodotorula* sp. *Rhodosporidium paludigenum* *Saccharomyces cerevisiae*

Sources: Camacho et al. (2019), Chauhan and Singh (2019), Wang et al. (2019a).

5.1.5.1 Nile Tilapia

Several studies have reported the effects of probiotics on inhibition of pathogens in fish such as Nile tilapia, rainbow trout, common carp, Atlantic cod, and sea bass juveniles. Nile tilapia (*Oreochromis niloticus*) were ranked as the second most produced finfish in global aquaculture (FAO 2020). Because of the high demand, they are usually cultured in intensive systems where the fish could be stocked up to 200 g per square meter cage (Mengistu et al. 2020), resulting in high risk of the spread of pathogens. Streptococcosis, a fish disease caused by the infection of *Streptococcus agalactiae*, is one of the important fish diseases affecting fish farming worldwide and can happen in all types of production systems (Jansen et al. 2019). The disease can cause fish abnormal swimming behavior, eye and skin hemorrhages, abscesses on the inferior jaw and the pectoral fin, and deaths eventually (Amal and Zamri-Saad 2011). Ninety percent of tilapias mortality was found after 2 days of *S. agalactiae* infection (Sirimanapong et al. 2018). Huge financial losses for the global aquatic industry due to the outbreak have been reported every year (Li et al. 2020). To overcome the problems, research of gut bacterial compositions and probiotics in inhibition of *S. agalactiae* in fish has been increasing because of the demand for producers and environment-friendly aquaculture.

The microbial community composition of the Nile tilapia (*O. niloticus*) and their antibacterial effects against *S. agalactiae* were reported. Differences in bacterial compositions were observed in the fish obtained from different farms. Bacteria in the phylum of Firmicutes and genus Clostridium was mainly detected in the Atlantic farm-raised tilapia, while the Llanos farmed fish showed the majority bacteria in *Cetobacterium* spp., *Clostridium* spp., and *Plesiomonas* spp. During continuous-flow competitive exclusion culture, *Cetobacterium* spp., *Clostridium* spp., and *Lactococcus* spp. were the most abundant genera identified after 2, 5, and 33 days of cultivation, respectively. In addition, *Cetobacterium* spp. and *Clostridium* spp. exhibited their inhibition ability by producing extracellular substances against *S. agalactiae* (Melo-Bolívar et al. 2019). Fifty-five isolates were found in the intestine of Nile tilapia in Thailand. Out of 55 isolates, there were 52 isolates identified as *Bacillus* spp. Only 22 isolates exhibited inhibition or colonization of *S. agalactiae*. Eight isolates with different degree of colonization and inhibition were identified as *B. amyloliquefaciens, B. subtilis, B. cereus, Bacillus altitudinis,* and *Bacillus thuringiensis*. They could survive under the lowest pH of 2 and the highest pH of 9, and in bile salts for 6 h (Sookchaiyaporn et al. 2020). Similarly, three species of *Bacillus* spp. were isolated from the tilapia intestine and identified as *Bacillus velezensis*, *B. subtilis*, and *B. amyloliquefaciens*. All of them could inhibit *S. agalactiae* and were resistant to heat, high acidity, and concentrated bile salts (Kuebutornye et al. 2020). The effects of mixed *B. subtilis* and *E. faecalis* on intestinal microbiology of farmed tilapia infected by *S. agalactiae* were observed. The fish that was challenged by *S. agalactiae* fed with no addition of the probiotics in the diet could not survive after 15 weeks. On the contrary, the infected fish fed with the mixed probiotics showed more than 60% of survival ratio. This was explained that it may be because the probiotics could alter the composition of the microbiome by increasing the diversity and enhancing resistance against pathogenic microorganisms. Exact sequence variants (ESVs) determined by using the ribosomal small-subunit V4 region of bacteria in the animals were used to represent composition of the microbiome. It was reported that higher ESVs of *Enterococcus* ssp. and *Bacillus* ssp. and lower ESVs of *Streptococcus* spp. were identified in the fish fed with probiotics. Only four genera of *Vibrio* spp. were found in the fish fed with mixed probiotics, but non-treated probiotic fish had seven genera of the bacteria. Besides, only the fish fed with probiotics had the microorganisms from the genera Synechococcus, Shewanella, Spirochaeta, Psychromonas, Psychrilyobacter, Kiloniella, and Allofrancisella, indicating more diverse gut microbial species than the control (Pan et al. 2021).

E. faecium were added into tilapia basal diets and fed to the fish at days 7 and 14. *E. faecium* increased final weight, weight gain and final biomass, as well as respiratory burst activity. The results demonstrated that *E. faecium* could promote fish growth conditions and maintain the defense mechanisms through immune systems (Tachibana et al. 2020). They could trigger functional mechanisms of intraepithelial T lymphocytes and IgA, macrophages, and dendritic cells, leading to production of nitric oxide and microbe digestion (Khalkhali and Mojgani 2017). The basal plant-based protein diet supplemented with *Bacillus pumilus* and protease were fed to tilapia for 84 days. These fish had better growth performance and feed utilization than the basal diet group. It was explained that *B. pumilus* and exogenous protease provided synergistic effects to the fish (Hassaan et al. 2021). The enzyme could enhance the probiotic activity by hydrolyzing substrates for the microorganism.

Simultaneously, the probiotic possibly improved the nutrient efficiency by stimulating the appetite, inhibiting any toxic compounds, producing essential metabolites, or changing the microbiota profile (Zorriehzahra et al. 2016; Sankar et al. 2017; Zhang et al. 2019). Moreover, the results indicated that *B. pumilus* and/or protease could enhance immune responses which could be related to their antimicrobial effects against the pathogenic bacteria in the gastrointestinal tract (Hassaan et al. 2021). Similarly, tilapia fed with diet containing *L. plantarum* showed higher weight gain than the control and increased lysozyme, tumor necrosis factor-alpha (TNF-α), and interleukin 1 beta (IL-1β) significantly (Mohammadi et al. 2021) This could be because *L. plantarum* supplementation probably increased beneficial colonic bacteria in the fish gut (de Sousa et al. 2019) or enhancing digestive activities of protease, lipase, and amylase. The probiotics could colonize to the intestinal epithelial cells, secrete digestive enzymes, and produce amino acids, fatty acids, and vitamins providing the nutrients for absorption (Liu et al. 2017). IL-1β and TNF-α were modulated through secondary metabolism of microorganisms present in the culture pond (Duan et al. 2001).

5.1.5.2 Rainbow Trout

Rainbow trout (*Oncorhynchus mykiss*) are popular coldwater finfish which are mainly cultured in Europe, America, and Australia. The global production of rainbow trout in 2018 was reported at 848.1 tones, ranked in the top 15 finfish species produced worldwide (FAO 2020). Alike tilapia production, due to intensification of the aquaculture, many rainbow trout farms have been experiencing unpredictable mortalities, caused by bacterial infection diseases such as streptococcosis (*S. agalactiae* and *Streptococcus iniae*), lactococcosis (*Lactococcus garvieae*), columnaris disease (*Flavobacterium columnare*), winter ulcer (*Vibrio viscosus*), enteric redmouth (*Yersinia ruckeri*), and Piscirickettsiosis (*Piscirickettsia salmonis*) (Dalsgaard and Madsen 2000; Toranzo et al. 2005). Several probiotic species have been introduced to the rainbow trout, such as *B. subtilis*, *B. licheniformi*, *Clostridium butyricum, Enterobacter amnigenus*, *Lactobacillus rhamnosus,* and *Pediococcus acidilactici* (Zorriehzahra et al. 2016). The major purposes were to prevent the fish from the infection, to promote the fish health, and to establish sustainable aquaculture practices.

The mixed 11 bacterial strains isolated from rainbow trout microbiota including *Aeromonas rivipollensis, Pseudomonas helmanticensis, Aeromonas rivipollensis, Pseudomonas baetica, Aeromonas hydrophila, Flavobacterium plurextorum* (strain 4517), *Acinetobacter* sp., *F. plurextorum* (strain 4519), *Delftia acidovorans, Flavobacterium* sp. (strain 4416), and *Pseudomonas* sp. could provide protection against *F. columnare* infection of the fish, while the fish in the germ-free condition died after infection with the pathogen for 48 h. Moreover, it was found that when the fish was treated with only *Flavobacterium* sp. (strain 4416), it could confer the protection against *F. columnare* effectively. Nevertheless, mixture of the other 10 strains did not show any effects. This indicated that *Flavobacterium* sp. (strain

4416) was a putative endogenous probiotic strain protecting the host form *F. columnare* infection. The researcher suggested *Flavobacterium* sp. (strain 4416), as an endogenous probiotic strain, possibly affected cell differentiation of the trout gut epithelium, resulting in stimulation of acute immune responses against the pathogen infection (Pérez-Pascual et al. 2021). Indigenous lactic acid bacteria, *Lactococcus lactis* and *Weissella oryzae*, isolated from the rainbow trout intestine could inhibit *S. iniae* and *Y. ruckeri* by producing extracellular substances, probably silver nanoparticles produced by *W. oryzae*, against the pathogen and increasing the number of beneficial bacteria in the fish, resulting in the improvement of intestinal gut microbiota balances (Mortezaei et al. 2020). *B. amyloliquefaciens* 131 and *Paenibacillus* spp. isolated from the healthy rainbow trout intestine were co-cultured and determined for their inhibitory effects against *Y. ruckeri*. The result showed that after coculturing at both 22 and 15 °C, *B. amyloliquefaciens* 131 and *Paenibacillus* spp. could completely inhibit *Y. ruckeri,* but not when they were incubated at 12 °C. Those two strains were tolerant to high concentrations of rainbow trout bile salts and acidic conditions, produced amylase, proteinase, and lipase, and had no pathogenic effects on the host during 20 days of culture (Medina et al. 2020). Similarly, autochthonous probiotics, *Lactobacillus delbrukei* subsp. *Bulgaricus* and *L. acidophilus* isolated from wild *Tor grypus* and *Citrobacter farmer* isolated from *Cyprinus carpio*, were supplemented to diets of juvenile rainbow trout to investigate their inhibitory activity against *Lactococcus garvieae* infection. In the in vitro study, it was discovered that all three strains had antagonistic activity against the pathogen. *L. garvieae* was more susceptible to *L. bulgaricus* and *L. acidophilus* rather than *C. farmer*. After feeding the *L. garvieae* infected fish with those probiotics for 4 days, *L. bulgaricus* and *L. acidophilus* could effectively reduce the fish mortality with survival rates of 63.71% and 51.56%, respectively (Mohammadian et al. 2019).

The effects of multistrain probiotics containing *B. subtilis, E. faecium, Pediococcus acidilactici*, and *L. reuteri* on growth performance and non-specific immune response of rainbow trout were studied. The probiotics were mixed with a basal diet and fed to the fish three times daily for 8 weeks. Sufficient amount of probiotic (1 g/kg) significantly improved growth and feed efficiency of the fish. Probiotics were likely to produce substances affecting the population of intestinal microorganisms of the fish. They also showed an ability related to antioxidant defense mechanisms by increasing the activity of glutathione-based enzyme, reducing the effects of poisonous compounds in the host. The bacteria reduced serum nitric oxide levels in the host, exhibiting the host defense immune mechanisms (Giannenas et al. 2015). Juvenile rainbow trout were fed daily with a standard diet containing *L. acidophilus* or *L. bulgaricus* for 60 days. It was found that both *L. acidophilus* and *L. bulgaricus* enhanced the growth performance parameters of the fish, including body weight gain, specific growth rate, and relative weight gain. Increase of digestive enzymes, serum lysozyme, and complement activities were also detected. The higher amylase, trypsin, alkaline phosphatase, and lipase activities in probiotics-treated fish contributed to the increase of feed utilization and growth performance, resulting from better nutrient digestibility (Mohammadian et al. 2019).

It was suggested that activity of alkaline phosphatase related to uptake of carbohydrate and lipid; if alkaline phosphatase activity was higher, more nutrients could be adsorbed (Calhau et al. 2000; Gawlicka et al. 2000). The lysozyme activity in the serum and the alternative complement activity indicated stimulation of the immunity system and innate responses to some infectious diseases (Díaz-Rosales et al. 2009). Upregulation of cytokine and growth gene expressions found in probiotics-treated fish showed stimulation of pro- and anti-inflammatory cytokines, responding to enhancement of the fish immunity system and disease resistance (Mohammadian et al. 2019). *L. plantarum* were orally administrated to rainbow trout for 72 days after yersiniosis vaccination. Feed conversion ratio, weight gain, and thermal growth coefficients were significantly increased, indicating improvement of growth performance. This could be contributed to cumulative actions of probiotics including balancing intestinal microbiota, enhancing digestive enzyme activities, and increasing nutrient digestibility and utilization. This was indicated by the increased protein and alkaline phosphatase in the probiotic-fed fish (Soltani et al. 2019). Additionally, it was mentioned that *L. plantarum* had a vital role in lowering stress levels which were bolstered by vaccination (Mohapatra et al. 2012). Lysozyme and alkaline phosphatase activities were also greater in the probiotic-treated rainbow trout than those with basal diets, showing the enhancement of immunocompetent cells by *L. plantarum* (Soltani et al. 2019).

5.1.5.3 Shrimp

Besides finfish, crustaceans have come into high demand for consumption. The production in culturally or artificially controlled environments has been increased. Farm-raised shrimp are one of the fastest-growing farmed foods. On the other hand, production of wild-caught shrimp has been declined (FAO 2020). Nowadays, environmentally friendly shrimp farms have gained attention from farmers and consumers. The application of probiotics in shrimp farming has been accepted and used worldwide. Lactic acid bacteria as probiotics played an important role in antibacterial activity against shrimp pathogens such as *Vibrio* spp., *Aeromonas* spp., *Staphylococcus aureus*, and *Escherichia coli*. Probiotic consortium (*Bacillus subtilis*, *Lactobacillus* sp., and *Lactococcus* sp.) isolated from white shrimp cultures and the tilapia intestine were determined for their capacity of colonization and completive exclusion against *Vibrio parahaemolyticus* and *Vibrio harveyi*. The result showed that the probiotic mixture could effectively inhibit the pathogens when the probiotic cell number reached 10^6–10^7 CFU/mL. The probiotics could produce antimicrobial substances, compete for available nutrients as well as for mucosal colonization, creating an unfavorable environment for the growth of pathogens (Quiroz-Guzmán et al. 2018). Lactic acid bacteria from morbid shrimp (*Litopenaeus vannamei*) culture and its natural ecosystem were isolated and identified as *Pediococcus stilessi*, *Lactobacillus fermentum*, *L. rhamnosus*, *Weissella cibaria*, *Enterococcus durans*, *E. fecalis*, *Streptococcus gallolyticus* and *Lactobacillus garvieae*. All nine bacteria were tested for their antagonistic activity against

Vibrio parahaemolyticus and *Vibrio campbelli* and the results demonstrated that *L. fermentum* showed inhibitory effects against both *V. parahaemolyticus* and *V. campbelli*, while other Lactobacillales could inhibit only *V. campbelli*. It was possible that the probiotics produced organic acids and some hydrolytic enzymes, reducing the growth of *Vibrio* spp., blocking biofilm formation, and competing for nutrients (Anas et al. 2021). *Pediococcus pentosaceus*, isolated from the intestine of freshwater prawn were mixed with the diet and fed to the prawn prior to being challenged with *Aeromonas hydrophila*. After 3 days of infection, the survival rate of the prawn was significantly higher than the control, which were 82.1 and 24.5%, respectively. It was also found that the microbiota profile in the prawn intestine was changed. Dominant genera were *Aeromonas, Lactococcus*, *Akkermansia*, and *Gemmata*. Interestingly, *Pediococcus* spp. were found in both control and treated groups but, there were less in the control than in the treated prawn. The intensity of *Aeromonas* was obviously lower in the treated-prawn group less the control. The result indicated that *P. pentosaceus* could compete with *A. hydrophila* to colonize the intestinal epithelium, creating barriers and blocking the adhesion of pathogen, as well as producing antibacterial agents such as pediocin (Xue et al. 2021). *Weissella cibaria*, lactic acid bacteria isolated from the shrimp gastrointestinal tract, was firstly reported for their antibacterial activity against *V. parahaemolyticus* and *S. aureus*, but not *E. coli*. The possible mechanism was *W. cibaria* secreting antibacterial compounds such as bacteriocin or inhibiting the colonization of pathogenic bacteria (Huy et al. 2020).

Growth performance and immune parameters of whiteleg shrimp (*Litopenaeus vannamei*) were determined when the shrimp was fed with Bacilli probiotics, *B. subtilis* and *B. licheniformis*, supplemented diets for 60 days. At the end of culture, the probiotics increased shrimp weight gain, specific growth rate, and feed conversion ratio as well as total protein, lysozyme, and hemocyte cell counts (Sadat et al. 2018). It was suggested that enhancement of growth performance was probably associated with the increase of accumulation of proteins in shrimp, including the increase of digestive enzyme activities, feed digestion, and absorption. Moreover, total protein in serum could represent the beneficial effects of probiotics toward immune systems of the shrimp (Yu et al. 2008). Likewise, activity of lysozyme, an antibacterial enzyme that destroys the cell wall of pathogens, could be improved by probiotic Bacilli, leading to the elevation of hemocyte cells and hematopoietic tissue (Aguirre-Guzman et al. 2009). Multiple-strain probiotics containing *Lactobacillus pentosus* BD6, *L. fermentum* LW2, *B. subtilis* E20, and *Saccharomyces cerevisiae* P13 were evaluated for their effects on growth performance and health status of white shrimp (*Litopenaeus vannamei*). The probiotics were mixed with the shrimp diet and fed to the shrimp for 56 days. After feeding, the result showed that the probiotics significantly affected the growth rate of the host. The body weight, percentage weight gain, and feed efficiencies were significantly increased when compared to the control. Regarding health status of shrimp, probiotics improved the activities of phenoloxidase and lysozyme, which were positively associated to the immune response of shrimp. The use of multiple strains of probiotic might provide synergistic effects and/or mutual inhibition affecting the health of the host

(Wang et al. 2019b). Pacific white shrimp were administrated with *Bacillus* sp. NP5, honey prebiotic, and symbiotic (*Bacillus* sp. + honey prebiotic) for 45 days and were determined for their growth performance. It was found that either probiotic, prebiotic, or synbiotic had higher specific growth rate, feed conversion ratio, and digestive enzyme activities of amylase, protease, and lipase of the shrimp than the control; prebiotic group showed greater effects than other groups. The researcher revealed that the improvement of growth performance was related to alteration of the intestine microbiota (Hasyimi et al. 2020). Probiotics and prebiotics possibly amplified diversity of the gut microorganism and the number of beneficial bacteria. This ensued the increase digestive enzyme activity and nutrient absorption (Anguiano et al. 2013).

5.2 Summary

The uncertainty regarding sustainability of fish meal in the long term, along with increasing prices and a growing world population, drive market trends toward alternative feed ingredient sources. Reducing fish meal inclusion levels in aquafeed and replacing fish meal with widely available and environmentally friendly sources of nutrients would be good for aquatic health and considered essential for the future development of the aquaculture industry. There are great possibilities in the utilization of microalgae, probiotics, yeast, and alternative protein sources to satisfy growth and immunity requirements for fish and shellfish aquaculture. Numerous such feed ingredients have been extensively used in the aquaculture industry. A comprehensive review of the literature regarding inclusion of the aforementioned alternative feed ingredients by replacement or addition showed generally favorable efficacy in aquatic animal feeding trials. The effects of feed ingredient inclusion on outcome parameters such as weight gain, growth rate, survival rate, feed conversion ratio, animal nutritional composition, and overall health were investigated. Key factors influencing successfulness of alternative feed ingredient inclusion in aquatic animal diets on outcome parameters were ingredient type/species, ingredient nutritional value, metabolism of ingredient, aquatic animal species, and inclusion level. While studies have typically focused on singular novel alternative feed ingredients, a multifactor approach of testing and optimization when combining ingredients such as microalgae, probiotics, and yeast among others may be beneficial to address gaps in aquatic animal growth, nutrition, and health. Investigation of value chain challenges in obtaining, processing, and cost-effectively utilizing novel alternative feed ingredients is also of great interest. While the application of novel feed ingredients for approaching aquatic sustainability has shown promise, the area of alternative aquafeed development is in the fast-growing stage and much assessment is still needed at lab, pilot, and industrial scales.

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Chapter 6
Molecular Closeness of Zebrafish and Human Platelets

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Abstract The platelets are the disc-shaped cells, which are known for their clotting action. The level of platelets and their function is important in maintaining the haemostasis of vertebrates. The chapters try to brief on the molecular closeness of human and zebrafish platelets. The platelets of human and zebrafish have similarities in various factors like the receptors Fli1, Fog 1, GATA-1(Zg1), NFE2, Runx1 present in the platelets and megakaryocytes. The similarities have also been seen in the presence of dense granules, disc shape and formation of pseudopodia like structure during the time of aggregation of platelets. The closeness like the presence of genes, including *runx1, miR-126, FOG1, GATA1, MASTL, Arghef3, NBEAL2* that are found to be either homologue or orthologue and their functional roles in platelets. The presence of epigenetic machinery like DNA methyltransferases, histone acetylation and alteration in miRNAs are also exist in the zebrafish as found in the humans. The chapter also briefs on the influence of environmental pollutants like benzo[a]pyrene, arsenic, nickel, cadmium, bisphenol A, polyfluorooctanoic acid and lead on platelets and development of zebrafish.

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6.1 Introduction

The platelets or thrombocytes are one of the blood cells that are produced using haematopoietic lineage. The platelets are needed for the coagulation process and any abnormalities in the platelet might lead to hyper- or hypocoagulation events that would end up in clotting diseases or bleeding condition, respectively. The platelet haemostasis is important for all the organisms that possess blood for their circulation. The zebrafish is one of the versatile animal models used in various studies. It is found that zebrafish share about 70% of homology among the genome of human being (Howe et al. 2013). The platelets of human and zebrafish have certain similarities like their shape, presence of thrombopoietin (Svoboda et al. 2014), GPIb, aggregation upon stimulation by ADP and von Willebrand Factor (Gregory and Jagadeeswaran 2002), dense granules and formation of pseudopodia on aggregation reaction (Jagadeeswaran et al. 1999; Morgenstern 1997).

The one major difference that exists between the human and zebrafish platelet is that the former is anucleated, which does not affect so much on its comparison. Various molecular similarities exist between the zebrafish and the human platelets like the transcription factors Fli1, Fog 1, GATA-1(Zg1), NFE2, Runx1 (Davidson and Zon 2004), the genes encoding these transcription factors along with the genes like mastl (Johnson et al. 2009), Arghef3 (Gieger et al. 2011) and NBEAL2 (Albers et al. 2011; Louwette et al. 2012). The similarities of these molecular factors also lead to the probability of similarity to the epigenetic mechanisms which are found to be true by certain works like the observation of Gavery and Roberts (2017) who found that the DNA methylation system is retained in the fish and shellfish and histone acetylation was confirmed to have a role in zebrafish development that have been identified using functional analysis according to the work done by Vastenhouw and Schier (2012).

Surfing along with the google search, PubMed, PubMed Central, research gate and science direct websites, approximately about 200 papers were referred to write the current chapter. The keywords used for searching are human and zebrafish thrombocytes, the similarity of human and zebrafish platelets, haematopoiesis in human and zebrafish, the epigenetic mechanism in human and zebrafish, role of environmental pollutants in affecting the platelets, environmental and epigenetic modifications in zebrafish. The presented chapter has been written to focus on the molecular closeness of platelets in humans and zebrafish, which initially describes the morphological and functional similarities along with the production of platelets in both human and zebrafish. This chapter then briefly describes the role of various genes like runx1, miR-126, FOG1, GATA1, MASTL, Arghef3, and NBEAL2.

The epigenetic mechanism and the environmental factors that are found to cause alterations in platelets are also briefed here. This chapter would give an idea about the similarities that exist between the human and zebrafish platelets with special importance to the genes that play role in the platelet haemostasis and then the ideas on the epigenetic mechanism which are yet to be explored more and remains an open field to research. The fact that the comparison is done between the human and zebrafish is to highlight the opportunities of using zebrafish as a model to study platelet homeostasis and also to protect the versatile organism zebrafish from any possible defects in its molecular factors.

6.2 Morphology and Aggregation of Zebrafish Thrombocytes

The zebrafish possess nucleated thrombocytes and the size of the nucleus is large when compared to its cytoplasm distribution, the sparsely distributed cytoplasm has a large number of vesicles, which acts as a passage to the cell surface (Jagadeeswaran et al. 1999). The human platelets have been well explored and have been found to contain no nucleus but mitochondria, dense tubular system, dense granules, alpha granules, peroxisomes and lysosomes (Morgenstern 1997). The vesicles present in the zebrafish thrombocytes are the open canalicular system, which is similar to that of dense tubular system, that are the means of propagation to the cellular molecules, and upon aggregation, both zebrafish and human platelets showed pseudopodia-like structures to connect to their nearby cells (Jagadeeswaran et al. 1999; Morgenstern 1997).

In a study conducted to decode the thrombopoiesis in zebrafish, an ortholog of human thrombopoietin gene tpo was identified and its overexpression was observed to impact the platelet production and its precursor production by increasing them (Svoboda et al. 2014). In the zebrafish thrombocytopoiesis pathways, both intrinsic and extrinsic, using transcription factors and thrombopoietin, respectively, are conserved as in mammals (Jagdeeshwaran et al. 2010; Lin et al. 2017). The thrombocyte aggregation in mammals initiates due to an injury or due to coagulants. In case of injury, the platelets are employed at the site of injury by binding to the platelet adhesive receptors like GPIb/IX that is in contact with collagen or von Willebrand Factor (Farndale et al. 2004; Andrews et al. 2003). The coagulants that stimulate the aggregation of thrombocytes are thrombin, ADP and thrombin A2 after binding to a protease-activated receptor or ADP receptor (Kahn et al. 1999; Daniel et al. 1998). Upon activation, the platelet release reaction starts where initially the α-granules and dense granules are secreted for the formation of a preliminary platelet plug which would seal the injured vessel and there is random production of thrombin (Fig. 6.1). Later the soluble fibrinogen is converted to fibrin that adds strength to the thrombus (Golebiewska and Poole 2015; McKee et al. 1972). Zebrafish was found to have the thrombin receptors, protease-activated receptor and ADP receptors on their thrombocytes and found to express the GPIb; they are also found to involve in aggregation reaction after stimulation by collagens, ADP and von Willebrand Factor (Gregory and Jagadeeswaran 2002).

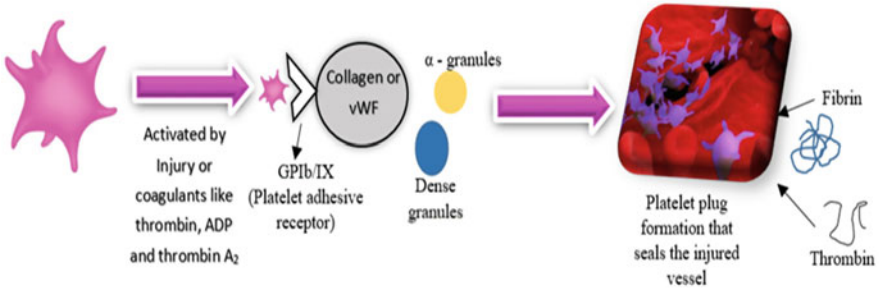

Fig. 6.1 Aggregation of platelets

Figure 6.1 represents the thrombocyte aggregation in mammal's initiates due to an injury or due to coagulants. In case of injury, the platelets are employed at the site of injury by binding to the platelet adhesive receptors like GPIb/IX that is in contact with collagen or von Willebrand Factor. The coagulants that stimulate the aggregation of thrombocytes are thrombin, ADP and thrombin A2 after binding to a protease-activated receptor or ADP receptor. Upon activation, the platelet release reaction starts where initially the α-granules and dense granules are secreted for the formation of a preliminary platelet plug which would seal the injured vessel and there is random production of thrombin. Later the soluble fibrinogen is converted to fibrin that adds strength to the thrombus.

6.3 Thrombocyte Formation in Zebrafish at Embryo Stage

It was found that the haematopoiesis in zebrafish embryos occurs as four waves among which the first two waves initiate 30 hpf (hours post fertilisation) and it is also known to be the primitive haematopoiesis occurring in the intermediated cell mass. Then starts the definitive haematopoiesis, i.e. third and fourth wave by which the erythromyeloid progenitor and haematopoietic stem cells are produced, the third wave occurs in the caudal haematopoietic tissue and begins at 24 hpf and also found high during 30 hpf. The fourth wave initiates 32–36 hpf within the endothelial cells of the ventral wall of the dorsal aorta similar to that of the mammalian aorta gonad mesonephros.

It is in the fourth wave the haematopoietic stem cells generated and occupy the caudal haematopoietic tissue and the adult haematopoietic organs kidney and thymus (Amatruda and Zon 1999; Davidson and Zon 2004). Handin's lab experimented on zebrafish embryo thrombocytes by using the CD41 promoter to express the green fluorescence protein (GFP) and they found the GFP labelled thrombocytes in circulation. To confirm that those cells are the thrombocytes, the thrombocyte aggregation assay and laser injury thrombosis assay were performed and they found the GFP labelled cells to form aggregates and confirmed that they are the

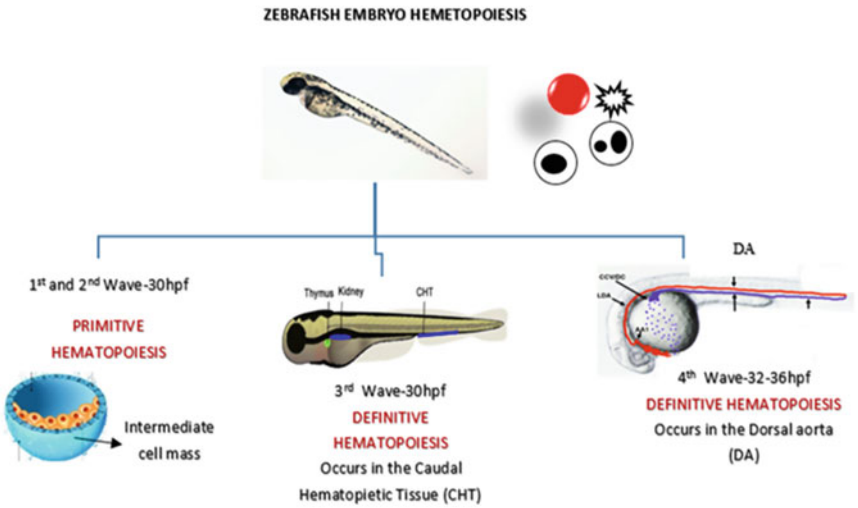

Fig. 6.2 Zebrafish embryo haematopoiesis

thrombocytes (Lin et al. 2001). The knockdown of c-mpl gene which is the receptor for cytokine thrombopoietin and scl gene resulted in the decreased level of GFP labelled thrombocytes that indeed proved the presence of c-mpl receptor on zebrafish thrombocytes (Lin et al. 2005). The c-mpl receptor mRNA was found in the thrombocytes after 42 hpf (Bertrand et al. 2008). The GFP labelled thrombocytes were absent in the ICM indicating that it is not present in the primitive haematopoiesis. However, the GFP labelled immobile thrombocytes were found between 42 and 48 hpf at the position intermediate to the aorta and caudal vein that corresponds to AGM (Lin et al. 2005). Figure 6.2 highlights the process of haematopoiesis in zebrafish.

Figure 6.2 represents the haematopoiesis that occurs in zebrafish during embryonic development. In zebrafish embryo occurs as four waves among which the first two waves initiate 30 hpf (hours post fertilisation) and it is also known to be the primitive haematopoiesis occurring in the intermediated cell mass. Then starts the definitive haematopoiesis, i.e. third and fourth wave by which the erythromyeloid progenitor and haematopoietic stem cells are produced, the third wave occurs in the caudal haematopoietic tissue and begins 24 hpf and also found high during 30 hpf. The fourth wave initiates 32–36 hpf within the endothelial cells of the ventral wall of the dorsal aorta similar to that of the mammalian aorta gonad mesonephros. It is in the fourth wave the haematopoietic stem cells generated and occupy the caudal haematopoietic tissue and the adult haematopoietic organs kidney and thymus

6.4 Molecular Factors Identified in Zebrafish Platelet Development

The transcription factors like Fli 1, Fog 1, GATA-1(Zg1), NFE2 and Runx1 that are present in the megakaryocytes were also identified in zebrafish (Davidson and Zon 2004). The runx1morpholino injected embryos of zebrafish lagged in normal circulation and the immature haematopoietic progenitors were found to be deposited in it (Kalev-Zylinska et al. 2002). The zebrafish with the CD41 promoter GFP also expressed RunX1 in its GFP + cells. The same type of zebrafish with the truncated or edited RunX1 developed GFP + cells indicating that there is a RunX1 independent secondary pathway existing (Sood et al. 2010). On the knockdown of the miR-126 key regulator of the negative regulator of megakaryopoiesis, c-Myb resulted in the fall of thrombocytes count and increase the erythrocytes count (Zhang et al. 2011). It also indicates that miR-126 is involved in the cell fate decision (Grabher et al. 2011).

The cofactor Fog1 interacting with GATA-1 and GATA-2 was identified to interact with erythrocytes and megakaryocytes differentiation which was found by injecting fog1morpholino injected CD41-GFP + embryos lacked the GFP + thrombocytes (Muntean and Crispino 2005; Amigo et al. 2009). It was identified that the fli-1 promoter gets stronger and gata-1 promoter gets weaker in mature thrombocytes which is vice versa in immature or young thrombocytes (Jagadeeswaran et al. 2010). The mastl gene knockdown decreased the thrombocytes in circulation (Johnson et al. 2009). On the Genome-Wide Analysis Study (GWAS) of genes nearby to the binding sites of Fli 1, Fog 1, GATA-1, GATA-2, Runx1 and scl of primary cells, the genes like march2, max, smox, pttg1lp, emilin1 and sufu were found which on knockdown decreased the thrombocytes count (Tijssen et al. 2011). On the meta-analysis of GWAS Geiger et al. identified about 68 genome loci and also four genes arghef3, ak3, rnf145 and jmjd1c which on silencing decreased both primitive erythropoiesis and thrombocyte formation. The gene tpma is an ortholog of gene tpm1 present in megakaryocytes, which on silencing resulted in thrombocyte count without alteration in erythropoiesis (Gieger et al. 2011). Nbeal2 and rgs18 were also silenced in zebrafish and was observed with a downfall in thrombocyte formation (Albers et al. 2011; Louwette et al. 2012) (Table 6.1).

Table 6.1 Chromosomal location of genes shared in common among humans and zebrafish

S. No.	Gene	Chromosomal location in humans	Chromosomal location in zebrafish
1.	*runx1*	21q	1
2.	*miR-126*	9	Paralogues of *miR-126* *miR-126a-* 8 and *miR-126b*-11
3.	*FOG1*	16	18
4.	*GATA1*	Xp11.23	11
5.	*MASTL*	10p 11–12	24
6.	*Arghef3*	3p14.3	–
7.	*NBEAL2*	3p21.1–22.1	16

6.4.1 runx 1 *Gene*

The runx1 gene is located on chromosome 21q (Song et al. 1999) of the human genome. It is termed as the master regulator of haematopoiesis, Runt-related transcription factor 1 (RUNX1). Different variants have been identified through its coding region, of which the homology domain mediating the binding and heteromerisation of DNA with core-binding factor-β (CBF-β) was found to be crucial for the Familial platelet disorder (Kamatchi et al. 1990). The mutations that have been found mostly in the runx1 gene are missense and continuous MYH10 expression that leads to the lower dense granule secretion in platelets and MYH10 expression has been used as a biomarker to diagnose the defect respectively (Stockley et al. 2013; Antony-Debre et al. 2012). The missense mutation is the case in which there is a single nucleotide change in the DNA sequence that can lead to a different amino acid in the protein encoded (Oliveira et al. 2013).

In the case of normal individuals, the RUNX-1 of runx1 silences the expression of MYH10 to switch from mitosis to endomitosis in the cell cycle that is a must for megakaryocyte differentiation (Lordier et al. 2012). In zebrafish, the runx1 gene is located in chromosome 1(zfin.org 2021). While affecting the Runx1 with antisense morpholino oligonucleotides resulted in the incomplete formation of blood vessels and deposition of immature haematopoietic precursors (Kalev-Zylinska et al. 2002). The Runx1 in zebrafish was subjected to low expression and high expression, which showed fluctuations in the haematopoiesis of myeloid lineage determining the fate of neutrophils in the embryonic stage (Jin et al. 2012). Thus the runx1 in zebrafish shows homology of being involved in the haematopoiesis as in the case of human being and it is still to be decoded on platelet haemostasis; however, its mutation is said to affect the blood cell haemostasis in zebrafish (Fig. 6.3).

Figure 6.3 represents the role of the RUNX1 gene in platelets formation. In the case of normal individuals, the RUNX-1 of runx1 silences the expression of MYH10 to switch from mitosis to endomitosis in the cell cycle that is a must for

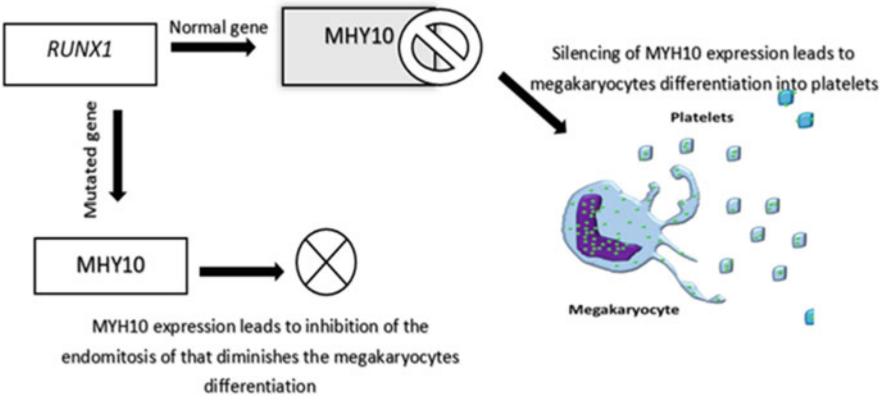

Fig. 6.3 Role of *RUNX1* gene in platelets formation

megakaryocyte differentiation whereas the above process is inhibited in the case of mutation in the RUNX1 gene

6.4.2 miR-126 microRNA

The small non-coding RNAs are called the miRNAs; they are involved in the regulation of mRNA translation and also help in the fine-tuning of protein expression (Bartel 2009). The platelets are found to have a different range of transcriptome consisting of about 700 miRNAs and more (Sunderland et al. 2017). The ADAM9 is a protease whose expression in the megakaryocytes is inhibited by the miR-126, by which the attenuation of the platelet adhesion to the collagen is inhibited (Cominetti et al. 2009).

It is also found that the inhibition of miR-126 leads to the decrease in P2Y12 receptor expression that is essential for platelet activation upon strong agonist stimulation by adenosine diphosphate release (Kaudewitz et al. 2016). miR-126 is located in chromosome 9 of humans (Fuentes et al. 2015). In zebrafish, there are two paralogues of the miR-126; they are miR-126a and miR-126b located in the chromosome 8 and 11, respectively (zfin.org, mirbase.org 2021). The miR-126 of zebrafish is found to have control over the c-mybproto-oncogene which on an increased level promotes the erythropoiesis while decreasing the thrombocytopoiesis (Grabher et al. 2011). Therefore it is conclusive that miR-126 has a potential role in controlling the expression of genes that are involved in platelet production and function in both the humans and zebrafish platelets (Fig. 6.4).

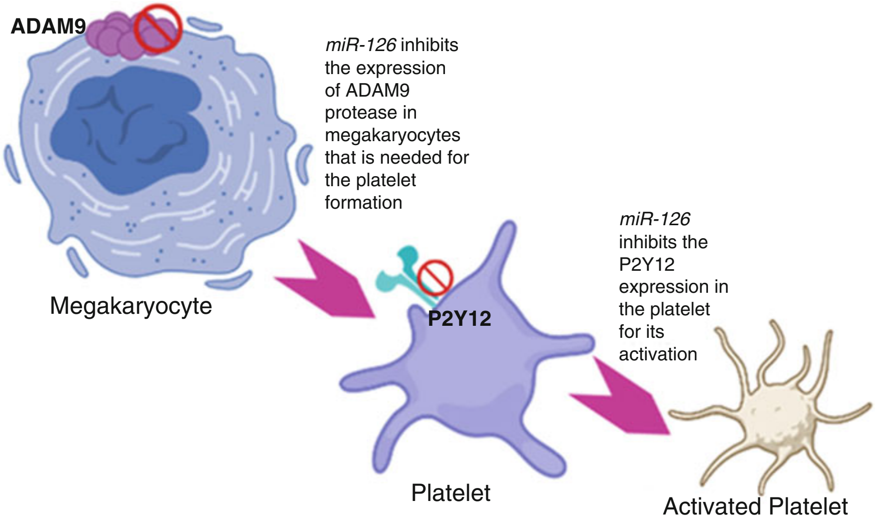

Fig. 6.4 Role of miR-126 gene in megakaryocytes and platelets

Figure 6.4 represents the role of the miR-126 gene in megakaryocytes and platelets. The ADAM9 is a protease whose expression in the megakaryocytes is inhibited by the miR-126, by which the attenuation of the platelet adhesion to the collagen is inhibited. The inhibition of miR-126 leads to the decrease in P2Y12 receptor expression that is essential for platelet activation upon strong agonist stimulation by adenosine diphosphate release

6.4.3 FOG1 *Gene*

The FOG1 gene is located in chromosome 16 of human beings. The fog1 gene is found to be located in chromosome 18 of zebrafish which was similar to the murine FOG1 (Walton et al. 2006). The importance of the FOG1 gene in humans is that its function along with GATA-1 is essential for differentiation of erythroid and megakaryocyte progenitors (Mancini et al. 2012). The FOG-1 is a nuclear protein in zebrafish platelets that enhances the binding of a transcription factor called GATA-1 which leads to the maturation of erythrocytes and megakaryocytes and it is found that the decrease in the expression of fog1 under normal expression of GATA-1 resulted in the anaemic and thrombocytopenic conditions in zebrafish while there was an increase in myeloid progenitor cells (Amigo et al. 2009).

In humans, the FOG-1 is the cofactor of GATA-1 and it is also called the friend of GATA-1. The direct interaction of FOG-1 and GATA-1 is essential for the αIIb gene expression that encodes for α integrin chain of the thrombocyte fibrinogen receptor αIIb/β3 (Wang et al. 2002). The αIIb/β3 is essential for platelets to in order to communicate with the fibrin at the time of clot retraction which is essential for the damage repair of the blood vessels after injury (Buitrago et al. 2020). Even though there is no determining sequence homology between the human and zebrafish FOG1 gene, its functional similarity is identical (Fig. 6.5).

Figure 6.5 represents the function of FOG 1 as a cofactor that is encoded by the gene FOG 1. The FOG 1 acts as a cofactor for GATA 1 and their direct interaction is needed for the expression of the αIIb gene that activates the α integrin chain of the thrombocytes. The α-integrin chain of the thrombocytes is the αIIb/β3 that interacts with the fibrin during the time of clot retraction. The αIIb/β3 interaction with fibrin is a must during the repair of injured blood vessels

6.4.4 GATA1 *Gene*

The X-linked gene GATA1 is located at X-chromosome Xp11.23 and encodes a transcription factor that is involved in the regulation of haematopoietic cell development that includes the erythrocytes, megakaryocytes, eosinophils, mast cells and dendritic cells (Ferreira et al. 2005). The transcription factor is a protein that binds to the DNA and has two zinc fingers and a transactivation domain. The two zinc fingers

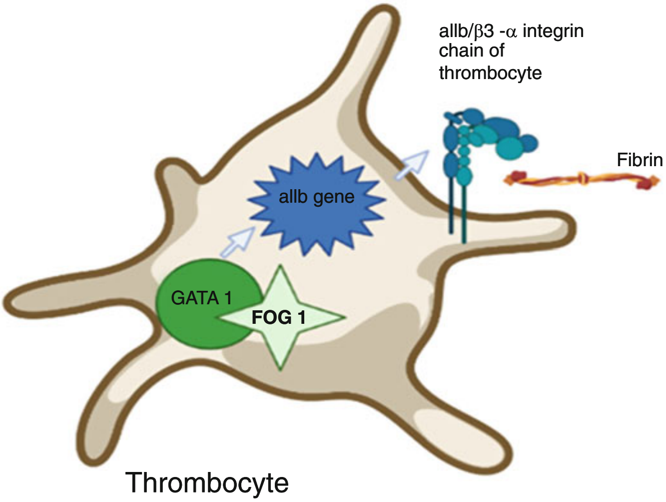

Fig. 6.5 FOG 1 function as a cofactor

are located at C and N terminal, respectively, while the C terminal zinc finger aids in the DNA-binding activity of GATA with its consensus sequence (Evans et al. 1988; Martin et al. 1989; Wall et al. 1988). The N-finger is critical in binding with the key cofactor FOG1 (Tsang et al. 1997). In the promoter regions of megakaryocyte expressed genes the binding sites for GATA 1 have been found (Visvader et al. 1992). In zebrafish, it is found that GATA 1 is essential in determining the fate of cells from erythroid and myeloid lineages during the time of embryogenesis (Galloway et al. 2005). The GATA 1 is located in chromosome 11 of zebrafish and it is also found that the gene is involved in its positive autoregulation (Kobayashi et al. 2001). It was also found that when the DNA-binding activity and transactivation properties of GATA 1 altered with the help of its sequence, it was found to affect the primitive haematopoiesis than the definitive one (Belele et al. 2009). Hence, it is clear that the GATA 1 of human and zebrafish is functioning similar with the help of its cofactor FOG-1 and its DNA-binding and transactivation properties (Fig. 6.6).

Figure 6.6 represents the impact of GATA 1 gene mutation. In zebrafish, it is found that GATA 1 is essential in determining the fate of cells from erythroid and myeloid lineages during the time of embryogenesis. The GATA 1 gene is involved in its positive autoregulation. It was also found that when the DNA-binding activity and transactivation properties of GATA 1 altered with the help of its sequence, it was found to affect the primitive haematopoiesis than the definitive one

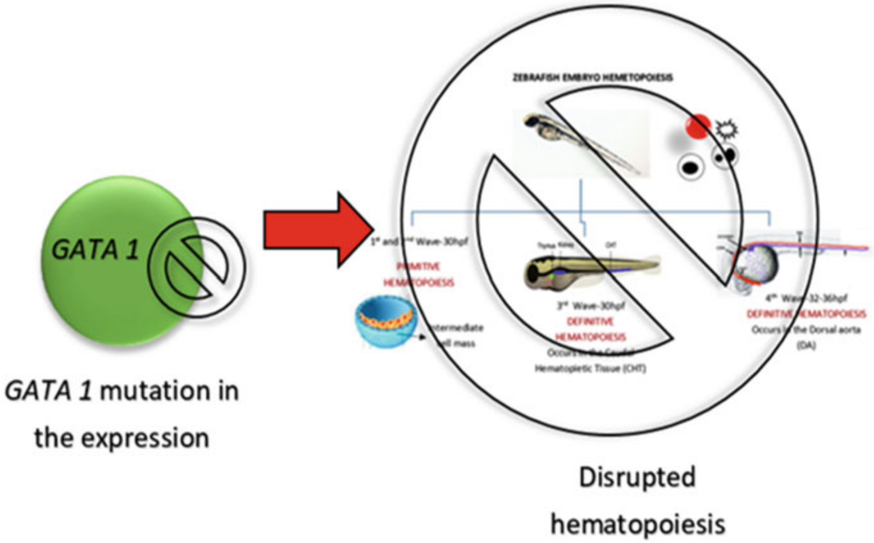

Fig. 6.6 Impact of *GATA 1* mutation

6.4.5 MASTL *Gene*

The MASTL gene locus is found in the mammalian cells at the locus of chromosome 10p 11–12 and it was found that its mutation resulted in nonsyndromic autosomal dominant thrombocytopenia which is also known as thrombocytopenia-2 (Gandhi et al. 2003). The patients with thrombocytopenia-2 were found to have incomplete differentiation of megakaryocytes with easy bruising and minor bleeding (Bithell et al. 1965; Drachman et al. 2000). The MASTL is microtubule-associated serine/threonine-protein kinase-like which is otherwise known as the Great wall because of its critical role in mitosis maintenance (Glover 2012; Lorca and Castro 2013). If there is the absence of MASTL, then there would be an increase in the activity of PP2A-B55 which results in the insufficient phosphorylation of mitotic proteins (Hurtado et al. 2018).

The missense mutation of the gene MASTL gene in the case of thrombocytopenia-2, it is assumed that alteration in the endomitosis of megakaryocytes or platelets would have led to its pathological condition (Gandhi et al. 2003; Drachman et al. 2000). In zebrafish, the MASTL gene is located in chromosome 24 and with the short time knockdown of this showed a decreased expression of thrombopoietin receptor, c-mpl, and CD41 platelet adhesion protein –gpIIb (Johnson et al. 2009). Therefore, it is conclusive that the MASTL gene plays a similarly significant role in platelet production in both the human being and zebrafish, while its mutation resulting in thrombocytopenic conditions in both cases (Fig. 6.7).

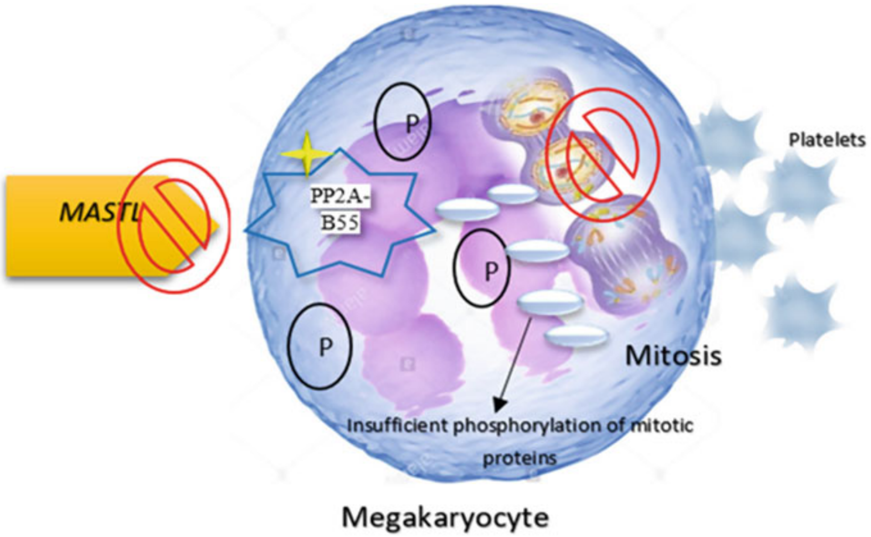

Fig. 6.7 Mechanism of how *MASTL* mutation leads to inhibition of platelet production

Figure 6.7 represents the impact of MASTL gene mutation in platelet production. If there is the absence of MASTL, then there would be an increase in the activity of PP2A-B55 which results in the insufficient phosphorylation of mitotic proteins. The missense mutation of the gene MASTL gene in the case of thrombocytopenia-2, it is assumed that alteration in the endomitosis of megakaryocytes or platelets would have led to its pathological condition.

6.4.6 Arghef3 *Gene*

The Arghef3 gene is located in the human chromosome 3p14.3 and its single nucleotide polymorphism was found to be in association with the platelet numbers and mean platelet volume (Zou et al. 2017). This gene is found to encode for the Rho Guanine Nucleotide Exchange Factor 3, which is an exchange factor found in platelets, Leukemic and Neuronal tissues otherwise known as XPLN (Arthur et al. 2002). It helps in the Rho GTPase switching from its inactive form of GDP bound state to its GTP bound state that is active and the gene is also one of the abundant guanine exchange factor found in the lineage of megakaryocytes and platelets (Astle et al. 2016; Eicher et al. 2016).

The expression of this gene is found to be high in platelets on the microarray and western blot analysis (Arthur et al. 2002; Simon et al. 2014). When the arghef3 was knockdown in zebrafish it was found to reduce the platelets counts but the mechanism is still to be decoded (Gieger et al. 2011). However the Arghef3 gene function is much studied in humans, it is still to be explored more in zebrafish but the gene shares a common behaviour of reduction in platelets when mutated among humans and zebrafish.

6.4.7 NBEAL2 *Gene*

The NBEAL2 is located in chromosome 3p21.1–22.1 of the human chromosome and it is found to be localised in the dense tubular system, i.e. endoplasmic reticulum in platelets by sucrose-gradient subcellular fraction analysis (Gunay-Aygun et al. 2011). The Gray platelet syndrome is a disorder that is characterised by large platelets which appear grey when viewed under light microscopy (Nurden and Nurden 2007). In the Gray platelet syndrome, the protein level in the megakaryocytes is found to be low; however, the endocytosed α-granules are not much affected (Weiss et al. 1979). The NBEAL2 gene is located in Chromosome 16 of zebrafish and when it was silenced, the platelets count in zebrafish was found to be low (Albers et al. 2011). Therefore the NBEAL2 gene is found to play a significant role in platelet formation in human and zebrafish platelets.

6.5 Epigenetics

The word epigenetics refers to the studies related to epigenetic modifications. Epigenetic modification is considered a temporary or permanent change that occurs to a gene expression in a particular generation without any alteration in the genetic material. There are various types of epigenetic modifications like methylation, acetylation, phosphorylation, ubiquitylation and sumoylation (Weinhold 2006) that lead to DNA methylation, histone modification, etc. DNA methylation is the covalent transfer of methyl group to the C5 position of the cytosine ring of DNA by DNA methyltransferases (Robertson 2005) and it is also one of the most studied epigenetic mechanisms. It has been found that fish and shellfish have genes that can encode the basic methylation machinery needed for DNA methylation (Gavery and Roberts 2017). It has been found that certain modified histones have been retained in both mammals and zebrafish in a non-random manner during the time of spermatogenesis while most of these proteins are replaced by protamines, indicating that these may play a significant role in transferring the epigenetic information to the embryos (Brykczynska et al. 2010; Wu et al. 2011).

6.5.1 DNA Methylation

The term DNA methylation refers to the concept of the addition of methyl group to the cysteine enzymatically and altered the expression of the gene without modifying the genetic code. The DNA methylation occurs with the help of enzymes belonging to the DNA methyltransferases (DNMT's) family, where DNMT1 and DNMT3a/3b are responsible for copying the pre-existing methylation patterns to the new strand from the pre-existing during mitosis and de novo methyl transferring, respectively (Gavery and Roberts 2017). In the gene bodies, DNA methylation was found to have a high level of expression (Jones 1999). Whereas, DNA methylation is found to be inhibited when located at the promoter region of the gene which is in association either by DNA-binding proteins or by physical blocking of transcriptional factors (Bell and Felsenfeld 2000).

The significant role of DNA methylation in mammals is that it maintains genomic stability using inhibition of the transposable elements (Maloisel and Rossignol 1998). DNA methylation is found to be important for embryonic development and cell type differentiation (Li et al. 1992). The DNA methylation in mammals was found to be in association with external factors apart from the internal cellular system like light exposure or photoperiod (Azzi et al. 2014), nutrition (Weaver et al. 2004) and toxin exposure (Dolinoy et al. 2006). The DNA methylation system is retained in the fish and shellfish and the DNA methylation patterns were also found to be uniform among the vertebrates which is the 'global' DNA methylation pattern that means most of the CpGs (cytosine located at $5'$ of guanine) are methylated except the CpG island region, which has the highest CpG concentration (Gavery and Roberts 2017).

6.5.2 Histone Variants

The histones are the component of a nucleosome belonging to chromatin. The chromatin is the structure that together constitutes the chromosomes. The chromatin plays a role in packaging the genome into the nucleus and using DNA accessibility controls the regulation of genes and other genomic regions (Cheung et al. 2000). The chromatin consists of the DNA in the nucleosome which has a histone protein made up of eight units of histone two of each H2A, H2B, H3 and H4 and the linker between the nucleosomes is the histone H1 (Gavery and Roberts 2017). The chromatin structure can be modified through histone variants and post-translational modification of histones that can influence either by enhancing or repressing the transcription (Berger et al. 2007). The changes in the chromatin can be brought either by the mitotic or meiotic pathway, which may convey the epigenetic mechanism. The histones are placed inside the nucleosome in a replication-dependent manner and each histone has its respective specialised functions (Henikoff and Smith 2015). The histone variants play important role in bringing about the short- or long-term responses to the environmental signals which make their role crucial (Talbert and Henikoff 2014).

The histones can be post-translationally modified initially, at their N-terminal tails which determines the degree of chromatin compaction which results either in euchromatin or heterochromatin based on the type and location of modification

(Lawrence et al. 2016). The modifications are also controlled by different enzymes belonging to different families like histone acetylases, histone deacetylases, histone methyltransferases and histone demethylases which are highly regulated during the time of embryonic development (Lin and Dent 2006). While some of the histone variants were found to be ‘Universal’ and present in most of the eukaryotes (Talbert and Henikoff 2010), some are conserved between the lower vertebrates and mammals, e.g. macroH2A which plays a role in the zebrafish development (Buschbeck et al. 2009). Histone acetylation was confirmed to have a role in zebrafish development that has been identified through functional analysis (Vastenhouw and Schier 2012).

6.5.3 Noncoding RNA

Even though most of the genome is transcribed, only a few portions of the transcripts encode for proteins and others remain as non-coding transcripts which are also known as non-coding RNA (ncRNA), which plays a major role in gene expression modulation. These non-coding RNAs are categorised according to their nucleotides size like the ones that are greater than 200 nucleotides as long ncRNA and lesser than 200 nucleotides are called small ncRNA and there are subtypes in small ncRNAs like microRNA (miRNA), short interfering RNA (siRNA) and PIWI-interacting RNA (pi RNA) (Gavery and Roberts 2017). The main role of the small ncRNA is inhibiting the protein synthesis either by blocking or primary transcripts degradation and they are found to be highly conserved (Castel and Martienssen 2013).

The long ncRNA are found to work either in cis or trans; while their mechanism is complex they are also found to be less conserved (Wang and Chang 2011). The genome stability, environmental plasticity and environmental development are the factors in which the ncRNA acts as a regulator in gene expression (Mercer et al. 2009; Bizuayehu and Babiak 2014). To silence or activate various parts of the genome the ncRNAs are found to interact with other epigenetic mechanisms like histone modification and DNA methylation (Peschansky and Wahlestedt 2014). Even though there are fewer works done on the ncRNAs in zebrafish, certain miRNAs have been found in them that can be affected due to epigenetic modifications.

6.5.4 Epigenetic Modifications and Platelets

As epigenetic modifications are a part of a lifecycle, the platelets also do not stand as an exception to such modifications. The platelets in a human being don’t have a nucleus and so the epigenetic studies on platelets were not given much importance until there was an initiation in exploring the mitochondrial DNAs for epigenetic impact. Certain studies have revealed that the modification of histone acetylation in the endothelial cells has played role in coagulation and thrombosis. The tissue plasminogen activator has a key role in the fibrinolytic process, whose stimulated secretion is essential for protecting the circulation from occluding thrombosis and their expression is found to be controlled by histone acetylation (Arts et al. 1995).

The histone deacetylase inhibitors were found to affect the expression of tissue plasminogen activator by H3 and H4 histone acetylation (Arts et al. 1995; Dunoyer-Geindre and Kruithof 2011; Larsson et al. 2012). Von Willebrand factor vWF which is found to play a significant role in maintaining the balance between blood clotting and bleeding was found to be regulated by histone acetylation (Peng et al. 2007). The mitochondria have their DNA which is found to be the genome copies, approximately about 17 kb that is circular and has 37 genes while 24 genes among this codes for tRNAs and rRNAs, then the remaining 13 genes are protein-coding genes (Baccarelli and Byun 2015). In a study conducted by Liu et al. (2021) it was found that short-term exposure to air pollution resulted in the hypomethylation of DNA methylation in the mitochondrial DNA of the platelet. Even though the platelet in humans lacks a nucleus, several genes are involved in its production and regulation and epigenetic modifications might alter those genes, which are still to be investigated. However, the zebrafish platelets are nucleated and having a brief idea on epigenetic modifications and the environmental modulators will help in maintaining the haemostasis of the platelets and fish.

6.6 Environmental Toxins as Epigenetic Modifiers in Zebrafish

6.6.1 *Benzo[*a*]Pyrene*

The benzo[a]pyrene is an environmental pollutant that is present in tobacco, coal tar, grilled meats and also in many foods. The benzo[a]pyrene is also found to be released in the wastewater by the industries associated with the smelting process, i.e. heating the ore to obtain metals like iron, steel and aluminium (Washington 2002; EPA 1984). Benzo[a]pyrene is one of the polycyclic aromatic hydrocarbons that is found to be a genotoxic carcinogen exhibiting tumourigenic potential in all the model organisms (Pogribny 2019).

When the developing embryos of the zebrafish were continuously exposed to the benzo[a]pyrene, there was a significant downfall in the DNA methylation compared to that of the control which was due to the decrease in methylation at the promoter region and increase in the mRNA transcripts particularly detected in the vasa genes (Fang et al. 2013). The vasa gene is found to be important for primordial germ cell migration and differentiation (Yoon et al. 1997; Knaut et al. 2000; Li et al. 2009a, b; Abbott et al. 2005). In a study conducted with the glutathione-S-transferase (GST)-M1 null genotype lymphocyte, the exposure to oxidant chemical benzo[a]pyrene was found to inhibit the platelet function (Onaran et al. 2000). In a study conducted among the workers who are exposed to benzo[a]pyrene, chances for thrombocytopenia and plasma coagulation factor abnormalities were revealed (Sroczyński et al. 1991).

6.6.2 *Arsenic*

Arsenic is an environmental toxicant and it is found to be distributed in rock, soil, water and air. It is a toxicant for both human and animal especially when it is found in the groundwater (Mandal 2017). Arsenic exposure is found to have series of cancer, neurological and cardiovascular diseases (Nemec et al. 1998; Rodriguez et al. 2002; Tchounwou et al. 2004; Hill et al. 2008). When the zebrafish embryos were exposed to sodium arsenite at a concentration of 2.0 mM, it was found to have neurological and cardiovascular malformations and the DNA methylation level also was found to be decreased when compared to the control, but eventually, the DNA methylation was found to be hyper during different developmental stages of the zebrafish embryo at the trunk and tail (Li et al. 2009a, b). The arsenic trioxide on administration was found to impair the platelet function and to decrease the number of platelets occurring in the circulation (Wu et al. 2014). It was found that in hyperglycaemic patients there was high exposure to arsenic-related vasculo-toxicity by which there is the alteration in the markers of platelets and megakaryocytes which might increase the chances of atherothrombotic risk (Newman et al. 2017).

6.6.3 *Nickel*

Nickel is one of the environmental pollutants that is released during the production of jewellery, coins, stainless steel, carbon particles, batteries, medical devices, Ni refinery, plating and welding (Salnikow and Zhitkovich 2008). The epidemiological studies in animal and cell culture have revealed that nickel compounds are carcinogenic, but their mechanism on carcinogenesis is not known (Doll et al. 1970; Kerckaert et al. 1996; Kuper et al. 1997; Miller et al. 2001). Various studies have demonstrated that the chromatin structure changes and epigenetic alterations are the initial events taking place during nickel carcinogenesis (Arita and Costa 2009). The phagocytosed nickel sulphide particles were found to affect the heterochromatin selectively which is through DNA methylation (Sen and Costa 1985).

According to a proposed model, the nickel might cause DNA methylation by substituting the Mg^{2+} ion in the phosphate background of DNA by Ni^{2+} that causes the condensation of the heterochromatin by triggering the de novo DNA methylation of tumour suppressor gene and senescence gene (Lee et al. 1995). The reason that the nickel targets the heterochromatin is predicted to be due to the higher concentration of Mg^{2+} ions at the heterochromatin and maybe because the heterochromatin forms the inner lining of the interface nucleus which might encounter the toxins before they reach the euchromatin (Costa et al. 1994; Conway et al. 1987; Borochov and Ausio 1984). Apart from the gene silencing by DNA methylation of nickel, there is also another epigenetic mechanism involved in its toxicity like loss in acetylation of histones H2A, H2B, H3 and H4, increase in the level of H3K9 dimethylation and ubiquitination in the H2A and H2B at the global level (Karaczyn et al. 2005; Karaczyn et al. 2006; Broday et al. 2000; Chen et al. 2006; Ke et al. 2006; Golebiowski and Kasprzak 2005; Klein and Costa 1997; Klein et al. 1991; Lee et al. 1995).

It is found that the nickel inhibits the activity of the histone acetyltransferase enzyme to decrease the level of histone acetylation and has no influence on the histone deacetylase activity (Kang et al. 2003). In a study done to examine the effect of Ni on human platelets, it was observed that the platelet aggregation due to collagen and the formation of platelet thromboxane B2 was significantly inhibited (Chen and Lin 2001). When zebrafish embryos were exposed to nickel at a concentration greater than 10 mg/L there was a delay in its hatching (Kienle et al. 2008).

6.6.4 Cadmium

Cadmium is a non-essential metal for humans and it is classified as a carcinogen by the National Toxicology Program; people are exposed to cadmium if they are employed in industries related to metals, batteries, and electroplating processes and also by tobacco usage (National Toxicology Program 2000; International Agency for Research on Cancer Monographs 1993). Cadmium exposure is very toxic to human because the human body does not have any mechanism to convert the cadmium to its less toxic form by metabolic degradation and it is also very less excreted (Waalkes 2003). Various studies have linked the occupational exposure of cadmium to carcinogenesis in lungs, prostate, kidney, liver, haematopoietic system, urinary bladder, pancreas and stomach (Waalkes 2000; Pesch et al. 2000; Hu et al. 2002; Waalkes and Misra 1996; Schwartz and Reis 2000; National Toxicology Program 2000).

Cadmium is found to be poorly mutagenic, and due to this, it is predicted to have a significant role in epigenetic and indirect genotoxic effect (Waalkes and Misra 1996). When the liver cells of the rat were exposed to an acute level of cadmium for about a week, DNA methyltransferases enzyme was inhibited that caused a hypomethylation, and when the exposure time was extended to 10 weeks, it led to hypermethylation of DNA and increased DNA methyltransferase activity (Takiguchi et al. 2003). Another study supported the above fact that the liver cells exposed to 10 weeks had a DNA hypermethylation at the global level with an increase in expression of DNMT3b Methyltransferase, DNMT activity, promoter hypermethylation and decrease in the expression of RASSF1A and p16 tumour suppressor gene (Benbrahim-Tallaa et al. 2007). A study has reported that the zebrafish embryos exposed to cadmium and nickel had a delay in hatching

(Aldavood et al. 2020). In a study conducted with rat, the cadmium was found to decrease the level of platelet aggregation (Kumar and Bhattacharya 2000).

The initial effect of cadmium on acute exposure zebrafish is the ion loss especially Ca^{2+} and Na^{2+} ions in adult and larvae stages (Alsop and Wood 2011). It is observed that Ca^{2+} binding to gills is antagonised by Cd^{2+} which leads to various effects and finally to death (McGeer et al. 2011). The cadmium exposure to zebrafish resulted in oxidative stress through altering the catalase and superoxide dismutase activity (Wang and Gallagher 2013). The cadmium oxide nanoparticles, when exposed to the adult zebrafish, cause liver tissue damage and oxidative stress induction (Balmuri et al. 2017). Certain observations have found that there is overexpression of genes that are playing role in protection against oxidative stress when there is exposure to cadmium (Arini et al. 2015). In the waterborne exposure to cadmium toxicity, there was found to be tissue accumulation of cadmium in the liver, kidney, gills and brain (Cambier et al. 2010).

6.6.5 Bisphenol A

Bisphenol A (BPA) is one of the synthetic forms of phenol used in the manufacturing of polycarbonate plastics and epoxy resins (Jalal et al. 2018). Exposure to BPA occurs because it leaches out from the containers which are made using BPA that contain beverages and food and enter the food particles it contains (Vandenberg et al. 2009; Kuo and Ding 2004; Munguía-López et al. 2005; Le et al. 2008). In a study conducted with zebrafish embryos, it was found that the BPA induced alterations in the yolk sac, pericardial oedema, hatching delay, deformation in the spine, and decreased heartbeat and eventually lead to death (Scopel et al. 2020).

A study with BPA exposure to zebrafish at environmentally relevant concentrations revealed that there was a reduction in the global DNA methylation level in the testes and ovaries leading to reproductive abnormalities in zebrafish through the oestrogenic mechanism by alteration in the DNA methylation enzymes (Laing et al. 2016). The BPA exposure to mice in the diet was found to have different changes in the gene expression like DNA hypermethylation, histone acetylation in the promoter region of Kcc2 which is an ion channel involved in the chloride ion excretion (Yeo et al. 2013). The BPA was found to influence the coagulation process through the formation of complexes with factor VII according to the study conducted in zebrafish (Chagas et al. 2020). It is found that the BPA in zebrafish causes gene-specific alteration of DNA methylation and decreases the dnmt1, dnmt3b3 and dnmt3b4a activities (Bouwmeester et al. 2016; Lombó et al. 2015; Chen et al. 2015).

6.6.6 Perfluorooctanoic Acid

The perfluorooctanoic (PFOA) acid belongs to synthetic chemicals of group perfluoroalkyl and polyfluoroalkyl substances and it is made up of eight carbon chain (Rashid et al. 2020a). The polyfluoroalkyl substances can be found in various household and industrial products like greaseproof papers, non-stick cookware, stain repellents, cleaners, carpets, aqueous film-forming foam and wetting agents (Kissa 2001; Kannan 2011). The PFOA is a highly persistent compound because it has carbon-fluoride bonds and the accumulation of PFOA might lead to adverse health defects (Buck et al. 2011). The presence of PFOA has been found in the dust (Scher et al. 2019), surface, drinking water (Guelfo and Adamson 2018; Liu et al. 2019), indoor and outdoor air (Goosey and Harrad 2012).

There have been accumulation and distribution of PFOA in tissues that have led to nephrotoxicity, neurotoxicity, genotoxicity, epigenetic toxicity, immunotoxicity, hepatotoxicity, reproductive and developmental toxicity (Shane et al. 2020; Kmecick et al. 2019; Wen et al. 2020a, b; Liu et al. 2021; Rashid et al. 2020a, b). When a human breast cancer cell line was subjected to PFOA, there were significant alterations in the expression patterns of DNMT1 and DNMT3A that has eventually led to the heterochromatin packaging changes (Liu et al. 2021). In a study conducted on human subjects with exposure to PFOA, it was found that there has been an accumulation of PFOA in the serum and platelets and there was also higher aggregation rates of platelets in comparison to control posing a threat to cardiovascular disease (De et al. 2020).

6.6.7 Lead

The lead is one of the naturally occurring heavy metal which has been mined for thousands of years (Hernberg 2000). There are different ways by which lead is exposed to the environment; they are through inhalation of air containing lead dust, ingestion of food and water containing lead and direct contact with soil that is contaminated by lead (EPA). The brain is particularly sensitive to the lead toxicity harmful effects, even though there are undesirable changes in all the organs of the body due to its exposure to lead (Sanders et al. 2009; White et al. 2007; Bellinger 2011). The unborn and younger children are more prone to lead toxicity because their small size and fast developing brain supports the fast absorption and retention of lead and the lead can also freely pass the placental barrier (Goyer 1990). Studies done on the human and animal models suggest that even the lowest concentration of lead is toxic to early development of brain (Kuehn 2012; Surkan et al. 2007).

In cultured neuroblastoma cells the lead exposure was found to inhibit the activity of IGF-1 stimulated methionine synthase which is involved in DNA methylation (Waly et al. 2004). There was about 20% decrease in the activity of the enzyme DNMT1 in adult brain tissues of animals exposed to lead, and on further investigation through microarray analysis, there was about 22 genes that had undergone alteration and 20 genes among them had enriched CpG sequences which are due to the lead-induced alteration in the expression of *DNMT1* (Wu et al. 2008). A research conducted with the mother's neonatal blood showed that high lead levels correlated with the DNA methylation changes at about 564 loci in their children's neonatal blood, suggesting that lead exposure during the time of pregnancy affects the foetal germ cell's DNA methylation status that leads to grandchildren's altered DNA methylation which tends to modify during the postnatal development (Sen et al. 2015).

Lead exposure is found to affect the blood coagulation activity through reduced nitric oxide, endothelial tissue injury, tissue plasminogen activator and hyper production of plasminogen activator inhibitor-1 (Vazir 2008). The animals which were subjected to chronic lead intoxication were found to have increase in their platelet count, platelet distribution width and mean platelet volume (Samuel et al. 2010; Mugahi et al. 2003; Golalipour et al. 2007). In a study conducted with zebrafish embryos to lead exposure resulted in altered vasculature in the somatic regions, motor neuron extension alterations both ventrally and dorsally from the spinal cord, Rohon-Beard Sensory neurons loss, and occurrence of apoptosis in increased areas which interprets that the lead exposure not only causes damage to the brain but overall most of the organs in the vertebrates (Roy et al. 2015) (Table 6.2).

6.7 Conclusion

This chapter has elucidated the morphological, functional and developmental closeness of the platelets in the human and zebrafish. There are a lot of similarities among the platelets of zebrafish and humans except for the presence of a nucleus. The process of aggregation and also the formation of platelets involves the presence of thrombin, ADP, vWF receptors along with the transcription factors and genes encoding them. The epigenetic mechanism and the environmental pollutants playing a role in it have reported their influence in platelets, which could affect the zebrafish platelets. Since the zebrafish is growing as a model for studies in the platelets there are only very few works done on its molecular side which stands a major field of research to explore.

Table 6.2 Environmental pollutants—impact on platelets and epigenetic modifications

S. No	Environmental pollutant	Impact on platelets	Organism	Ref	Epigenetic modification	Ref
1.	Benzo[*a*]pyrene	Inhibition in platelet function	Human lymphocyte culture	Onaran et al. (2000)	Hypomethylation	Fang et al. (2013)
2.	Arsenic	Decrease in platelet count and impairing its function	Human	Wu et al. (2014)	Hypomethyla-tion in embryonic stage of zebrafish. Later becomes hyper	Li et al. (2009a, b)
3.	Nickel	Inhibition in platelet aggregation	Human	Chen and Lin (2001)	DNA methylation and histone acetylation	Lee et al. (1995), Kang et al. (2003)
4.	Cadmium	Decrease in the platelet aggregation	Rat	Kumar and Bhattacharya, (2000)	Hypo- and hypermethylation	Takiguchi et al. (2003)
5.	Bisphenol A	Influences coagulation process	Zebrafish	Chagas et al. (2020)	Hypermethyl-ation and histone acetylation	Yeo et al. (2013)
6.	Perfluorooctanoic acid	Increase in the platelet aggregation rate	Human	De et al. (2020)	Alteration in the expression of DNA methylation enzymes	Liu et al. (2021)
7.	Lead	Increase in the platelet count, platelet distribution width and mean platelet volume	Rats	Okediran et al. (2010)	DNA methylation	Waly et al. (2004)

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Chapter 7
Neuroendocrinology of Fishes

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Abstract The chapter includes information regarding neuroendocrinology in fishes particularly on the hypothalamohypophysial system, the organization of the telencephalon, preoptic region, hypothalamus, central neurohormones, hypophysiotropic peptides, steroid feedback regulation of stimulating and releasing hormones [luteinizing hormone (LH), *follicle*-stimulating hormone (FSH) and gonadotrophin-releasing hormone (GnRH)], hypothalamic neurotransmitters, hormonal targets of hypothalamus and pituitary as well as neuroendocrinology of fluid intake and fluid balance.

Keywords Fish hypothalamus · Endocrine regulation · Teleost · Fish neuroendocrinology

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7.1 Introduction

The secretory cells in the teleost fish brain were suggested by Scharrer (1928). The hormones secreted in the pars nervosa of the pituitary is governed by glandular nerve cells. Several studies revealed the presence of a hypothalamohypophysial system that links the endocrine and the central nervous systems (CNS) to regulate a wide variety of vital process. The hypothalamus, neurohypophysis and adenohypophysis are the three main areas of the hypothalamohypophysial system in fish. The hypothalamus is the part of the diencephalon; the adenohypophysis is the non-neuronal structure of the gland and the neurohypophysis is developed from the ventral diencephalon and characterizes the neural compartment of the pituitary (Pogoda and Hammerschmidt 2007). The cell bodies are present in the preoptic area of the nerve terminals and the pituicytes make up the neurohypophysis. Pituicytes have a supportive function. The neurohypophysis is divided into the pars nervosa and median eminence in non-teleost (Lagios 1968) and Elasmobranchs (van de Kamer and Zandbergen 1981). Adenohypophysis receives secretory products from hypothalamic neurons that have a network of blood capillaries which is the portal system. The portal system and median eminence are not found in teleost fishes. The diffusion distance between the region of adenohypophysis and hypothalamic neurons is reduced; hence the hypothalamic neurons control the adenohypophysis.

The endocrine system of the fishes consists of the thyroid gland, pituitary gland, corpuscles of Stannius, adrenal gland, urohypophysis, ultimobranchial glands, pineal gland and pancreatic islet. Thyrotropins release *thyroid-stimulating hormone (TSH)* that regulates the synthesis and secretion of triiodothyronine (T3), thyroxine (T4) and calcitonin and thyroid growth. Gonadotrophs triggers *luteinizing* hormone (LH) and *follicle-stimulating* hormone (FSH) hormones and thus control oogenesis, gonadal hormones and spermatogenesis. Somatotrophs trigger growth hormone (GH) and increase basal metabolic rates and growth of the fish body. Lactotrophs trigger prolactin (PRL) release that helps in melanogenesis and osmoregulation. Adrenocorticotropic hormone (ACTH) is triggered by corticotrophs and regulates corticotropin from the adrenal glands. Pigmentation and melanophores are regulated by melanocyte-stimulating hormone (MSH) and melanin-concentrating hormone (MCH) in the body and skin. Saltwater balance, mating/laying eggs and osmoregulation are regulated by oxytocin and arginine-vasotocin (AVT).

In bony fish, the thyroid arises as a median evagination from the floor of the pharynx. The thyroid hormones (TH) help in osmoregulation, migration, maturation, scale and bone development. The adrenal cortex and medulla are placed in two separate regions. Cortisol secretion, sodium retention and water metabolism are the responsibilities of adrenal glands. Hypocalcin is secreted by the corpuscles of Stannius, which regulates calcium balance. Calcitonin also regulates calcium levels that are secreted by ultimobranchial glands in the fish body. Urotensins control the metabolic regulation that is secreted by urohypophysis present at the end of the spinal cord. Insulin that regulates carbohydrate metabolism is produced by the pancreatic islets located in gut walls. Melatonin that controls photosensory or secretory functions is secreted by the pineal gland. In fish, epinephrine, norepinephrine, and dopamine are produced by the chromaffin cells that present in the adrenal glands.

7.2 Telencephalon

The organization of the telencephalon is highly distorted in teleost fish. Evagination is the process through which the telencephalon develops at the earlier steps for the development of telencephalon in most of the vertebrates such as amniotes, cartilaginous fish, lampreys, and amphibians. As the telencephalon protrudes and expands, the lumen of the neural tube subsequently forms the telencephalic ventricles. The telencephalon comprises olfactory bulbs and telencephalic hemispheres. The telencephalic hemispheres are divided into two parts as ventralis telencephalic or subpallium and dorsalis telencephalic or pallium. The area ventralis telencephalic is structured into nuclei (Wullimann and Mueller 2004). In contrast, the dorsalis telencephalic is the largest region that exhibits a large number of histological distinct zones. The dorsal area is divided into three periventricular zones (pars dorsalis, pars medialis and pars lateralis) which is based on cytoarchitectonic criteria. The ventral area of the teleost telencephalon is a non-everted part that is rostrally placed on the preoptic area that is divided into precommissural and postcommissural nuclei. Precommissural nuclei are divided into lateral, dorsal and ventral whereas the post commissural portions contain supra commissural, post commissural, central, endopeduncular and intermediate nuclei.

7.3 Preoptic Area

The preoptic area is found in the hypothalamus. It is the functional and structural continuum with the basal forebrain. This area is responsible for thermoregulation. From the thermoreceptors in the skin, hypothalamus and mucous membrane it receives nervous stimulation. The preoptic periventricular recess is surrounded by the preoptic region that is located in between the anterior commissure and optic chiasm. The large neurosecretory cells of the magnocellular preoptic nucleus are further subdivided into magnocellular, gigantocellular and parvocellular parts. It is like an inverted plane, in the longitudinal plane, with a nucleus, rostrally located vertical rod and dorsocaudally extending horizontal rod. The larger dorsocaudal cells constitute the giganto- and magnocellular parts and the smaller rostroventral cells compose the parvocellular parts. The magnocellular and parvocellular preoptic nucleus is the parts of the preoptic area and the parvocellular preoptic nucleus is divided into posterior pars and anterior. The pars and anterior is known to be preoptic parvocellular nucleus, the division of magnocellular is called the preoptic nucleus and posterior pars, periventricular nucleus, in goldfish (*Carassius auratus*), two salmonid species (Peter et al. 1991) and killifish (*Fundulus heteroclitus*). It is subdivided into the posterior and anterior periventricular nucleus.

7.4 Hypothalamus

Hypothalamus is found in caudal to the preoptic area under the thalamus that contains preoptic neuroendocrine fibres and hypothalamic fibres. It has densely stained and packed cells that surround the infundibular recess. Three main regions of the teleost hypothalamus are paired inferior lobes, periventricular region and tuberal region that is medially located separated by a deep ventral sulcus from the tuberal hypothalamus (Meek and Nieuwenhuys 1998). Hypothalamus is subdivided into ventral, caudal and dorsal according to other authors (Wullimann et al. 1996). Most of the median tuberal portion of the hypothalamus is constituted by caudal and ventral zones.

Periventricular cell population bordered by laterally migrated nuclei is displayed by all three subdivisions. Two laterally migrated populations, the anterior tuberal nucleus and the lateral hypothalamic nucleus, and two periventricular cell populations, the ventral and dorsal, occupy the rostral area of the hypothalamus region (Braford and Northcutt 1983). The hypothalamus of the teleost fish comprises Gomori-negative nucleus lateralis tuberis and Gomori-positive nucleus preopticus. The nucleus lateralis tuberis is situated caudally to the hypothalamus, and further it has been subdivided as pars rostralis, pars medialis, pars lateralis and pars ventrolateralis. In different teleost fishes, the nucleus lateralis tuberis subdivisions vary. The preoptic nucleus consists of pars magnocellularis and pars parvocellularis located on both sides of the preoptic recess. The preopticohypophysial tract is formed by the axons, which is originating from the preoptic nucleus. This tract penetrates through the pituitary gland and terminates in the neuro intermediate lobe.

7.5 Central Neurohormones

Isotocin is the nonapeptide homologous to mammalian oxytocin among bony fishes (Acher 1996). It belongs to the arginine vasopressin-oxytocin family of peptides and is exclusively produced in the preoptic area. Isotocin is produced in the pars and anterior and magnocellular in all fish species. The isotocin neurons cluster collectively to display the contact between the processes in the rainbow trout's magnocellular region. For local neuronal circuitry, the isotocin neurons can use electrical or chemical synapses. It plays an important role particularly in the aspect of reproductive behaviour and in fish reproduction. In rainbow trout (*Oncorhynchus mykiss*) and goldfish, it stimulates the release of ACTH from the pituitary. In the form of a non-covalent complex, the secretory granules store each hormone with its associated neurophysin, before secretion. By exocytosis, this complex is released into the blood causing spontaneous dissociation. Neuropeptides, when released into the circulatory system, act as hormones, whereas in the CNS, it plays the role of neuromodulators as well as neurotransmitters. The hormones are stored in secretory as a non-covalent complex with its associated neurophysin. This complex is released into the blood by the exocytosis process.

The AVT, one of the main neurohypophysial hormones which is made by nine amino acid peptide (a nonapeptide), that belongs to the arginine vasopressin-oxytocin family of peptide and are homologous to arginine-vasopressin (AVP) and oxytocin in mammals (Acher 1996). It modulates the social behaviours of lower vertebrates (Goodson and Bass 2001). Their primary role is in the endocrine control of the vascular function, saltwater homeostasis (McCormick and Bradshaw 2006) and several physiological processes (Balment et al. 2006).

AVT is synthesized by preoptic neurons that are released into the vascular system by travelling through the preoptico-hypophysial tract via neurohypophysial axon terminals. The magnocellular and parvocellular neurons of the preoptic nucleus exclusively have the AVT-immunoreactive neurons. AVT-immunoreactive neurons in rainbow trout and many other fish species simultaneously project towards the extrahypothalamic and pituitary regions including the ventral telencephalon, mesencephalon and thalamus. In other vertebrates, these multiple projections are not known. Under different physiological challenges, these multiple projections are essential for coordinated control of central and peripheral output through the organization of the electrical activity (Saito et al. 2004). AVT neurons cluster together in the magnocellular of goldfish, eel (*Anguilla* sp.) and rainbow trout by soma-somatic apposition beside the ventricular wall. The function could be communication by changing somatodendritic peptide release or local field potential (Saito et al. 2004).

The MCH is a circulating cyclic heptadecapeptide that mediates the change of colour in the teleost fish (Kawauchi et al. 1983). It was initially purified from chum salmon pituitaries. The changes in the refractive index are mediated by the central accumulation of pigmentary organelles. This allows cryptic camouflage by making the scales appear paler (Kawauchi and Baker 2004). When the fish moves into a pale background, MCH is released into the fish blood in teleost fish. Some positive fibres of the MCH axons penetrate the pars distalis, which regulates the synthesis or release of adenohypophysial hormones (Baker and Bird 2002; Pandolfi et al. 2003). The lateral hypothalamic nucleus or the nucleus lateralis tuberis of fish contains the neuronal cell bodies that produce MCH.

7.6 Hypophysiotropic Peptides

Cholecystokinin (CCK) or gastrin immune reactive neurons belongs to the family of peptides (Chandra and Liddle 2007; Rehfeld et al. 2007) that is produced in the nervous system. This octapeptide of the C-terminus (Trp-Met-Asp-Phe-NH_2) of CCK is the same in chicken, mammals, turtle and frog and is a well-conserved structure during evolution with the substitution of one amino acid in the fish (Peyon et al. 1998). Longer CCK peptides such as CCK 22, 33 and 58 are identified in the circulation and peripheral tissues (Rehfeld et al. 2007). In goldfish, the CCK is generally distributed in the hindbrain, forebrain and midbrain (Himick and Peter 1994). The inferior hypothalamic lobes and ventrolateral and ventromedial hypothalamus of goldfish have prominent, highly concentrated CCK/gastrin-immunoreactive perikarya and fibre systems (Himick and Peter 1994). In the hypothalamus of the goldfish brain, CCK mRNA that is similar to CCK-immunoreactive is widely expressed (Peyon et al. 1999). A large bundle of CCK/gastrin-immunoreactive fibres that originates in the central hypothalamus enters the hypophysis towards the neurohypophysis. In the preoptic recess proximal pars distalis (PPD), these large bundles of CCK/gastrin-immunoreactive fibres branch into smaller bundles and subsequently single fibres. The immunoreactive fibres separate neuro- from adenohypophysis by terminating on the basal lamina (Batten et al. 1999). The CCK-immunoreactive fibres have a strong relationship with GH release (Batten et al. 1999).

After the administration of amphetamine and cocaine in the rat, the cocaine- and amphetamine-regulated transcript (CART) mRNA expression was elevated in the rat (Douglas et al. 1995). A 116 or 129 amino acid having 27 amino acid signal peptides is the product of CART gene splicing in rat that results in pro-peptide do either long arm (102) or short arm (89) residues (Douglas et al. 1995). Rat long CART (55-102) or rat short CART (42-89) is released as a result of the pro-peptide process that depends on the precursor length (Dylag et al. 2006). Widely distributed CART immunoreactivity localized within the neuroendocrine territories is observed within the brain of catfish. Throughout the PPD, CART-immunoreactive terminals in neurohypophysis and long fibres were detected with a high concentration of thyrotropes and somatotropes. In fishes, four neuropeptides such as a binding protein (CRF-BP), urotensin I (UI) and two G-protein coupled receptors includes the corticotropin-releasing factor (CRF) (Bernier 2006) that plays an important role in the coordination of stress response. It can be modulating the activity of adenohypophysial ACTH and MSH cells; the CRF system regulates the pituitary-adrenal axis along with other physiological process regulation (Flik et al. 2006). In hypophysiotropic regions of the brain that include telencephalon, olfactory bulb, tuberal hypothalamus and preoptic area of zebrafish as well as a white sucker, the CRF is widely expressed (Alderman and Bernier 2007). A major site of CRF production is the preoptic area. Neuronal CRF circuits in the preoptic area differ depending on the species. In the vicinity of the ACTH cells along with of ACTH-releasing factor in the pars distalis of the sea bass is placed the CFR fibres. MSH secretion in teleost fish is stimulated by CRF.

Isolated from the porcine intestine, the 29 amino acid long N-terminal peptide, Galanin, is processed proteolytically from prepropeptide with a galanin message-associated peptide. This neuropeptide exhibits considerable differences in functional coupling and signalling process by binding to three different G-protein coupled receptors. It is usually distributed in the peripheral and CNS and has multiple biological effects including metabolism and feeding, water-intake and osmotic regulation, sleep regulation, nociception and reproduction (Lang et al. 2007). The pituitary of many vertebrate groups contains galaninergic fibres that can help in the regulation and secretion of the pituitary gland (Anglade et al. 1994; Jadhao and Pinelli 2001). The occurrence of galanin-immunoreactive terminals in the central region of neurohypophysial was demonstrated in ultrastructural studies in fish. Some fibre is interlocked into PPD that ends in basal lamina opposite to GH, TSH, PRL, gonadotropins (GTH) and ACTH cells. ACTH and PRL adenohypophysial cells are directly innervated by galanin fibres. Somatolactin (SL), GH and PRL cells colocalize with the galanin-immunoreactive fibres. Confined galanin receptors are present in the region occupied by the PRL cells in the adenohypophysis particularly in the rostral part (Moons et al. 1991; Batten et al. 1999).

Gastrin-releasing peptide (GRP) is a set of peptides that are illustrated by a highly preserved C-terminus that is vital for many biological processes. It consists of 27-amino acid that is a part of the bombesin (BBS) and neuromedin B family of peptides. The exogenous BBS and endogenous GRP are structurally similar that reflects the functions of each other. Gastrointestinal and CNS is widely distributed with BBS/GRP peptides (McCoy and Avery 1990) which when administrated intraperitoneally or centrally acts as potent anorexigenic substances in fishes and mammals (Flynn 1991). Gut motility and visceral activity in fishes is regulated by BBS-like peptide. BBS-immunoreactive fibres are present in the posterior hypothalamus, tuberal hypothalamus, ventral telencephalon, preoptic area and assumed feeding centre associated areas in goldfish (Himick and Peter 1995).

The GnRH has an amide function at the carboxy terminus and pyro-glutamate modification in the amino terminus. GnRH is a decapeptide that has a cyclic structure and has been characterized into 24 molecular isoforms (Kah et al. 2007). GnRH is grouped into three types, namely, GnRH type I, GnRH type II and GnRH type III (White et al. 1998). GnRH type I is the hypophysiotropic GnRH variants, which includes mGnRH, cfGnRH and pjGnRH and sbGnRH. GnRH type II consists of cGnRH-II, which is localized in the midbrain. GnRH type III includes sGnRH (Lethimonier et al. 2004). The GnRH peptide is distributed from the olfactory bulb, throughout the ventral telencephalon in the forebrain (Pandolfi et al. 2005).

Studies suggest that the forebrain cGnRH-II in goldfish doesn’t initiate ovulatory LH surge in comparison with the GnRH mRNA levels with LH levels at the time of ovulation and spawning (Canosa et al. 2008). GnRHs are classified into four types, which includes GnRH1, GnRH2, GnRH3 and GnRH4. The GnRH1, GnRH2 and GnRH3 are present in teleost fish, whereas the GnRH4 represents the lampreys. The structure of GnRH varies across vertebrate species, whereas in GnRH2 and GnRH3 the amino acid sequences are preserved. The GnRH may be considered the hypophysiotropic hormone when synthesized and secreted in the preoptic area of the hypothalamus. GnRH has additional neuroregulatory and neuromodulatory roles and is necessary for reproduction.

In teleosts, synthesis and secretion of LH and FSH are controlled by gonadal steroids. Actions at the level of the hypothalamus and pituitary involve the secretion of LH and FSH by sex steroid feedback regulation. The levels of GnRH and other neuroendocrine factors are affected by the effects on LH and FSH synthesis in the pituitary, testosterone and 17β-oestradiol that control LH synthesis and secretion.

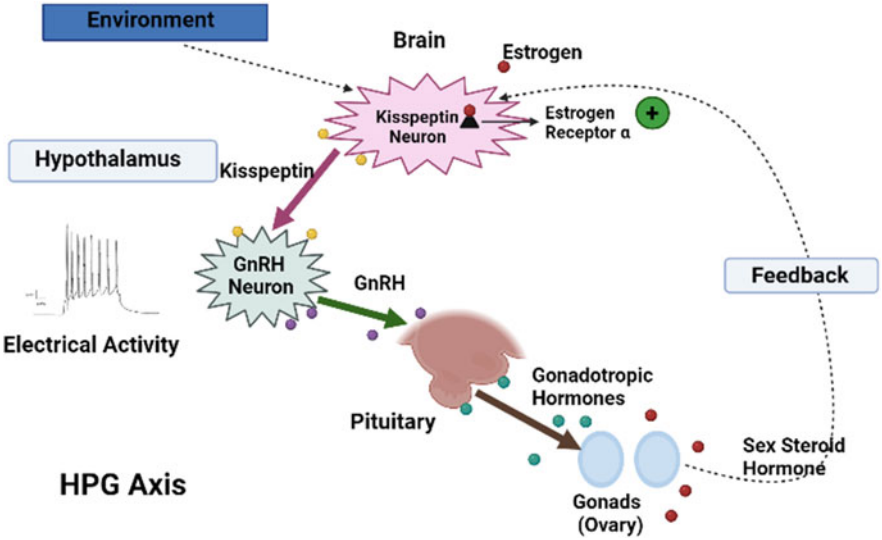

Fig. 7.1 Schematic representation of central regulation of reproduction in fishes. HPG axis: hypothalamic-pituitary-gonadal axis; GnRH; gonadotropin-releasing hormone

Through oestrogen receptors, the effects of testosterone are mediated through the receptor of androgen or result from metabolism to oestrogens. The synthesis and secretion of LH and FSH are implicated by maturation-inducing steroid 17, 20-β-dihydroxy-4-pregnen-3-one, corticosteroid and cortisol. These effects are secondary to the dominant actions of testosterone and 17β-oestradiol (Fig. 7.1).

Pituitary adenyl cyclase-activating polypeptide (PACAP) and growth hormone-releasing peptide (GHRH) have numerous similarities in structure and function. These belong to the family of glucagon/vasoactive intestinal peptide (VIP) and secretin superfamily of peptides (Sherwood et al. 2000). Molecules other than PACAP are formed by tandem exon duplication and gene duplication, whereas PACAP is a conserved, ancestral molecule from which other molecules are subsequently formed (Sherwood et al. 2000). Around 88-97% of the amino acid sequence is identical to PACAP across vertebrates whereas only 32-45% of amino acid sequence identity of GHRH exists between non-mammalian and mammalian vertebrates (Vaudry et al. 2000). GHRH stimulates the GH and it regulates physiological functions such as adaptation to different salinity growth and somatic growth by binding to growth hormone receptor (GHR). GH has diverse effects in vertebrates with multiple targets. It influences metabolism. The behaviour of fish is altered by GH by increasing appetite, aggression, and swimming activity and by reducing anti-predator behaviour. By the action of the peripheral mediator on the fish brain, the swimming activity is increased by GH. A secondary peripheral factor affects appetite. Immune functions that include non-specific defence such as haemolytic, cytotoxic, phagocytic and lysosome activities are enhanced by GH. Ceruloplasmin levels are elevated by a specific defence of immunoglobulin production by GH. In fish models, gametogenesis, gonadal steroidogenesis and sexual maturation are contributed by the interaction of GH with the gonadotropic axis. The PRL and SL belong to the class I helical cytokines family that forms a family of pituitary hormones along with GH. The pituitary hormones signal via specific receptors, finally activating a similar intracellular signalling cascade. Except in the Chondrichthyes and Agnathans, both hormones have been identified in almost all classes of vertebrates whereas SLs are found only within the Osteichthyes. In the pituitary gland of teleosts, GH, SL and PRL are localized in three distinct areas such as PPD, pars intermedia and rostral pars distalis, respectively.

PRL has seven various functions such as growth and development, reproduction, immunoregulation and protection, endocrinology and metabolism, water and electrolyte balance, brain and behaviour and actions associated with disease conditions. Fish need to have PRL osmoregulatory actions to reside in water with different salinities. PRL, SL and GH interact with specific single transmembrane-domain receptors for exerting their biological effects. Hypothalamic neurohormones regulate the secretion and synthesis of the pituitary hormones. The hypothalamic neurohormones are classified generally into two categories such as releasing and inhibiting hormones. The presence of a highly specific receptor, which belongs to the G-protein coupled receptors (GPCR) on the target cell in the pituitary, determines the action of each hypothalamic neurohormone. GPCR are seven transmembrane segments that bind to either activate or inhibit G proteins.

7.6.1 Functions of Prolactin (PRL)

Water and Electrolyte Balance PRL stimulates the ion retention in teleosts, thereby preventing the water influx to the osmoregulatory organs for freshwater adaptation. Gills, skin, kidney, urinary bladder, gastrointestinal tract and opercular membrane are the principal osmoregulatory organs in fish. Fish acclimated to freshwater has high PRL cell activity than those in seawater. PRLRs expression ubiquitously in the osmoregulatory organs in euryhaline teleosts helps to adapt themselves for the sudden osmotic changes in the surrounding.

Growth and Development Somatotropic activity in tilapia was observed to cause stimulus of hepatic IGF-I mRNA expression and to cause an elevation in [H3] thymidine and [S35]sulphate incorporation into branchial cartilage. In climbing perch liver, PRL inhibited many enzymes involved in fatty acid biosynthesis. In juvenile coho salmon, lipid depletion was observed after PRL treatment. During embryogenesis and after hatching, the PRL gene was detected. Somatotropic action of PRL in teleosts was supported by the widespread tissue distribution of PRLR mRNA and protein in the developing pituitary gland during its embryonic and larval period.

Immunoregulation In several teleost fishes, the immune function is influenced by sex steroids, cortisol and pituitary hormones including PRL. Phagocytosis, respiratory burst activity, and leukocyte mitogenesis are found to be stimulated by PRL and also increase the plasma IgM titres. Circulating lymphocytes, spleen and head of tilapia contains PRLP mRNA. The level of expression of PRL and PRL receptors were established to the higher concentration of salt in salt water (particularly, in seawater) acclimation fish than in freshwater fish, suggesting that the osmoregulatory action and the immunomodulatory actions of PRL are independent of each other.

Behaviour Administration of PRL in sexually mature male three-spined sticklebacks stimulates the parental fanning behaviour. Mucus is produced in tilapia by inducing the transformation of the skin gland by PRL for the nourishment of the offspring. In seawater eel, drinking water behaviour is affected by the administration of PRL into the fourth ventricle by inhibiting the water intake.

Reproduction Steroidogenesis is stimulated by PRL in the testes and ovary. During sexual maturation in salmonids, the plasma PRL and pituitary mRNA levels are elevated. In fishes, PRL release is stimulated by GnRH and E2 in both in vitro and in vivo models (Weber et al. 1997). In some teleosts, PRLR mRNA was detected in gonads suggesting the role of PRL in reproductive processes in fish.

7.6.2 Functions of Somatolactin (SL)

The SL is involved in adaptations to the background and decreased illumination, smoltification, adaptation to environmental changes, stress responses and control of some physiological aspects of reproduction, calcium and phosphate metabolism regulation, energy metabolism, growth and acid-base balance in fishes (Fig. 7.2). GHR1 expression was found to be higher in the skin consistent with SL functioning in chromophore regulation in rainbow trout and tilapia. SL levels in the plasma are not affected by the background colour in the rainbow trout. Water temperature causes seasonal changes in the SL secretion than to photoperiod. In teleosts, SL play a vital role in body colour regulation by the recognition of a gene, which is responsible for medaka colour interfere (ci) mutant. A significant increase in the number of leukophores and a decreased amount of visible xanthophores were exhibited by the medaka SL-deficient mutant colour interfere. During morphological body colour adaptation to different backgrounds, the SL transcription was dramatically changed showing the involvement of SL in chromatophore development.

Suggesting the metabolic function, tilapia GHR1 and rainbow trout SLR were highly expressed in liver, muscle and fat. Sea bass SL was injected into juvenile gilthead sea bream and found that it decreased the respiratory quotient by increasing the oxygen uptake and carbon dioxide output, but it does not modify the excretion of nitrogen-ammonia and circulating amount of IGF-I. The activity of hepatic acetyl-coenzyme A carboxylase was inhibited by SL, supporting the involvement of SL in the enhancement of lipid metabolism and energy homeostasis. Proopiomelanocortin (POMC)-expressing neurons, endogenous melanocortin antagonist expressing

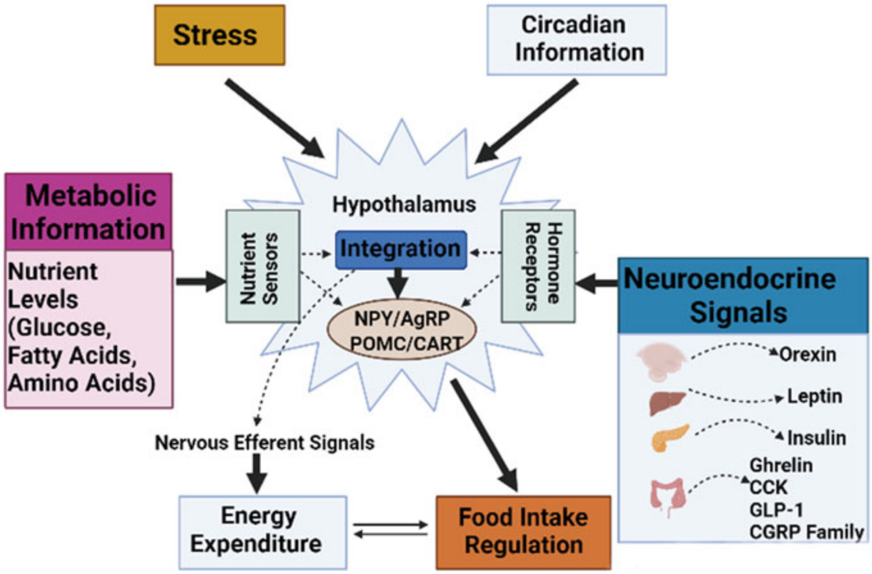

Fig. 7.2 Metabolic pathways of food intake. *NPY* neuropeptide Y, *AgRP* agouti-related protein, *POMC* proopiomelanocortin, *CART* cocaine- and amphetamine-regulated transcript, *CCK* cholecystokinin, *GLP-1* glucagon-like peptide-1, *CGRP* calcitonin gene-related peptide

neurons, agouti-related protein (AgRP) and central melanocortin receptor-expressing downstream targets of these neurons comprise the central melanocortin system. Three main domains containing MSH peptide are exhibited by a complex precursor encoded by the POMC gene. The α-MSH is the N-terminal sequence of ACTH that is found in the second domain (Cerda-Reverter et al. 2003). The pituitary produces POMC and its post-transcriptional modification occurs in a tissue-specific way. ACTH and β-lipoprotein are generated by the proteolytic cleavage by proconvertase 1 (PC1) in the corticotropes in the RPD. α-MSH and β-endorphin are generated by proconvertase 2 (PC2) and proconvertase 3 (PC3) in the melanotropes (Castro and Morrison 1997). A wide range of physiological functions are carried out by the melanocortins ACTH and MSH by binding to G-protein coupled receptor family. At the time of stress, the expression of POMC in magnocellular is upregulated while the function of ACTH in the brain of fish is unknown so far (Metz et al. 2004).

The neuropeptide tyrosine (NPY) family of peptides exhibits carboxy termination (C-terminal) amidation that consists of 36 amino acid. It is classified as three different peptides such as tyrosine-tyrosine peptide (PYY), pancreatic polypeptide (PP) and the NPY. Non-tetrapod vertebrates produce two kinds of peptides (NPY and PYY), whereas tetrapod species has all three peptides (PYY, PP and NPY). The NPY and PYY are synthesized in teleost fish, but PP is not synthesized (Sundstrom et al. 2008). The endocrine secretion of adenohypophysial cells is controlled by the involvement of NPY including GH and LH (Cerda-Reverter et al. 1999) in several fishes. Gonadotropes (LH-FSH), GH and melanotropes have direct contact with NPY-immunoreactive neurosecretory vesicles (Pontet et al. 1989). Seasonal variations that support the regulation of the reproductive axis are exhibited by the neurohypophysial NPY innervations. NPY-immunoreactive gold particles that carry neurosecretory axons are seen occasionally in association with the GnRH-containing cells in the PPD. An important centre for the sex-steroid signal processing is the NPY neurons present in the central (Sakharkar et al. 2005).

Orexin or hypocretins is produced from a common precursor of the incretin family (de Lecea et al. 1998). They are excitatory neuromodulatory peptides. This exerts its function upon binding with G-protein receptors that showed a variable affinity for hypocretin and different distribution in CNS; the hypocretin 1 and 2 exert their biological functions (Sutcliffe and de Lecea 2000). In goldfish, orexins increase their locomotor activities (Nakamachi et al. 2006). Fasting increases the levels of the mRNA of orexin in the hypothalamus (Nakamachi et al. 2006) whereas hypocretins stimulate the intake of food (Novak et al. 2005). Insomnia-like phenotype is induced in zebrafish by the overexpression of orexin (Prober et al. 2006). Disruption in sleep/wake behaviour is disrupted in zebrafish that lacks a functional orexin receptor (Yokogawa et al. 2007).

Somatostatin (SS), also known as growth hormone inhibiting hormone (GHIH), is a peptide hormone and an effective inhibitor of basal and stimulated GH secretion in the teleost (Canosa et al. 2007). The peptide consists of three different SS precursors that are encoded by a variety of genes in teleost (Canosa et al. 2007) that includes PSS-I, PSS-II and PSS-III. The PSS-I is the most conserved form that has an identical amino acid sequence and codes for SS-14 at the C-terminus. PSS-II encodes for a variable length of SS proteins between 22 and 28 amino acids. PS-III encodes a peptide that bears the amino acid proline in the second place of 14 amino acid C-terminals. Numerous physiological processes are coordinated by SS and somatostatin receptors (SSTR) interaction. It plays an important role in regulating the metabolism and growth by inhibiting the release of hormones such as IGFs and GH in teleost fish. Thyrotropin-releasing hormone (TRH) may also stimulate several pituitary hormones such as GH (Canosa et al. 2007), PRL (Barry and Grau 1986) and MSH (Lamers et al. 1991) in teleost fish. It is the primary hypothalamic releasing factor that may be characterized chemically from the hypothalamus of sheep and pig (Guillemin, 1970). Its role in pituitary TSH release control is well established in tetrapods. In carp, TRH stimulates GH and PRL secretion and MSH in trout and goldfish. The adenohypophysis of the teleost fish produces a glycoprotein, thyrotropin by the action of TSH that secretes TH by stimulating the thyroid gland. TRH influences the thyroid activity and TSH cell activity in fishes.

7.7 Hypothalamic Neurotransmitters

7.7.1 Glutamate and Gamma-Aminobutyric Acid (GABA)

The main excitatory neurotransmitter in the CNS of vertebrate (Trudeau et al. 2000) is considered glutamate that is an important regulator involved in LH, PRL and GH control (Bellinger et al. 2006). Gamma-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the CNS with a significant role in pituitary hormone secretion control (Martyniuk et al. 2007). A single enzymatic step that is catabolized by the glutamic acid decarboxylase (GAD) enzyme results in the synthesis of GABA. GAD is used as a marker for GABAergic fibres and cell bodies (Anglade et al. 1999). Telencephalon and olfactory bulbs of goldfish have GAD-immunoreactive cell bodies. LH secretion is regulated by GABA (Trudeau et al. 2000). GABA-containing cell bodies are found in the hypothalamus in tuberal and preoptic regions in the diencephalon.

7.7.2 Dopamine

Dopamine (DA) is one of the major neurotransmitters, which belongs to catecholamines in the CNS of the vertebrate that possesses hypophysiotropic functions. The dopaminergic system in the fish is extensively considered by using antibodies to identify the enzyme system involved in DA synthesis, tyrosine hydroxylase (Smeets and Gonzalez 2000; Rink and Wullimann 2001). In the posterior tuberculum and adjacent hypothalamic regions of fish, dopaminergic neurons are localized in the highest concentration (Ma 2003; Ma and Lopez 2003). In nuclei that are associated closely with ventricle and recesses, a large number of dopamine-containing neurons have been found. Dopaminergic neurons are located in the tuberal hypothalamus, the ventral regions of the preoptic area and olfactory bulbs. The dopaminergic neurons that innervate gonadotropes originating in the rostral region of pars and anterior are responsible for steroid hormones (Kah et al. 1986). D1 and D2 receptors are two DA receptor subtypes that are two distinct membranes that belong to the GPCR superfamily. The D1 receptor mediates the GH release and adenylate cyclase stimulation in the goldfish pituitary, whereas the D2 receptor inhibits PRL release in mammals and inhibits adenylate cyclase activity. In goldfish, there is significant hypertrophy and nuclear enlargement in cells of PRL was induced by the injection of DA agonists. A rise in the quantities of rough endoplasmic reticulum and reduce in the quantities of the secretory granules in PRL cells were induced by the treatment with DA in tilapia.

7.7.3 Serotonin

Serotonin or 5-hydroxytryptamine (5-HT) consists of the monoamine with neurotransmitters DA and NA. This is an indoleamine neurotransmitter that has both neuroendocrine (Trudeau 1997; Canosaet al. 2007) and behavioural functions (Johansson et al. 2004). 5-HT system contains two main localizations in the brain region of the teleost fish such as one anterior and posterior localization. The response of neurotransmitters during gonadal development in yellow snapper was analysed using 5-hydroxy indole acetic acid (5-HIAA) and found that both 5-HIAA and 5-HT were not detectable at any gonadal development in the pituitary. It works as a neuromodulator with multiple functions in fish and other vertebrates. The function of fish reproduction is modulated by serotonin through a variety of pathways including through peripheral (gonads) and central (preoptic-hypothalamic area and pituitary) actions.

7.8 Endocrine Targets of the Hypothalamus and Pituitary

7.8.1 Sex Steroid Production in the Fish Brain

The regulation of reproduction and energy balance is an important role of gonadal steroid hormones. Steroid production in gonads is stimulated by pituitary tropic hormones, GTH. These steroids control the secretion of tropic hormones to adapt their activity to the current physiological situation by feedback onto the neuroendocrine systems. The brain itself is a steroidogenic organ that produces several steroids with their functions less understood. Two enzymes that convert testosterone into dihydrotestosterone and oestradiol are the product of 5-α reductase and the cyp19a1b, P450 aromatase B (A or B), respectively. P450 aromatase B is evidence of steroidogenic enzymes for brain expression in fish. The presence of P450scc is required to cleave the lateral chain of cholesterol to form pregnenolone. There are two key steroidogenic enzymes including cytochrome P450c17 (CYP17) and 3-β-hydroxysteroid dehydrogenase/D4-D5 isomerase (3βHSD) performing further metabolization of pregnenolone. The 3βHSD is involved in dehydrogenation and isomerization of pregnenolone to progesterone. Hydroxylation of C21 steroids (17-α-hydroxylase activity) is caused by CYP17 followed by the cleavage of the two-carbon side chain. The C19 steroids dehydroepiandrosterone or androstenedione are generated. Histological differentiation of gonads is achieved by the steroid biosynthetic capacity in the brain. The asynchronous peak was shown by mRNAs of these genes, indicating the formation of oestradiol locally in the forebrain as well as the midbrain after 120 days of hatching, in the black porgy. In the zebrafish brain, oestrogen production might be associated with neurogenesis. By the action of oestrogen 2-hydroxylase, the oestrogens can be metabolized into catechol-oestrogens. Oestrogen effects on neuroendocrine and behavioural functions are

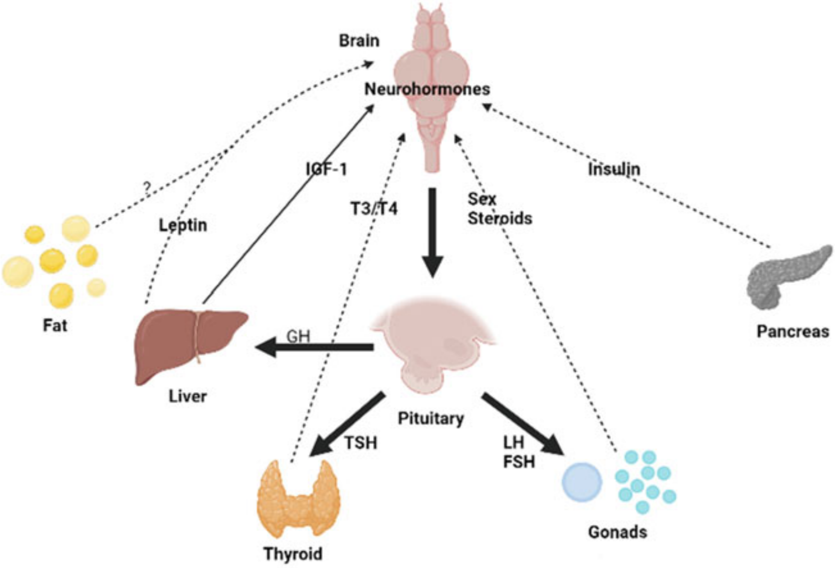

Fig. 7.3 Schematic representation of brain-hormone relationships in fishes. *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *TSH* thyroid-stimulating hormone, *GH* growth hormone, *IGF-1* insulin-like growth factor-1, *T3* triiodothyronine, *T4* thyroxine

mediated by potentially bifunctional, oxidized 2-hydroxy oestrogen molecules. Tyrosine hydroxylase activity is non-competitively inhibited by catechol-oestrogens through modifying catecholamine metabolism in fishes (Fig. 7.3).

7.8.2 Metabolic Hormones

Sensing of metabolic signals by neuroendocrine circuits that involve neuropeptides, cocaine and amphetamine-regulated transcript, KiSS or GnRH ensures the signal between the reproductive axis and energy status through several neuropeptide hormones. Mandatory signals are required for normal gonadal function and pubertal maturation that is emerged by kisspeptins, which is encoded by the gene named KiSS-1 and its receptor GPR54. Changes in fertility may be linked to disturbed energy balance in fish are explained by the negative feedback inputs on the GnRH systems through the KiSS neurons.

7.8.3 Leptin

Leptin levels have been roughly proportional to fat stores that are produced by the adipose tissue. It stimulates the appetite centre, which is located in the ventromedial nucleus of the hypothalamus to regulate food intake. Leptin works by stimulating α-MSH activity and by inhibiting AgRP and NPY. The brain uses leptin as a parameter to assess the energy levels to proceed with highly demanding reproductive function. In fish, the liver is the primary source of leptin.

7.8.4 Insulin-Like Growth Factor and Insulin

The hormone insulin regulates the glucose homeostasis in the vertebrates that are produced from proinsulin precursor molecule of the pancreas under the action of prohormone convertases. It is also involved in survival factor during early morphogenesis by expressing in the developing brain. The proliferation and cell survival of the developing retina and brain are stimulated by the unprocessed proinsulin. Insulin is secreted from Brockmann bodies in the teleost fish as well as in the pituitary gland and the brain of tilapia but with low concentration. GH stimulates the formation of IGF-I and it binds to specific IGF receptors present especially on bone, liver, muscle, kidney, lungs, skin, brain, cartilage, hippocampus, cerebellum and olfactory bulb. Embryonic growth and development are regulated by IGF-I signalling by promoting the progression of cell survival and cell cycle. GH cells release IGF-I that serves as a mediator of a negative feedback system. The formation and secretion of pituitary hormones are regulated by local IGF-I in a paracrine or autocrine manner. It stimulates the proliferation of endocrine cells and prevents apoptosis. Depending on the reproductive stages, IGF-I affects the sGnRH-induced GTH subunit gene expression differently.

7.8.5 Receptors for Thyroid Hormone

Pleiotropic effects on growth, differentiation, metamorphosis and reproduction are exerted by TH through thyroid hormone receptors (TRs). TH belong to the superfamily of nuclear receptors and there are two major forms as thyroxin and tri-iodothyronine. They are iodinated derivatives of tyrosine. Thyroxin contains four iodine residues whereas tri-iodothyronine, the most potent and the major biological TH, contains only three iodine residues. The feedback of TH to the brain pituitary is due to some of these effects. In fish and amphibians, it plays an important role in metamorphosis and parr-smolt transformation on salmon. In teleost fish, there are two forms of TH, as TRα and TRβ regulate the development, growth and metabolism. Hindbrain patterning is disrupted by the overexpression of TRα1 form during embryogenesis that causes the repression of retinoic acid receptors in hox gene expression control.

7.9 The Neuroendocrinology Regulation of Fluid Intake and Fluid Balance

7.9.1 Mechanism of Fluid Exchange and Balance

A thin respiratory epithelium separates fish body fluids from the surrounding area. The osmolarity range of these environments ranges from a few to 1000 mOsm kg-1. Regulation of their body fluids autonomously of the surrounding area for their survival is necessary. Maintenance of the composition of extracellular fluid and its volume during the continuous process of osmotic gain of water from the dilute environment associated with a stable diffusion loss of major fluid ions, in particular, Na^+ and Cl^- are the major challenges for fish in fish water. For the excretion of water in large volumes of urine, the glomerular kidney filters the blood and carries out tubular reabsorption of ions and other solutes. The reabsorptive ability of the ion by the fish urinary bladder supports this process. Dietary intake and gut absorption support the balance of the ion losses (Fig. 7.4). The ion actively taken up from the environment by the cells rich in mitochondria in the gill leaflets also supplements for balancing ion loss. H^+-ATPase with synergetic action of Na/K-ATPase facilitates the active uptake (Lin et al. 2000).

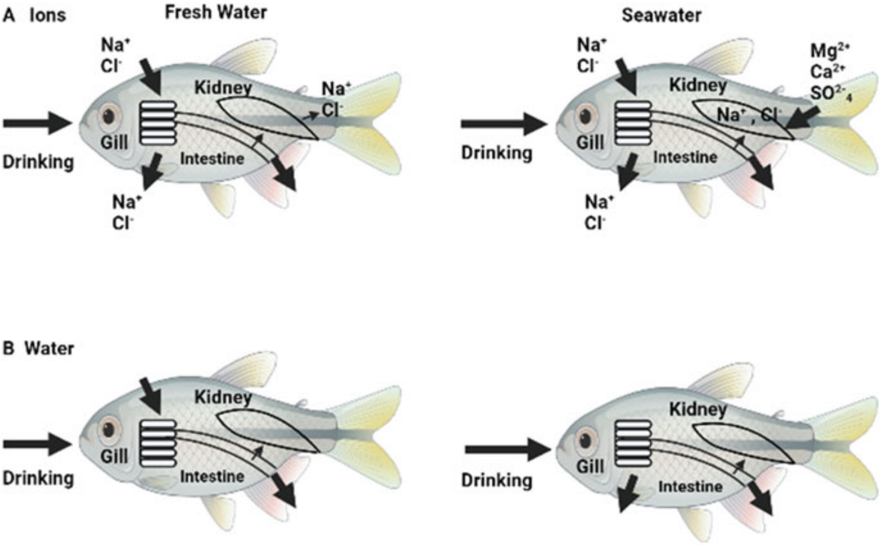

Fig. 7.4 Movement of ions (**a**) and water (**b**) between environment and body in starved teleost fish

7.9.2 Regulation of Fluid Intake

The oral intake of free water, water from the food, water released by the oxidation by cell metabolism of carbohydrates, proteins and lipids are the main sources by which animals gain body fluids. The oral water intake of terrestrial animals and fish differ greatly as fish live in water. To avoid overhydration, the freshwater teleost fish frequently drinks only a little amount of water through the gills by the osmosis process. Water must be ingested orally to compensate for the osmotic loss in marine teleost fish.

7.10 Hormonal Regulation of Drinking in Fish

7.10.1 Hormones That Induce Drinking

Neural mechanisms play an important role in the regulation of drinking habit in the fishes. The dynamic component of the renin-angiotensin system (RAS) is the best known dipsogenic hormone, Angiotensin II (ANG II) and it induces abundant water intake in all vertebrates. In euryhaline species, on moving into hypertonic waters the ANG II increases the drinking habit. In various stenohaline species that are restricted to live in either saline water or freshwater, the ANG II did not induce drinking. The water can passively enter into the oesophagus, and through the coordinated movement of muscles concerned with swallowing, the ANG II induces a burst of drinking. Therefore, in saline water fish, in constant drinking, the ANG II may not be concerned. The infusion of antiserum induces the removal of free ANG II from plasma, but the drinking rate was not suppressed by ANG II in saline water eels. The increased bradykinin in plasma or captopril treatment results in the inhibition of drinking.

Papaverine, a relaxant of the smooth muscles, may activate the endogenous RAS. In flounder, it also increases drinking rates. To induce drinking, increased circulation of ANG II acts on the target cells in the brain that lack the blood-brain barrier as shown in birds and mammals. After "decerebration" that is the removal of the complete forebrain and most of the midbrain, the ANG II was still effective, or rather more effective in the eel. Another circumventricular organ in the hindbrain, the area postrema (AP) that faces the fourth ventricle, is the most possible site of the mechanism of ANG II (Mukuda et al. 2005).

7.10.2 Hormones That Inhibit Drinking

Natriuretic Peptides (NPs) In teleost fish, the Natriuretic peptide family consists of seven members as B-type, four C-type NPs such as CNP1, CNP2, CNP3 and CNP4, atrial and ventricular NP such as atrial natriuretic peptide (ANP), BNP, and Ventricular NP (VNP). VNP is majorly produced in the heart and C-type NPs are synthesized in the brain, which lacks a C-terminal "tail" sequence extending from the intramolecular ring having common structural characteristics. In the eel, ANP and VNP are potent anti-dipsogenic hormones. The abundant drinking of saline water eels was almost terminated at 3 pmol kg min-1, and at a rate of 0.3–-3 pmol kg min-1, ANP dose-dependently reduced the drinking rate when introduced into the circulation. The infusion takes place at 3 pmol kg min-1, in which the plasma endogenous ANP concentration was increased to the level. CNP was 50 times more potent in drinking inhibition than in elasmobranch with the stimulatory effect of ANG II. In saline water eels, plasma ANG II concentration is decreased by dose-dependent ANP infusion which amplifies its anti-dipsogenic effect.

To survive in the hypotonic saline water environment, it is indispensable for teleost fish to drink saline water. As fish lives in water, overdrinking is a susceptible factor; when they are in seawater it leads to hypernatremia. In less than 1 min in feedback to the elevated Cl^- ion concentration in saline water, vigorous drinking occurs after the transfer of eels from freshwater to saline water. The excessive and acute intake of water stopped in 15 minutes and suppressed drinking was continued at a decreased rate than the constant saline water drinking rate for a few hours. An inhibitory signal, such as stomach distension, is an inhibitory signal formed after the initial robust drinking that causes such transient inhibition, whereas the inhibition time course coincides with a short-term increase in ANP concentration in plasma once after the transfer of saline water. When encountering saline water, ANP likely suppresses excessive drinking to enhance an abrupt increase in Na^+ concentration in plasma and to encourage fish to adapt to saline water. In freshwater and saline water adapted eels, there is no difference in plasma ANP concentration. To maintain plasma Na^+ concentration in saline water eels higher than in freshwater, the excessive drinking in seawater adapted eels are chronically inhibited by ANP, because ANP antiserum introduction to remove the ANP that is circulating resulted in increased plasma Na^+ concentration and cause a surge in drinking.

Ghrelin Ghrelin is a 19–28 amino acid residue long, a linear peptide with fatty acids addition that includes decanoic acid, octanoic acid and so on at the third Thr or Ser residue that has been identified in two species of elasmobranchs and 11 species of teleosts. In the rainbow trout and channel catfish, two different genes have been identified. GH secretion was stimulated when ghrelin was administrated into the periphery.

When ghrelin was injected in the eel into regions such as the brain and periphery, it was found to have potent anti-dipsogenic actions on body fluid regulation. Likewise when ghrelin was injected into the fourth ventricle of saline water eels, ghrelin is even more potent than ANP. Swallowing is inhibited by the action on the AP by ANP. In the ventricular surface, a change in the potential to cross the ependymal layer was observed when ghrelin is acylated with fatty acid. In many vertebrate species including fishes, ghrelin has stimulatory actions on GH production and orexigenic effects. During fasting and energy metabolism, the changes in plasma ghrelin concentration were measured. In plasma, ghrelin exists in active form which is acylated and the inactive form which is non-acylated. At a rate of 50 fmol $\text{mL}^{-}1$, the acylated form of ghrelin circulates in eels. Once after 6 h of the transfer of eels from freshwater to saline water, the concentration increases. The GH/insulin-like growth factor-I (IGF-I) axis is stimulated and the in vitro GH secretion is increased by homologous ghrelin in the tilapia.

Bradykinin Bradykinin is the active linear nonapeptide hormone and final product of the kallikrein-kinin system (KKS) that have strong inflammatory and cardiovascular functions. There are two kallikrein types such as tissue KKS and plasma KKS. The low molecular weight kininogen associates with the tissue kallikrein to secrete kallidin, [Lys0]-bradykinin, whereas the high molecular weight kininogen and plasma kallikrein together act to produce bradykinin.

The RAS and the KKS are closely related because they share ACE for activation and inactivation of the system, respectively. The ACE is also known as kininase II which degrades bradykinin. Bradykinin degradation is inhibited by the treatment of captopril to inhibit ACE. When administered as a bolus or introduced at a rate in a way such that the arterial pressure is unchanged, the homologous [Arg0]- bradykinin acts as a potent anti-dipsogenic hormone in the eel. Plasma ANG II concentration was increased when [Arg0]-bradykinin was injected into the circulation. Bradykinin injection increased the ACE activity that may cause an elevation in plasma ANG II concentration. In anti-dipsogenic effect, [Arg0]-bradykinin is more potent than bradykinin or [Arg0]-des-Arh9-bradykinin (Fig. 7.5).

7.10.3 Other Hormones That Regulate Drinking

Hypertensive substances are anti-dipsogenic and hypotensive substances are dipsogenic. In the eel, β-adrenergic agonist, such as histamine, acetylcholine and isoproterenol, that acts as vasodepressor aredipsogenic, while vasopressor α-adrenergic agonist, such as adrenaline or noradrenaline, oxytocin, AVT, and uropygial extract (probably urotensin II), are all anti-dipsogenic. In saline water adapted eels, the intestinal pentapeptide, a VIP and CCK depressed the drinking. In saline water eels, when injected centrally, serotonin, GABA, AVT, PRL, noradrenaline and VIP obstruct drinking along with ghrelin and ANP, while isoproterenol, acetylcholine, and substance P intensified drinking along with ANG II. When fish migrate to hyperosmotic media, in salmonids and teleosts, the cortisol and GH boost the drinking rate and cortisol and GH are important saline water adapting hormones.

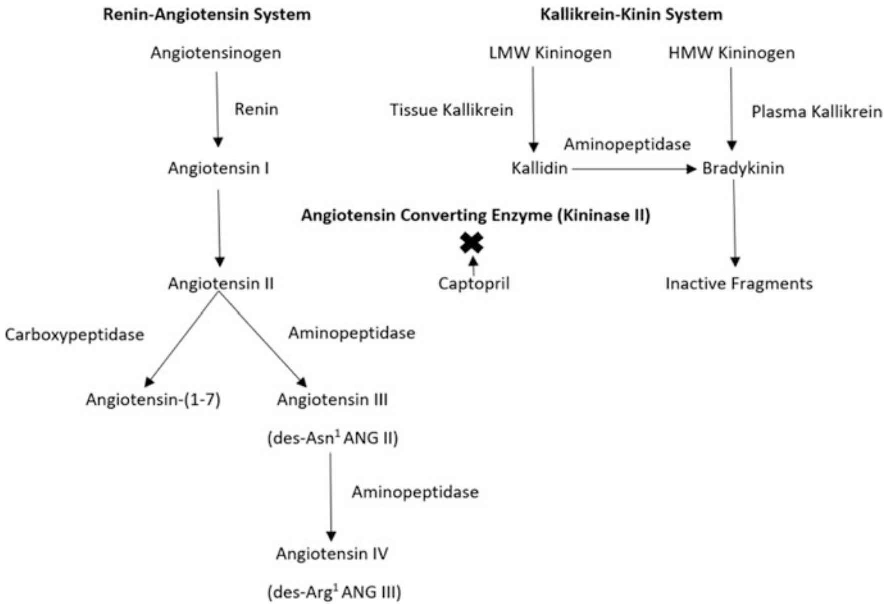

Fig. 7.5 Cascade components of Renin-Angiotensin and Kallikrein-Kinin and its relationship with the ACE. ANG I to ANG II conversion is inhibited by captopril by inhibiting the ACE, but the truncation of bradykinin into its inactive fragments are reduced by the inhibition of this enzyme. *LMW* low molecular weight, *HMW* high molecular weight

7.10.4 Neural Mechanisms of Drinking in Fish

In saline water eels, copious drinking is not influenced by the forebrain removal and the majority of the midbrain. Hindbrain regulates drinking in fish. In eels whose cerebrum is removed, ANG II acts on the hindbrain because the ANG II-induced drinking is observed to be survived. For the act of drinking, the tenth cranial nerve, the vagus nerve plays an important role as a bilateral transverse section of the vagus nerve terminate both induced drinking by ANG-II and saline water-induced drinking.

The different skeletal muscles, third brachial, fourth brachial, oesophageal sphincter, opercular, pharyngeal, the sternohyoid, different skeletal muscles and oesophageal body muscles are involved in swallowing movements that are supplied by glossopharyngeal and/or vagus nerve. The different regions of the glossopharyngeal-vagal motor complex (GVC) which elongate anterior-posteriorly through each side of the medulla oblongata give rise to each muscle. The upper oesophageal sphincter muscle (UES) is composed of skeletal muscle which functions as a gate for ingestion that is compressed by cholinergic control. Catecholamines inhibit the GVC neuronal activity which affects swallowing, indicating adrenergic innervations from the commissural nucleus of AP and/or Cajal. Water is blocked from entering into the oesophagus when boosted UES is constricted constantly by stimulatory signals through the vagus. The GVC neurons that transmit signals to the UES are inhibited when the adrenergic innervations to the GVC are activated, resulting in UES relaxation followed by water ingestion.

7.10.5 Fluid Balance Regulation

The loss and build-up of ions and water are balanced through dietary uptake and exchange with the surrounding environment; by which the body fluids are regulated in fish. To establish the body fluid osmolality and absolute volume balance of intracellular and extracellular fluid compartments, a close relation between ion balances with body water content is observed. The transporting epithelia of the gill, kidney, rectal gland and gut contributes to the fluid balance.

7.10.5.1 Fluid Balance by Arginine-Vasotocin (AVT)

In all jawed vertebrates, two neurohypophysial hormones homologous to mammalian vasopressin and oxytocin are present. AVP in mammals is replaced by AVT in all non-mammalian species (Acher 1996). AVT is concerned with the regulation of body fluid composition and volume. Rather than a reduction in blood volume, increased osmolality is also a strong stimulant for AVT secretion in the euryhaline flounder, *Platichthys flesus*. To the osmotic challenge as well as long-term adaptation, AVT contributes acute feedback. In the initial days after the fish is transferred between saline water and fresh water, the expression of hypothalamic AVT mRNA surges, plasma AVT levels are raised and pituitary AVT content reduced. In the initial hours after the fish enter into freshwater, the plasma AVT concentration falls, and when fishes are transferred to saline water, the plasma AVT concentration elevates along with the elevated plasma osmolality. Once after 2 days of fish kept in concentrated saline water (130%), the amount of the expression of AVT mRNA in plasma and AVT mRNA in the hypothalamus were notably elevated. In fish, both renal and extra-renal target tissues are involved in the AVT actions that contribute to body fluid balance. Studies in freshwater eels reported an antidiuresis or a clear fall in urine flow alongside a lower, non-pressor dose of AVT while high doses of AVT associated with a pressor response produce a diuresis.

7.10.5.2 Fluid Balance by Renin-Angiotensin System (RAS)

Throughout the vertebrates, osmoregulatory mechanisms are modulated by one of the major endocrine systems, the RAS. Gill that produces a notable amount of converting enzyme and the kidney that produces the hormone renin are involved in the regulation of RAS activity and themselves being the target tissues for the active ANG II. RAS is activated by hypotension or hypovolemia that is usually caused by salinity transfer or haemorrhage and other challenges that causes hypotension and hypovolemia. Teleost fish acclimatize the movement in between the media of contrastive salinities. The inter-renal product cortisol is essential for long-term acclimatory adjustments, including mitochondria-rich cell functions and gut transport capacity. The level of plasma cortisol and ANG II are parallelly changed when the sea beam was adapted to variable salinities, whereas in flounder, the ANG II introduction elevates the level of plasma cortisol. Captopril, which is a converting enzyme, when administrated, blocks the production of cortisol following freshwater to saline water transfer of eels.

A drop in GFR and antidiuresis is caused by the exposure of fish to dehydrating conditions. During dehydration, a direct renal effect of ANG II and RAS activation cause vasoconstriction of renal microvasculature. When there is a drastic loss in blood volume or a drop in blood pressure, the RAS is considered to contribute to blood pressure. The unique elasmobranch mineralocorticoid, 1-hydroxycorticosterone secretion is stimulated by the involvement of indirect osmoregulatory actions. In elasmobranchs and in teleosts, the presence of macula densa and juxtaglomerular cells, along with RAS is involved in the glomerular filtration rate control.

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Chapter 8
Common Bacterial Fish Diseases and Approaches on Molecular Techniques for Characterization and Early Detection of Pathogens

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Abstract Cultural and serological tests are the earliest methods for the detection of diseases. The histological studies also help for the identification of responsible pathogen causing the disease. Following these have several disadvantages, i.e. laborious and time-consuming, but over 15 years, molecular methods are supportive for early disease diagnosis. Fishes in the asymptomatic stage of the disease can be diagnosed, reducing their dependence on antibiotics. The development of antibiotic resistance among bacterial strains is yet another difficulty that needs to be addressed. At the same time, the prevention of fish diseases also improves socio-economic status among aquaculture farms. These features are essential for initiating early treatment, prevention and control of the disease. The diagnostic methods include microscopic examination of cultures bacterial cells, biochemical tests for bacteria, histopathological examination of fish tissues, and serological tests. The subsequent methods such as polymerase chain reaction (PCR), restriction enzyme

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digestion, probe hybridization, enzyme-linked immunosorbent assay (ELISA), in-situ hybridization, and microarray are applied in disease diagnosis. So far, molecular methods had led to an improved understanding of genetic characteristics, virulence factor, pathogenicity and host-pathogen interactions. This chapter is the comparative analysis of molecular methods for detecting bacterial fish disease. Such advancements in the techniques gain the scope for novel vaccine development and antibiotics for treatment in the prevention of diseases caused by bacterial fish pathogens.

Keywords Bacteria, Fish disease · Culture methods · Serological test · Molecular diagnosis

8.1 Introduction

Aquaculture is cultivating and harvesting fishes, considered as a part of agriculture worldwide. Despite the growing global population, aquaculture provides food to satisfy the hunger of humankind. It is considered as most important in improving the socio-economic status of fish farmers. Improving food production is another objective of aquaculture to compensate for the hunger of human society. Under normal environmental conditions, the surface and internal parts of the fish is loaded with numerous bacteria. There exists a high probability for the existence of normal flora, commensals and opportunistic pathogens. Fishes are susceptible to diseases by several microorganisms like bacteria, fungi, virus and parasites. Next to parasitic diseases, bacterial diseases are the second most common in aquaculture fishes. The poor water quality and poor nutrition for the fishes are the main determining factors. A diverse range of bacteria belonging to the following genus *Vibrio*, *Aeromonas*, *Flavobacterium*, *Edwardsiella*, *Yersinia*, *Renibacterium*, *Streptococcus*, *Lactococcus* and *Mycobacteria* are responsible for diseases (Sudheesh et al. 2012). Early signs of fish infection show spots, lumps, missing scales and frayed fins. The most common behaviour changes are inappropriate buoyancy, listing, and loss of appetite or increased respiratory effort. The infected fish may show some abnormalities and symptoms such as improper feeding, cloudy eyes or ulcers subsequent irregular breathing and death.

The major fish disease is caused by the bacterial genus *Vibrio*, which is a gram-negative rod-shaped bacterium causing vibriosis. The pathogenic species of *Vibrio* are *Vibrio anguillarum*, *V. harveyi*, *V. ordalii*, *Aliivibrio salmonicida* and *V. vulnificus*. They cause haemorrhagic septicaemia and winter ulcer. The second important genus is Aeromonas, which is classified into two groups. The first group is non-motile, psychrophilic species, i.e. *Aeromonas salmonicida*. This is an obligate fish pathogen causing furunculosis. The second group is motile, mesophilic bacteria characterized as human pathogens, i.e. *Aeromonas hydrophila*.

A. hydrophila causes motile Aeromonas septicaemia (MAS), haemorrhagic septicaemia, “ulcer disease”, or “red-sore disease” generally known as septicaemia (Paniagua et al. 1990). There are over 30 species under genus Flavobacterium. The following species *Flavobacterium psychrophilum*, *F. branchiophilum*, and *F. columnare* are recognized as severe pathogens of catfish and salmonids. Genus *Edwardsiella* is a gram-negative bacterium that lives in the intestines of animals. *Edwardsiella tarda* and *E. ictaluri* are identified as important pathogens of fish; these two species are found to be closely related by sequencing. *E. tarda* is reported to cause a disease called systemic haemorrhagic septicaemia whereas *E. ictaluri* cause enteric septicaemia in catfish. *Yersinia ruckeri* is a pathogenic fish species responsible for causing enteric redmouth (ERM). *Renibacterium salmoninarum* cause slow progressive systemic bacterial kidney disease (BKD) in salmonid fishes. The list of bacterial pathogens causing diseases in fish, symptoms produced and detection methods are presented in Table 8.1.

Other microbial diseases in fishes are caused by *Aphanomyces invadans* (epizootic ulcerative syndrome); epizootic haematopoietic necrosis virus; *Gyrodactylus salaris*; HPR-deleted or HPR0 infectious salmon anaemia virus; haematopoietic necrosis virus; koi herpesvirus; red sea bream iridovirus; salmonid alphavirus; spring viraemia of carp virus and viral haemorrhagic septicaemia virus. The severity of fish diseases results in mortality and causes economic losses in aquaculture. The proper care and good hygiene are considered as the important factors reducing the disorders or diseases. In such conditions, disease in fishes can need proper detection. There exist high possibilities for transmission of infection from infected fish to other population in a fish tank. The contaminated water in the fish tank and infected fish can transmit the disease to humans. Finding out an infected fish from a population is a difficult task, requiring a large number of samples to be tested.

8.2 Methods Followed for Detecting Fish Pathogens

Before collecting the fish samples for isolation of pathogens, the fishes are observed for the appearance of symptoms, which is considered as the first step. Later, other methods that are followed includes classical and molecular methods. The classical methods are preferred due to low cost but it is laborious and time-consuming. The basic steps for the identification of bacterial pathogens infecting fishes are represented in Fig. 8.1.

8.2.1 Microscopic Methods

Microscopy is the very basic and important method used for identifying the morphological structures of bacteria after isolation. The first microscopic method followed usually is Gram staining, which gives information about the shape and size and also distinguishes it as gram-positive or gram-negative. Acid-fast staining is done to differentiate acid-fast and non-acid-fast bacteria. This test is necessary for the identification of *Mycobacterium* sp. infecting fishes. Some gram-positive

Table 8.1 List of bacterial fish pathogens causing disease in particular hosts, symptoms produced and detection methods

Bacterial pathogens	Disease	Symptoms	Host	Diagnostic method
Vibrio harveyi	Vibriosis, infectious gastroenteritis	Intestinal necrosis, anaemia, ascetic fluid, petechial haemorrhage	Shark, abalone, red drum, sea bream, sea bass, cobia, and flounder	PCR-based identification
Aliivibrio salmonicida	Haemorrhagic septicaemia; winter ulcer	Haemorrhages on the body, mouth and fins	Atlantic salmon, cod	PCR-based identification
V. anguillarum	Vibriosis	Ulcers, abdominal distension, petechial flesh rot, lethargy, appetite loss, necrosis, erythema, haemorrhage, boils, lesions and death	Salmon, rainbow trout, turbot, sea bass, cod, eel and ayu	Ribotyping, pulse field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD), repetitive element-based PCR (REP-PCR), Enterobacterial repetitive intergenic consensus (ERIC-PCR)
V. vulnificus	Vibriosis	Necrotizing soft tissue infection or primary septicaemia	Eels, tilapia	Real-time PCR
P. plecoglossicida	Pseudomoniasis	Haemorrhagic ascites	Ayu	Slide agglutination test, DNA-DNA hybridization
Pseudomonas anguilliseptica	Pseudomoniasis, winter disease	Petechial haemorrhages of the skin, peritoneum and liver	Sea bream, eel, turbot, and ayu	Slide agglutination test
Aeromonas salmonicida	Furunculosis	Furuncles, haemorrhaging, enlarged organs, erratic swimming, and lack of feeding	Salmon, trout, goldfish, koi and a variety of other fish species	PCR-based method
A. hydrophila	Motile Aeromonas septicaemia (MAS), haemorrhagic septicaemia, "ulcer disease", or "red-sore disease"—septicaemia	Swimming abnormalities, pale gills, bloat and skin ulcerations	Salmonid and nonsalmonid fish, sturgeon, tilapia, catfish, striped bass, and eel	Triplex PCR assay
F. branchiophilum	Bacterial gill disease	Loss of appetite, lethargy and no response to stimuli.	Salmonid and nonsalmonid fishes	Indirect fluorescent antibody test (IFAT), ELISA, PCR

Flavobacterium psychrophilum	Coldwater disease	Tissue erosion, jaw ulcers, inflammation and behavioural issues.	Salmonids, carp, eel, tench, perch, ayu	Fluorescent in-situ hybridization (FISH), quantitative PCR (qPCR)
F. columnare	Columnaris disease	Frayed and ragged fins	Cyprinids, salmonids, silurids, eel, and sturgeon	Real-time PCR
Edwardsiella tarda	Systemic haemorrhagic septicaemia	Loss of pigmentation, exophthalmia, opacity of the eyes, swelling of the abdominal surface, petechial haemorrhage in fin and skin, and rectal hernia	Salmon, carps, tilapia, catfish, striped bass, flounder, and yellowtail	Co-agglutination test
E. ictaluri	Enteric septicaemia	Multiple petechial haemorrhages, depigmented ulcers	Catfish and tilapia	Bacterial culture, immunofluorescence, ELISA, PCR
Yersinia ruckeri	Enteric red mouth (ERM)	Loss of appetite, lethargy, swimming abnormalities, petechial haemorrhages	Salmonids, eel, minnows, sturgeon, and crustaceans	ELISA, agglutination test, immunofluorescence antibodies tests, restriction fragment length polymorphism (RFLP), loop-mediated isothermal amplification (LAMP), ERIC-PCR, qPCR
Renibacterium salmoninarum	Bacterial kidney disease (BKD)	Exophthalmia, blebs and blisters, white or yellowish haemorrhages	Salmonids	PCR
Streptococcus iniae	Streptococcosis, meningoencephalitis in tilapia	Lethargy, dorsal rigidity, erratic swimming behaviour, death	Yellowtail, flounder, sea bass, and barramundi	IFAT, PCR
Streptococcus parauberis	Streptococcosis	Haemorrhages, cornea opacity, spinning near the water surface, erosion of the caudal fin, exophthalmos	Turbot	Real-time PCR
Streptococcus phocae	Streptococcosis	Exophthalmia, ventral petechial haemorrhages	Atlantic salmon	Biochemical methods, PFGE

(continued)

Table 8.1 (continued)

Bacterial pathogens	Disease	Symptoms	Host	Diagnostic method
Photobacterium damselae	Photobacteriosis	Creamy-white granulomatous nodules or whitish tubercules	Striped bass, yellow tail, Gilthead Sea bream, sea bass, sole,	Slide agglutination test
Piscirickettsia salmonis	Piscirickettsiosis	Anaemia, kidney necrosis, an enlarged spleen, haemorrhaging, nodules, lesions	Salmonids	Real-time PCR
Lactococcus garvieae	Lactococcosis or enterococcosis, haemorrhagic septicaemia	Anorexia, lethargy, loss of orientation, bilateral exophthalmia	Rainbow trout, sea bass, yellowtail and eel	Fluorescent antibody test, ELISA, multiplex PCR
Mycobacterium	Mycobacteriosis	Loss of weight, skin ulceration, granuloma	Sea bass, turbot, and Atlantic salmon	qPCR
Nocardia salmonicida	Nocardiosis	Nodules, multiple skin ulcers	Sockeye salmon	Culture methods, PCR

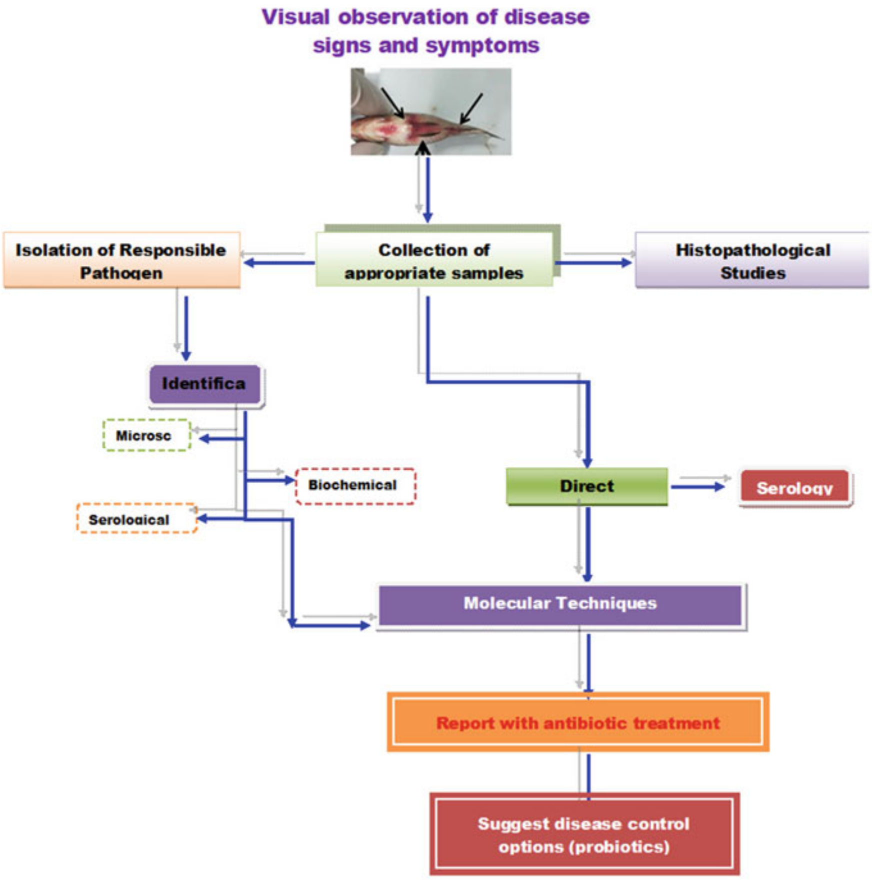

Fig. 8.1 Basic steps for identification of bacterial pathogens

bacteria produce dormant heat-resistant structures called endospores are visualized by endospore staining. Other structures of bacteria like capsule and flagella can also be identified under the light microscope. The most advanced microscopic techniques are also available to study the internal structures of bacteria. The electron microscope is one of their advanced microscopic techniques. The two types of electron microscopes are scanning electron microscope (SEM) and transmission electron microscope (TEM). Other microscopes involved are fluorescent microscope and phase-contrast microscope, which improved for better pathogen identification. Advanced microscopic techniques allow the detection of fish pathogens even from the environmental samples.

8.2.2 Culture Methods

Culture methods are most preferable and widely used for the isolation and identification of bacteria responsible for the disease. Isolation of a pathogen from the infected fish is the primary step for disease diagnosis. Identification protocol involves several staining procedures, biochemical tests, and use of selective and differential media for further confirmation (Pazos et al. 1996). The time required for a single test takes 24–48 hours. The high laborious work and time-consuming are considered as drawbacks of traditional methods. Additionally, there still exists confusion in the confirmation of bacterial species belonging to the same genus (Shewan and McMeekin 1983). Sometimes, DNA homology and protease variability are also required. Classical disease detection focuses on the identification of virulence factors.

A bacterial disease of fishes in aquaculture is largely treated with antibiotics. Most researchers are interested in studying the development and characterization of antibiotic resistance among bacteria in aquaculture. Antibiotics are chemical substances produced by one group of bacteria and act against another group of bacteria. Antibiotics such as streptomycin, amoxicillin, ciprofloxacin, penicillin and more have a cidal or static action against bacteria. Due to the illegitimate use of antibiotics, residues of antibiotics accumulate in the ponds of integrated fish farms which are rarely cleaned. Such selective pressure in an environment makes bacteria develop and spread resistance against antibiotics, which is also a potential threat to human beings. In the gut of cultured fishes, *Enterococcus* sp. and *Aeromonas* sp. are found to develop resistance against antibiotics.

8.2.3 Histological Diagnosis

The thin section of tissues is taken, stained and subjected to microscopic examination to study the morphological changes due to diseases caused by pathogens. The changes in the tissue structures represent the type of disease. The specific pathogens are also detected in tissue sections by immunohistochemical methods. Immunohistochemical methods are followed to detect viral diseases in fish. Histological methods facilitate the pathogenesis of infectious diseases causing cellular changes. This technique also clarifies the progression of non-infectious diseases.

8.2.3.1 Preparation

Aqueous fixative is used to prepare thin tissue sections. The purpose of the fixative is to maintain the originality of the cellular and tissue structures, without undergoing any further changes during preparation and staining. The fixation step should be done fast and is essential to prevent the self-enzymatic destruction of tissues after the death of fishes in a short period. The tissues are dehydrated to remove water from the tissues using alcohols and cleared. Next, the molten paraffin wax is used for embedding the tissues and cooled to toughen the wax. Before mounting on a glass microscopic slide, microtome thin sectioning is necessary. The wax is removed before staining from the tissue sections.

8.2.3.2 Staining

Haematoxylin and Eosin is the most preferable method for staining the tissues after clearing and rehydration. This method clearly distinguishes the tissue components. Gram's stain is used to differentiate the gram-positive and gram-negative bacteria based on the cell wall (peptidoglycan) and lipid composition of bacteria. For identification of fungal infection in fish tissues, the Periodic Acid-Schiff (PAS) staining specifies the presence of certain carbohydrates. The positive reaction is indicated as a pink/red colour.

8.2.4 Serological Tests

Serological tests are based on the detection of a particular antigen or antibody in the infected fish. They are mainly developed to detect the presence of bacterial or viral pathogens. Nowadays they are also used for the diagnosis of fungal and other parasitic infectious agents. The tests are based on the response of antibodies against particular antigens or the identification of pathogens. The serological tests use serum, which contains antibodies. The host immune system produces antibodies, i.e. immunoglobulins (gamma globulin proteins) to neutralize the foreign antigens. They are released into the bloodstream and also present in other body fluids. The purpose of all the antibody-based tests is the identification of pathogens in infected fish tissues. These methods have several advantages and disadvantages. The advantages are specific, cost-effective and can be used as both diagnostic and confirmatory tests. The limitations of serological tests are false-positive results.

Various tests are involved to detect the presence of various antibodies. The diagnostic procedures involving antigen-antibody tests are crucial in identifying fish diseases. Immunodiagnosis is considered important over traditional methods because it can detect the diseases in the subclinical or carrier state of infection. The use of monoclonal antibody is a great development, which increased the accuracy of results and the pathogenesis of fish diseases can be studied. These include agglutination and precipitation tests. The precipitation tests show antigen and antibody reactions; a bivalent antibody reacts with multivalent antigen. This react shows the visible precipitates when antigen and antibodies are mixed in the right proportion. The advanced serological tests are ELISA, latex agglutination test and fluorescent antibody test. An agglutination test is followed to identify unknown antigen present in the sample reacts with known antibody.

8.2.5 Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA is an important method to detect the presence of specific antibody from the sample. It involves the use of 96 microtiter plate to produce highly efficient results. The bottom of the wells is coated with antigen to detect the presence of a specific antibody. The blood samples are used for the separation of serum, in which the antibodies are present. If the antigen and primary antibody binding occur, the secondary antibody coated with enzymes like horseradish peroxidase or alkaline phosphate binds with the primary antibody. The unbound antibodies are removed by washing after the incubation period. The antigen-antibody binding is confirmed by adding a colourless substrate (chromogen) that reacts with enzymes to produce the coloured product. The amount of antibodies present in the sample is proportional to the intensity of colour formed and the optical density of each well is read in the ELISA plate reader.

8.2.6 Dot-Enzyme-Linked Immunosorbent Assay (Dot-ELISA)

Dot-Enzyme-Linked Immunosorbent Assay (Dot-ELISA) detects the presence of antigen in the fish sample. The information related to the use of ELISA for detecting fish pathogens is very little. *Edwardsiella tarda* is the most common bacterial pathogen causing mortality in fishes and is identified by Dot-ELISA. This particular technique is rapid and confirm the presence of a particular antigen of this pathogen in dead and putrefied fish. The pathogen is detected in the liver, kidney and spleen tissues. Edwardsiella ictaluri is also detected using this technique by developing a monoclonal antibody. In the 96-well microtiter plate, nitrocellulose membrane (NCM) is coated and small antibodies are immobilized onto it. The antigen is sandwiched between two antibodies that have two different epitopes of the same antigen. The antigen present in the sample first reacts with the coated antibody on NCM, later with the secondary antibody coated with enzymes like horseradish peroxidase. When the chromogenic substrate is added after incubation, colour development occurs. The intensity of colour developed in the spot is read in the ELISA reader and is proportional to the concentration of the antigen.

8.2.7 Latex Agglutination Test

The latex agglutination test allows the detection of antigens or antibodies present in the body fluids. Monospecific polyvalent antibodies are used to detect Aeromonas hydrophila by this method (Pani Prasad 1999). Usually, an antibody (or antigen) is coated on the surface of latex particles which is known as sensitized latex. When a sample containing the specific antigen (or antibody) is mixed with the milky-appearing sensitized latex, it causes visible agglutination.

8.2.8 Fluorescent Antibody Test

The fluorescent antibody technique is a qualitative test that detects the bacterial antigen by binding it coated on a microscopic slide. The antibodies are tagged with fluorescent dye to detect the bacteria. Some bacteria that are difficult to isolate using culture media can be detected by this method. There are two methods of fluorescent antibody test, namely direct and indirect method. In the direct method, if the antigen is present antibody binds with specificity and produce sensitive results. The advantage of this technique is even a single cell can be labelled and possible to visualize cells in natural environments. Sometimes, it produces false-positive results due to cross-reactivity and hence requires further confirmation of results is a disadvantage. This method is followed to detect the bacterial kidney disease caused by *Renibacterium salmoninarum* in salmonid fish (Sanders and Barros 1986).

8.2.9 Molecular Methods

The serological tests are very specific and it is quite difficult if the pathogen levels are very low in the environmental samples. Under such conditions, molecular-based DNA detection methods are more reliable. The culturing methods are not needed for detecting the DNA from environmental samples. In addition to culturable bacteria, several non-culturable bacteria exist in the fish tanks or aquarium. The exact nutrient composition of those non-culturable bacteria is not yet determined, but still can be studied based on molecular methods.

Molecular biology methods are highly useful in the identification of cultivable and the non-cultivable pathogens. Advantages of molecular methods over culture methods include time-consuming, less laborious, high specificity and reliability (Altinok and Kurt 2003). The pathogens can be detected with high specificity and sensitivity from the samples such as water, sediments, blood and tissues of fishes using PCR methods. Fresh and frozen samples are also used for analysis.

8.2.10 Polymerase Chain Reaction

The presence of nucleic acids in trace amounts of dead microorganisms can be detected in molecular methods. PCR methods are applied to amplify the small amounts of DNA in particular regions with the help of two primers and a DNA polymerase enzyme. Once the amplification is complete, the products of PCR usually 150–3000 basepair (bp) length produced are separated in agarose gel electrophoresis for further identification (McPherson et al. 1991).

The construction of primer involves several tools because the primers should be highly specific and sensitive for the successful amplification of a DNA segment. For specific binding of primer to the DNA, the length of the primer is an important factor to be considered preventing the non-specific binding. The requirements of PCR include target DNA purified from the infected fish tissue, primers, dNTPs, DNA polymerase and a thermal cycler to produce multiple copies of target DNA. For every run of PCR in a thermal cycler, denaturation, annealing of primer and polymerization steps are taking place for DNA amplification. Recently, the PCR and in-situ hybridization methods are developed for the detection of fish pathogens. The most common pathogens of fish such as *Aeromonas hydrophila* (Cascon et al. 1996); *Aeromonas salmonicida* (Hiney et al. 1992); *Renibacterium salmoninarum* (Brown et al. 1994); *Photobacterium damselae* subspecies *P. piscicida*, formerly *Pasteurella piscicida* (Aoki et al. 1995, 1996) and *Vibrio anguillarum* (Hirono et al. 1996) are detected by molecular methods. Forward PCR, RT-PCR, one cycle, nested, reverse cross blot hybridization and quantitative PCR are the DNA-based methods currently used for the identification of pathogens. In addition to previously listed bacterial diseases, the following diseases caused by parasites (PKX) (Morris et al. 1997, 1998), viruses (infectious salmon anaemia virus), and fish rickettsia (*Piscirickettsia salmonis*; Alday-Sanz et al. 1994) are also detected. Even the species of Genus *Mycobacterium* viz., *M. marinum*, *M. fortuitum* and *M. chelonae* are identified by reverse cross blot PCR. Quantitative PCR is used in the detection of *F. psychrophilum* from the environmental samples and fish tissues.

The standardization of PCR is mandatory for the identification of specific pathogen species. The factors influencing the output of PCR include the extraction methods and primers used. The positive and negative controls are important for the interpretation of results. Many reports are available for the detection of fish pathogens by PCR-based techniques and obviously require proper validation (Hiney and Smith 1998).

8.2.11 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

RT-PCR technique uses an enzyme called reverse transcriptase to reverse transcribe mRNA sequences into complementary DNA (cDNA). cDNA is then used as target DNA and amplified using PCR. RT-PCR is used for the quantification of mRNA. There are two ways of RT-PCR, one-step PCR and two-step PCR. One-step RT-PCR kit contains RNA, primers, reverse transcriptase, DNA polymerase and buffer reagents. The reaction mix is added to the PCR, and in the first cycle, the cDNA synthesis takes place. The reverse transcriptase gets inactivated in the second cycle at the denaturation step. The cycle repeats to produce amplified PCR products.

The two-step RT-PCR has two steps. The first step is the reverse transcription and uses a non-specific primer whereas the second step is amplification by PCR and involves the use of the specific primer. RT-PCR is effective in determining the levels of gene expression helps in detecting the specific mRNA (Koo and Jaykus 2000). The RT-PCR is used to detect mRNA in very few quantities whereas for larger quantities other two methods that are commonly followed are Northern blot analysis and RNase protection assays. The RT-PCR is the most effective, sensitive and specific for the detection of fish infections. The prokaryotic DNA has highly conserved sequences of 16S rRNA which rarely undergoes mutation and resembles the characteristics of ancestors. These gene sequences serve as a marker for the molecular identification of pathogens. Such types of sequences are also present in eukaryotic microorganisms. PCR methods are followed for the identification and amplification of such specific genes. A well-designed RT-PCR produce sensitive and accurate results, which is essential for the investigation of fish diseases.

8.2.12 Multiplex PCR

Multiplex PCR is a more advanced method of PCR, in which more than one target sequence is amplified. It improves the efficiency of time, laboratory effort and cost (Williams et al. 1999). The two categories of multiplex PCR include single template PCR and multiple templates PCR. The first type uses a single template genomic DNA and several pairs of forward and reverse primers, finally amplifies the specific regions in a template. The second type uses multiple templates and primers in the same tube. The drawback of this method is the use of multiple primers may allow cross-hybridization with each other and the possibility of missing primers with other templates. The designing of primer parameters such as primer length, melting temperature and specificity determines the efficiency of PCR reactions. Successfully, it can be applied in gene deletion analysis, quantitative analysis, mutation analysis and RNA detection. By following this method, the identification of bacteria, fungi and viruses is easy. According to the type of pathogen identification, suitable PCR methods have been opted.

8.2.13 Restriction Fragment Length Polymorphism (RFLP)

Genetic mapping is done based on the presence or absence of restriction enzyme sites, which is the principle of RFLP. In addition, insertion or deletion of restriction site occurs due to mutation. The four steps of RFLP are DNA extraction, fragmentation, separation of DNA in gel electrophoresis and band visualization. The DNA is isolated and purified initially. If the DNA samples are available in fewer quantities, PCR can be applied for further amplification. Restriction enzymes or restriction endonucleases are enzymes that cleave the DNA at specific sites. There are three types of restriction enzymes, namely type I, type II and type III restriction endonucleases. Due to high specificity in recognition of short sequence of DNA and cut at particular sites, type II endonucleases are used in molecular biology and genetic engineering experiments. The recognition sites may vary from six to eight base pairs (bp). Even change in a single nucleotide leads to loss or gain of recognition site, and ultimately change the number of fragments produced. The digested DNA fragments are separated in gel electrophoresis based on size and observed for restriction patterns in RFLP. The difference in the RFLP patterns helps to differentiate one species from the other. The mutation points can be detected at a low cost. The unique restriction patterns produced is the identity of this technique. RFLP-PCR is simple and rapid. DNA isolation for RFLP analysis is a time-consuming and labour-intensive process but the amplification of DNA can be done in less time for more samples in PCR.

8.2.14 Amplified Fragment Length Polymorphism (AFLP)

It is a PCR-based technique that uses a genetic multilocus marker. It is used for amplification of a selective segment of DNA digested with restriction enzymes of the whole genome (Vos et al. 1995). After DNA restriction, sticky end fragments are ligated to the digested fragments. It involves both RFLP and PCR amplification. The banding patterns of AFLP show variations in the restriction sites or the intervening regions. In this techniques DNA fragments produced may vary from 50 to 500 per reaction, separated by Polyacrylamide Gel Electrophoresis (PAGE) and are considered as dominant markers. A short oligonucleotide primer of 18–24 base-pair length is used to anneal the target DNA. Fluorescent labelled primers are used for the detection of fragments rather than radiolabelling. This technique can be applied for prokaryotes and eukaryotes and helps to identify the closely related or genetic similarity of the same species, based on this construction of a phylogenetic tree can be made. It is also used to assess the genetic variation among the same or different species. The advantage of following this technique is to detect multiple loci, easy for genotyping, sensitive, reproducible and highly reliable. The fragments of AFLP are analysed by the automatic sequencer. AFLP is one of the most used markers in population genetics. The difficulty in obtaining a high molecular weight and pure form of DNA to run AFLP is one of the disadvantages. In addition, it is highly laborious and time-consuming. So, the isolation of the DNA step is considered more important.

8.2.15 Random Amplified Polymorphic DNA (RAPD)

The RAPD is also a PCR-based technique for identifying genetic variation. The DNA fragments are randomly amplified using short primers. This single primer serves as forward and reverse primer, i.e. arbitrary primers, and can be able to amplify fragments from 1–10 genomic sites simultaneously. The RAPD requires the use of Taq DNA thermostable polymerase, dNTPs, magnesium and reaction buffer. The method is so fast and does not require prior sequencing of DNA. The steps of RAPD involves denaturation, primer annealing and primer extension. Different size of fragments is produced from non-identical DNA samples. Huang et al. (1994) applied the RAPD method to study the fungus, Astacus astacica using plaque in crayfish. This method can be able to group many fungal pathogens, which helps to trace out the epidemiological status (Oidtmann et al. 1999). Aphanomyces is an oomycete fungus responsible for causing Epizootic Ulcerative Syndrome (EUS) leading to 100% mortality in fish, which is studied by RAPD analysis. Severe impacts are shown in fish farms and wild fish in Asia (Lilley et al. 1997). Other pathogens can be identified by RAPD. The RAPD is a useful technique; the first step is the development of specific primers or probes to study bacteria.

8.2.16 In-Situ Hybridization

In-situ hybridization is a technique that employs the use of single-stranded DNA or RNA chemical or radiolabelled probe binds with the target sequence to form complementarily in the chromosomes. It is also used to detect the nucleotide sequence in tissues or tissue sections or cells. In-situ hybridization is used for the detection of *Mycobacterium* spp., *F. psychrophilum*, *R. salmoninarum* and infectious salmon anaemia virus (ISAV). The most preferable samples for this method are fresh or frozen samples. Even fixed samples are involved in analysis using in-situ hybridization. Compared to antibody-based methods, both PCR and in-situ hybridization are efficient for the detection of the parasite PKX because the antibodies are dependent are lifecycle (Morris et al. 1998). When the pathogen levels are extremely low in the samples like environmental samples, in-situ hybridization and PCR methods are considered more suitable and efficient.

The samples are histopathological tissue sections fixed with formalin to make them intact. The initial step of in-situ hybridization is deparaffinization of the tissue section, protease treatment, post-fixation in paraformaldehyde, the addition of probes, high-temperature DNA denaturation, hybridization, washing and detection of signals. The probes are frequently radiolabelled, which allows detection of infected cells in the tissue. Intracellular parasites are also detected by following this technique. The probes are small (~300 bases) enabling them to reach the target cells by penetration.

8.2.17 Dot Blot or Slot Hybridization

DNA of the pathogen present in the clinical samples are subjected to lysis for DNA extraction; after denaturation two strands of DNA are formed. The DNA fragments are then fixed onto a nylon membrane in a dot or slot-like pattern and fixed. The single-stranded DNA fragments coated on the membrane are allowed hybridization with radiolabelled probes. The probes may be either ssRNA or ssDNA sequences that are complementary to the single-stranded target sequences. The probe is usually labelled with radioactive or non-radioactive substances, which serves as a reporter molecule. Non-hybridized probes are washed away and the positive result is the formation of hybridized duplexes. This technique is very simple since a single membrane can be reused for testing several other samples. A single specimen is sufficient for testing several organisms on the same membrane. If the probes are radiolabelled, the detection was carried out.

8.2.18 DNA Microarray

The other names for DNA microarray are DNA chip or biochip. The test samples use DNA sequences that are labelled with fluorescent dyes. DNA microarray follows various ways to detect DNA or RNA sequences. The DNA microarrays include cDNA microarrays, oligonucleotide microarrays, Bacterial Artificial Chromosome (BAC) microarrays and Single Nucleotide Polymorphism (SNP) microarrays. DNA microarrays help to measure gene activity and genotyping. The DNA sequences present in the sample binds or hybridizes to a region in the microarray is detected by the fluorescent array method and the data analysis is made with the use of the computer. A large collection of microscopic DNA spots is attached to a solid surface. Each spot contains small synthetic oligonucleotides or cDNA sequences or small PCR products in picomole (10–12 moles) quantities known as probes. The probes are designed for specific genes. The long oligoprobes are 50–70 m and the short oligomers are 25 m. Each spot will be about 100-200 μm in size and contains sufficient binding sites to sufficiently represent differences in expression patterns. If the test sample DNA have complementary sequences, form hybrids with the probe. Microarrays are coupled with PCR for species-specific polymorphism in one or more genes when the test DNA sequences are available in limited quantities. The washing step is done to remove the unbound or unhybridized DNA sequences. The intensity of fluorescence is detected using the computer.

The protein microarrays are necessary to study the protein expression and profiling of protein interactions. The tissue microarrays are followed to study the histological sections of normal and diseased tissues. The cellular responses are studied by cellular microarrays. An antibody microarray (proteomics) detects the presence of antibodies, proteins, lipids and protein expression within the cells. In carbohydrate microarrays, various oligosaccharides or polysaccharides fixed onto a solid support.

It is used to detect multiple gene expression at the same time. An additional application of microarray is to genotype multiple regions of the genome. This technology rapidly detects particular functional genes or single nucleotide polymorphism in fishes that are related to diseases or growth and speed up the drug discovery process (Zhang et al. 2009). It also helps in the identification of new genes in fishes living in natural populations, host-pathogen interaction and ecotype. Such researches are mostly studied in salmonid, zebra fishes and other Indian major carps, catfish and prawns (Roy 2017). The advantage of microarray technology is less expensive than DNA sequencing. The uses of microarrays are expression analysis, comparative genomic hybridization and mutation analysis. DNA microarray is the best option than traditional nucleic acid hybridization because the genetic sequences of fish pathogens are available in the Genbank; hence the construction of complementary oligonucleotide sequences are possible and inserted into microarray chip for the identification of the particular fish pathogen from fish populations. The most advantageous feature of microarray is its extremely short analysis time. Even during the asymptomatic disease stage, disease diagnosis is possible in the future using this technology.

8.2.19 Genome Sequencing

The size of the bacterial genomes varies from species to species and even among the strains of same species. The size ranges from 0.6 to 10 megabases (Mb). The smallest bacteria are Mycoplasma which has the genome size of about 530 kb. The sequencing of genomes helps to identify the phylogenetic relationship between responsible pathogenic species and non-pathogenic species. This helps to identify the lifecycle of pathogens interacting with hosts and targets for drug discovery can be assessed. The genomes of bacteria exist in various forms such as circular, linear chromosomes and plasmids. The genes responsible for pathogenicity, expression of virulence factors and antibiotic resistance are coded in the plasmids. Through conjugation the genes for pathogenicity may get transferred to closely related species that are generally non-pathogenic. Calculating the % G + C content and constructing a phylogenetic tree clearly states the closely relatedness between two species. The details of pathogenic bacteria species of fish that are genome sequenced were listed by Sudheesh et al. (2012).

Genome sequences of four major fish pathogenic vibrios, *V. anguillarum, V. ordalii, Aliivibrio salmonicida,* and *V. vulnificus* have been completed and published (Gulig et al. 2010; Naka et al. 2011). The whole genome sequencing of two species *E. tarda* and *E. ictaluri* was completed and published. Comparative genome analysis reveals that these two species are closely related to each other. The multilocus sequence typing recently carried out for *Y. ruckeri* was distantly related with other species belonging to same genus. Based on 16S rRNA phylogenetic analysis reveals that *R. salmoninarum* was grouped under actinomycetes subdivision, related to the taxa *Arthrobacter, Micrococcus, Cellulomonas, Jonesia,* *Promicromonospora, Stomatococcus*, and *Brevibacterium.* The morphological and biochemical taxonomic similarity exists among these organisms. The genus *Streptococci* and *Lactococcus* are gram-positive cocci shaped bacteria that cause disease in the fish and humans. The complete genome sequencing was studied for two strains *S. parauberis* and *L. garvieae,* identified as zoonotic pathogen and published (Reimundo et al. 2011; Morita et al. 2011; Nho et al. 2011). The information on responsible virulence genes were identified based on the complete genome sequencing, which helps for developing vaccines and drugs against fish diseases.

8.3 Conclusion

The fishes are easily prone to diseases when grown in aquaculture. The most common diseases are caused by bacteria and leading to severe fatality. The number of bacterial species causing infection in fishes is keeping on increasing. The scientific techniques are developed to study the pathogenicity of bacteria and they are relevant to fish diseases. Diagnostic methods are necessary for the early detection of diseases. Any technique followed for detecting pathogens should satisfy several criteria; simple, reliable, reproducibility, accuracy, specificity and cost-effective. The sequencing of the complete genomes of pathogens is allowing understanding the structure, steps to improve the diagnosis and control of pathogens. The serological methods are still in use for detection due to their simple procedure, reliability and cost-effectiveness. The results are completely based on immune response against the antigen/antibody. Sometimes, false-positive or false-negative results may occur. The molecular biology methods are highly advanced, specific and fast. Therefore, molecular biology can be a routine tool in the search for improved methods of diagnosis and control of fish pathogens and the epidemiology of infectious fish diseases.

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Chapter 9
The Different and Basic Functions of Organ Systems of Fishes

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Abstract This chapter includes the most important information regarding fish biology particularly on characteristics, classification, condition factor, reproduction, muscular system, brain, reproductive system, digestive system, integumentary system, circulatory system, fish ecology, etc. Fish biology incorporates many confined disciplines, of which the investigation of morphology is indistinguishably identified with the investigation of the life of the organisms. The different shapes and sizes are the most important features to understand the variety in these life forms and the morphological characters may be similar in species frequently identified with the changed environmental elements.

Keywords Fish biology · Fish physiology · Agnatha · Chondrichthyes · Osteichthyes · Bioacoustics

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9.1 Introduction

Fish biology includes information about the developmental characteristics and physiological requirements of fish throughout their life. Fishes play a significant role in human life, by providing a rich amount of protein, and are of important economic sustenance to various nations. Fishes are a group of digitless aquatic animals (in both freshwater and marine water) with gills and skulls. They are mainly categorized into four types: jawless fish, bony fish, cartilaginous fish (such as sharks and rays) and hagfish. It is important to recognize the population dynamics of the fishes, their growth and reproduction, the age and size at which they can offspring; the transience rates and its reasons, as well as their biological processes.

Fish biology research comprises three main themes:

1. Early life history
2. Ecophysiology
3. Growth and reproduction

It additionally provides insights into conservation biology, ecosystem functioning, population dynamics, as well as aquaculture innovation.

9.2 Fish

Fish are aquatic, craniate, gill-bearing, limbless cold-blooded vertebrate animals. They are found in both fresh and saltwater. Fishes are the first vertebrates identified with Jaws. Fishes are members of the phylum Chordata. Ichthyology is the branch of biology that deals with the study of fish. Fish have the ability to communicate through underwater environments with the help of acoustic communication. The size, shape, habits and habitats of fishes differ from each other. The general structure is given in Fig. 9.1.

9.2.1 The General Characters of Fishes are as Follows

1. The body of the fish has been divided into three parts, which includes head region, abdomen region and tail region.
2. Fishes are living in both freshwater and marine water.
3. Fishes have a bilaterally symmetrical body.
4. It has a triploblastic body derived from three embryonic cell layers.
5. Fishes are internally segmented organisms.
6. Many fishes are usually in spindle shape and few species are dorso-ventrally depressed, laterally compressed, globe-like and also snake-like.
7. Fishes are found in a variety of colours.

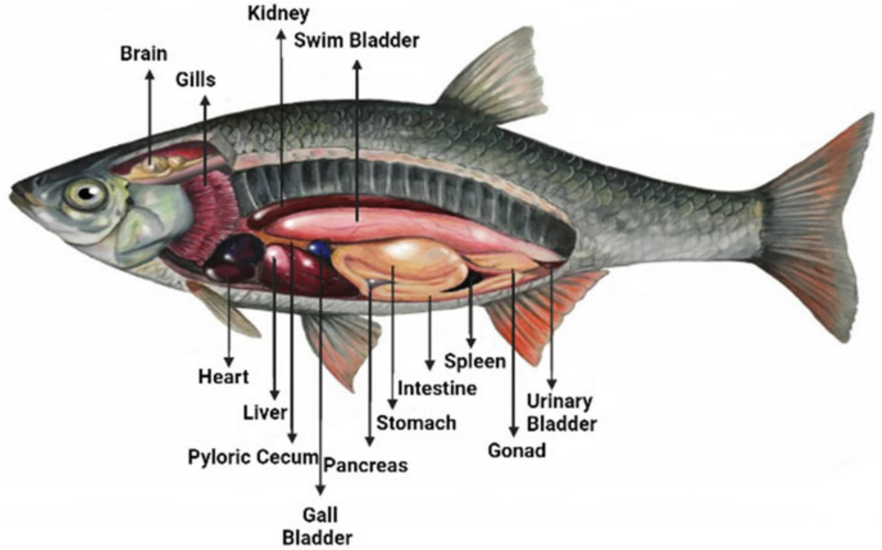

Fig. 9.1 Schematic representation of fish

8. The size of the fishes ranges from 1.25 centimetres to 20 metres in length.
9. The body of the fishes is enclosed with bony plates and scales.
10. Fishes have fins and they are used for swimming and balancing. These fins may be supported by structures known as rays which are of two types, spiny and soft rays.
11. There are two types of fins, namely paired and unpaired fins.

9.2.2 Classification

The fishes are classified into three. They are:

Agnatha—These fishes are jawless.
Chondrichthyes—These fishes are cartilaginous fish.
Osteichthyes—These are bony fish.

9.2.2.1 Agnatha

Agnatha is a jawless fish.

Phylum: Chordate
Subphylum: Vertebrata

It consists of both cyclostomes and conodonts and ostracoderms (which are extinct) species. The oldest fossils of an agnathan were found in the Cambrian, and two groups of them, namely, lampreys and hagfish, still survive today.

Characteristic of Agnatha

- Absence of jaws.
- Absence of paired fins.
- Bony scales and skin plates are absent in the living species and these are present in the ancient species.
- Gill pouches are present.
- In the digestive system, the stomach is absent.
- They also lack the internal skeleton system.

LAMPREYS: They are found in fresh, cool, and coastal waters of all continents except in Africa. The body is eel-shaped, with scaleless, smooth skin along with well-developed caudal and dorsal fins. It contains disk bearing horny teeth that surround the mouth. They have developed eyes with a single nostril on the head. The length of adult lampreys ranges from 15 to 100 cm (6 to 39 inches).

HAGFISH: The body of the hagfish is nearly cylindrical with scaleless soft skin along with a single nostril overlying the mouth at the anterior end and a low caudal fin around the tail. A few types of these fishes have gills open to the surface through isolated pores though in others the gills open to a typical pipe, which in the end opens to the surface through a solitary pore. They have minimal eyes; it is covered by the skin. The length of the grown-up Hagfishes goes from 40 to 80 cm (around 15–30 in).

9.2.2.2 Chondrichthyes

Chondrichthyes is a type of jawed fishes, which is having a cartilaginous skeleton. These types of fishes incorporate an assorted gathering of fishes including skates, chimaeras, rays and sharks. Mostly, these are all part of marine fishes. It is remembered for the division Gnathostomata as they have jaws.

Phylum: Chordate
Subphylum: Vertebrata

Characteristics of Chondrichthyes

- Endoskeleton is made up of cartilage.
- The skin of the fish is enclosed with tiny tooth-like constructions. It is said to be placoid scales.
- The fins of the fishes are without rays.
- The mouth is ventral.
- The air bladder is absent.
- The fertilization of the egg is internal and most of the species are viviparous.

- The notochord is present throughout life.
- They are cold-blooded animals (poikilotherms) and not able to maintain the internal body temperature.
- The heart is made up of two chambers, one is auricle and the other is ventricle.

9.2.2.3 Osteichthyes

Osteichthyes is the types of jawed fishes, which is having a hard endoskeleton. These are the biggest class among vertebrates and incorporate a different gathering of marine water and freshwater hard fishes. This is a class of types remembered for the division Gnathostomata that incorporates every one of the vertebrates having jaws. These are subdivided into two groups, which includes (1) Sarcopterygii (Lobe-finned fish) and (2) Actinopterygii (Ray-finned fish).

Phylum: Chordate
Subphylum: Vertebrata

Characteristic of Osteichthyes

- Their endoskeleton is made up of bones.
- It contains numerous vertebrae and has a more or less bony skeleton.
- In the skin the mucous glands and embedded dermal scales are present.
- The fins paired are present.
- The presence of jaws.
- Gill arches support the gills and are protected by the operculum.
- The mouth is terminal.
- The skin is enclosed by bony dermal scales and is known as cycloid, ctenoid or ganoid scales.
- The heart is made up of two chambers, auricle and ventricle. In lungfishes, the structure of the heart is made of three chambers (two auricles and one ventricle).
- They are cold-blooded animals (poikilotherms) and not able to maintain the internal body temperature.

9.3 Bioacoustics of Fish

Bioacoustics is a field of science that incorporates both science and acoustics. Acoustics is a part of physical science that deals with the investigation of mechanical waves in solids, fluids and gases including subjects like sound vibration, infrasound and ultrasound. Bioacoustics incorporates sound creation, hearing and correspondence utilizing sound (Popper and Hawkins 2019).

Fishes utilize an assortment of tangible frameworks to detect their surroundings and impact. Of the different faculties, hearing assumes an especially significant part for fishes in giving data, frequently from a great distance, from all around these creatures. This data is in every one of the three spatial measurements, regularly conquering the impediments of different faculties like vision, contact, smell and taste. The sound is utilized for communication between the fishes for mating conduct, and also for the identification of prey and predators, direction and movement as well as living space choice. Consequently, something that meddles through the capacity of a fish for identifying and reacting to organically important sounds may diminish the endurance and wellness of people and populations (Moulton 1963; Popper and Hawkins 2019).

Acoustic communication in fish includes the transmission of acoustic signs starting with one individual of animal categories then onto the next. The creation of sounds as a mode for communication among fish is regularly utilized with regard to mating behaviour, feeding and aggression. The sounds radiated by fish can change contingent upon the species and upgrade included. They can create either stridulatory sound by moving parts of the skeletal framework or can deliver non-stridulatory sounds by controlling particular organs, for example, the swim bladder (Parker 1902; Popper and Hawkins 2019).

All fish species have inner ears for the detection of sound. The ear empowers the fishes to adjust within the three-dimensional space. This detects the movement and the capacity of the vestibular region of the internal brain and ear. The hearing includes the internal ear auxiliary structure, for example, the swim bladder. Recognition of the movement of water is one of the important capacities of the lateral line. The production of sound includes a wide variety of structures (Popper and Hawkins 2019).

9.4 The Integumentary System

The integumentary framework comprises the outermost layers called skin and its subsidiaries. It frames the protective covering of the body and plays various significant capacities in the fishes. It additionally gives protection from injuries to the body and diseases and helps in respiratory, excretory and osmoregulatory activities. The skin subsidiaries play a significant role in the metabolic pathways of fishes. In a portion of the fish species, uncommon designs like the poisonous glands, electric organs, and phosphorescent organs are additionally integumentary subsidiaries.

9.4.1 Organization of Skin

The skin of the fish is made up of two layers, an internal dermis and an outer epidermis. The epidermis involves a couple of covering of praised separated epithelium. The epidermis has an ectodermal beginning. Important layers contain columnar cells that shape the layer germinatum in which the cells are dividing by mitotic process to restore the destroyed cells. The shallow layer of dead cells known as stratum corneum which is found in the terrestrial vertebrates is not available in fishes.

The dermis is mesodermal in beginning and involves connective tissue, veins, nerves and cutaneous receptors, colour cells. The strands of connective tissue are nearly corresponding to the surface. The cells associated with colour (pigment cells) is identified in the external layer of the dermis. The thin upper layer of the connective tissue is said to be layer spongeosum and the thicker lower layer is known as layer compactum. A lot of cylindrical mucous cells are dispersed along with the epidermal cells and it is also extending up to the dermis. These cells are releasing a glycoprotein called mucin and it blends with the water to make slimy thick mucus that may cover the entire body. The mucous cells are originated from the layer germinatum. The shape, size and number of mucous cells may differ in various kinds of fishes.

9.4.1.1 Functions

1. At the time of swimming, mucous coat skin helps the fish in reducing the body friction in the water.
2. These shield and protect the body from the attack of parasites, growth, microbes and different microorganisms.
3. The body is protected by denticles, plates or scales (Exoskeleton) which are important derivatives of the skin.
4. It aids in osmoregulation and repairs surface wounds.
5. The skin is capable of absorbing nutrients which is in dissolved form in the water.
6. It has the capability to cure skin injuries.
7. Due to the high vascular dermis skin, they act as the respiratory organ in climbing perch and eel.
8. In certain species the glandular cells alter into poisonous organs and it has been utilized for defence and protection.
9. Electric organs (accessory organs) found in fish are also skin derivatives that are effective in defence.
10. Various colour patterns present in the dermis is due to the presence of different chromatophores.

9.4.2 Scales of Fishes

The scales of the fish are formed from derivatives of the skin that protect and cover the body surface. The majority of fishes are covered with exoskeleton made of scales, which are absent in some like catfishes. Acipenser and Polyodon species are found to have plates or scales on the limited regions of the body.

9.4.2.1 Classification of Scales

The scales are classified into four types based on their structure, which include placoid cycloid, cosmoid, ctenoid (bony ridge scales) cycloid and ganoid (rhomboid). Placoid scales are characteristics of Chondrichthyes; in higher teleosts ctenoid scales are present; ganoid scales are confined to bony fishes. Growth rate and age are determined with the help of ctenoid and cycloid.

Cosmoid Scale

Crossopterygii and Dipnoi fishes are found with cosmoid scales. Vitrodentine (external layer) is enamel-like and thin. The middle layer is composed of cosmine which is tough, non-cellular and made up of dentine-like substance and also contains many numbers of tubules and expanding chambers. Isopedine is a vascularized bony substance that forms the internal layer. Isopedine material is helpful in scale development and it is added from below. Alive fishes do not have cosmoid scales and meagre cycloid scales are found in living Dipnoi.

Ganoid Scale

Ganoid scales are found in primitive actinopterygians and are known as ganoid fishes. Ganoid scales have different structures and various forms. The external layer is made of ganoine which is inorganic and enamel-like. Different expanding tubules and cosmine make the middle layer. The deepest layer is made of lamella bone and it is thick. The inclusion of new layers in the upper and lower layer develops the scale formation.

The center, cosmine layer is lost in this type and the rigidity or thickness is diminished. Well-developed ganoid scales are present in the holostean and chondrostean fishes. Ganoid scales are larger, isolated and bony scutes that are present in Acipenser fishes and are available at most prominent places in five longitudinal rows. The scales are clean, harder and rhomboid plates fitting edge and these lines form a shield in Lepisosteus. The thickness and rigidity are diminished and the centercosmine layer is lost. The ganoin layer is not present in scales of Amia; they are slender and are analogues of cycloid scales.

Placoid Scale

The placoid scales are trademark highlights in sharks. They are composed of flat trident spine and rhomboidal basal plate. The basal plate is free-formed and made of material similar to the bone called calcified trabeculae and having a hole in the middle. They are permanently fixed in the compactum layer of the dermis using

Sharpey's fibres (connective tissue filaments). The spine to its greater extent is made of the hardest material called dentine and these are covered with vitrodentine (hard layer). There are different little plates found on the outside of the spine that are orchestrated in transverse rows.

The spine goes through the epidermis and laxum layer and part of it is projected into water. The body scales are arranged in reverse in spines and clasper scales are arranged forward. In the spine, the basal plate has a hole that prompts a mash cavity. The mash has a nerve fibre, scleroblasts (supply route), dentine-forming odontoblasts and some alveolar tissue. These scales are made with the mesodermal layer cells of the dermis. The placoid scales are correlative to teeth present in vertebrates and dentine nature.

Ctenoid and Cycloid Scales

The ctenoid and cycloid scales may also be called bony ridge scales. Most of the teleostean fishes have this type of scales. Bony ridge scales are flexible, thin and transparent structures because of the void of the first and middle layer found in other types of scales. These scales exhibit ridges alternating by grooves, which may usually form concentric rings. Locus develops first and it is the central part. The development and origin of ctenoid and cycloid scales are comparable. Cycloid scales are approximately round and thick in the centre and thin at the margin. They produce a protective cover and also projects in an imprecating way. The anterior area is found surround by the skin. Chromatophores are sometimes seen at an uncovered portion of the scales. These are established in teleostean fishes like Cirrhina, Barbus, Labeo, Catla, etc.

The ctenoid scales are circular and have more or less secreted free edge which helps in distinguishing them from cycloid. The fishes found with spiny fins have ctenoid scales. Intermediate forms between the ctenoid and cycloid are present in various parts of the body. Perciformes fishes including Perca, Anabas and Gobius commonly have ctenoid scales. The ctenoid and cycloid scales are developed by the multiplication of cells in the form of dermal papilla. In scale formation, the epidermis doesn't have a role. Generally, the scale is first formed in the caudal peduncle and develop in the other parts of the body. The ridges are deposited after the papilla secretes the central part.

9.5 The Muscular System

The locomotion in bony fishes is aided by its muscular system. The fish muscles are made up of myotomes that are made up of myosepta and myomeres. Its purpose is to facilitate movement by the contraction of wave-like or sinusoidal movements and oscillation of the segmented muscles. The myomeres are the segmented muscles that are separated from each other by the myoseptum. The myotome is the bundle of

Table 9.1 Comparison of red and white muscles

	Red	White
Capillary bed	Extensive	Sparse
Myoglobin	Extensive	Sparse
Fibre density	Sparse	Extensive
Muscle mass	Sparse	Extensive
Rate of fatigue	Slow	Fast
Glycogen	Sparse	Sparse
Metabolic emphasis	Aerobic	Anaerobic
Muscle performance	Efficiency	Power
Swimming type	Slow cruising	Burst swimming
Car analogue	High gear	Low gear

Source: Rhodes University Ichthyology Department.

myomeres separated by the myoseptum. The horizontal and vertical midline of the fish body has connective tissues called septa that separate the muscles to right and left regions and dorsal and ventral regions, respectively. Epaxial muscles are the muscles of the upper half of the fish body, whereas hypaxial is the muscles of the lower half (Altringham 1994). Two main types of muscles in fishes for locomotion are red and white muscles. Red muscle is specialized for slow speed sustained swimming and white muscle facilitates short bursts of speed. The colours of these muscles are related to the amount of haemoglobin present in the muscles (Altringham and Ellerby 1999) (Table 9.1).

Red muscle fibres or slow muscles function for sustained and energy-efficient swimming that are confined to narrow strips along the lateral line of the fish body. They form a thin lateral superficial sheet immediately under the skin. It has plenty amount of haemoglobin present in the muscles. Red muscle diameter is smaller (24–45 μm) and constitutes less than 10% of the total fish body musculature with a high capillary supply. Red muscles contain high amounts of glycogen stores, mitochondria and lipid droplets with a high myoglobin concentration and aerobic metabolism (Altringham and Johnston 1990).

White muscle fibres or fast muscles have reduced blood flow causing reduced oxygen availability. White fibres make up the underlying mass of the myotome. They have thicker diameters than the red muscles (50–100 μm) with fewer capillaries. It has a little amount of haemoglobin present in the muscles. The metabolism is anaerobic (glycogen to lactate conversion), therefore producing 2.7 times greater tension than that of red muscles. They are energetically waste; therefore they are most efficient in short quick bursts of movement (escape response and prey capture). Due to fewer mitochondria, lipid droplets and less myoglobin concentration, they are not efficient for sustained swimming. The main edible part of the fish myotome is composed of white muscle fibres. Between families and strains of fishes, the quantity of the muscle fibres varies. These are all influenced by several environmental causes including light and temperature regimes, exercise and diet (Altringham and Shadwick 2001).

9.6 The Brain and Nervous System

In association with the ventricular surface region of the neural tube, the neurons are produced which prompts brain development in the vertebrates. In some cases, neurons are generated in the neighbouring sub-ventricular zone. Cell bodies move out of the ventricular region towards various directions that outcome in the arrangement of one or the other cortex or nuclei that are essential to separate utilitarian reasons. A cortex consists of multiple distinct numbers of neurons that present in the different layers and hence receive afferent connections to provide efferent projections. The receptive part of a few or all neurons of a layer and their dendrites expand to another population territory that reads the input arriving there (Butler 2000).

A nucleus is a group of neurons that is located deep inside the cerebral hemispheres and brainstem of the central nervous system. The receptive parts are limited in the border of the nucleus. Local, internal connectivity is present between the neurons of both cortexes as well as nuclei via axons of their local circuit neurons. They are long, either cortical or nuclear in structure, efferent projections to another group of neuron cells generally inhibitor that is away some distance. Across all vertebrates and fishes, several nuclei are present which migrates to different degrees then finally develop in size. Different aggregates and varieties that separates into numerous distinctive synapse and neuromodulator aggregates, happen by the variety in morphological highlights of dendritic of different neurons. The other factors that contribute to the variability are the number of efferent and afferent nuclei and cortical areas at distinct regions along the neuraxis. Alar plate-derived structure development contributes to most of the variation, while the basal plate-derived structure has only a lesser degree of variation. Alar plate derivatives give rise to cortical nuclei (Butler and Hodos 2005).

The fish brain is divided into five parts from the anterior to posterior region. The telencephalon or forebrain is the anterior region of the brain. The primary functions of the fishes are related to the receptive and passage of olfactory stimuli. Cranial nerve I or the olfactory nerve connects from the nostrils to the olfactory lobe situated in the brain. The size of the olfactory lobe is large in lampreys and hagfishes, moderately large in catfishes and huge in hammerheads (Sphyrnidae) that depend heavily on odours when foraging (Ebbesson 1980).

In between the forebrain and the midbrain lies the diencephalon or tween brain. It correlates the incoming and the outgoing messages that regard homeostasis and the endocrine system. A hollow, vascularized, invaginated pineal body is located on the dorsal side of the diencephalon connected by a narrow hollow stem. The neurosensory cells are present in the pineal that resembles the cone cells in the retina. It is light-sensitive in some fishes that involve circadian and seasonal clock dynamics and colour change. The light sensitivity helps the fishes for navigation in the migrations in between oceans. It also helps fishes to change their colour matching the background (Huesa et al. 2009). The midbrain or mesencephalon is the site of vision. Impulses from the eyes reach the brain by the cranial nerve II or optic nerve. The midbrain is the region of correlation centre of incoming messages from sensory receptors (Nieuwenhuys et al. 1998).

The posterior portion of the brain and the anterior portion of the spinal cord consist of the brainstem, medulla oblongata or the myelencephalon. It is the supply centre for the entire sensory system except for the smell (Cranial nerve I) and vision (Cranial nerve II). It also contains the respiratory and osmoregulatory centre in bony fishes and controls somatic and visceral functions (Wullimann and Vernier 2007).

9.6.1 The Peripheral Nervous System

The vertebrate cranial nerves are similar to those of the fishes. There are ten cranial nerves in fishes. The cranial nerve I is the olfactory nerve which is the sensory nerve that connects the olfactory bulb with the olfactory lobes. The second cranial nerve is related to the optic nerve that connects the portion of the retina with the optic lobes. The third cranial nerve is related to the oculomotor, the fifth cranial nerve is associated with trochlear and the sixth cranial nerve (abducens) is the somatic motor nerves that are supplied to six striated muscles in the eye. The sixth cranial nerve innervates the external rectus, the fourth cranial nerve, the superior oblique and the third cranial nerve, the other four eye muscles. The parts of the lateral line system are innervated by the sixth and tenth cranial nerves. The fifth cranial nerve, the trigeminal is the mixed somatic sensory that serves the anterior region of the head. The seventh cranial nerve (facial) and the eighth cranial nerve (acoustic) joins together to form the acousticofacialis nerve that is further divided into four groups of mixed nerves. This nerve serves the branchial and temporal regions of the head. The ninth mixed nerve glossopharyngeal, provides the gill region that frequently fuses with the ninth cranial nerve, vagus which is connected to the viscera as well as the body lateral line (Bertrand et al. 2003).

9.7 The Circulatory System

Fish have a closed type of blood circulatory system. The circulatory system is involved in regulating the metabolism of food, preserving repairing and destroying the pathogens causing infection. Except for the lungfish, the circulation of blood from the body of the fish to the gills is accomplished by single circulation. In a single circulation, the heart exchanges the blood with carbon dioxide-rich blood and a low concentration of oxygen (Bushnell et al. 1992).

Teleost contains blood volume ranging from 1.5% to 3% of total body weight. The fish blood consists of plasma and various other cells such as leukocytes and erythrocytes. The plasma contains ions such as Na^+, Ca^{2+}, and K^+ and some micro-organic molecules such as fatty acids and urea that is dissolved in water. The spleen, the Leydig organ and the epigonal organ are the organs in sharks and rays where the blood cells are produced. In teleost fish, the spleen and the kidneys are the organs that produce the blood cells. Erythrocytes collect oxygen at the gills and transport the oxygen into different sites of the body and then receive carbon dioxide from the same cells which they release to the external water through the gills (Farrell and Jones 1992).

The heart is located below the pharynx and behind the gills that have four chambers located one behind the others. The four chambers of the fish's heart are atrium, sinus venosus, ventricles and bulbus arteriosus. Sinus venosus is the starter blood collecting chamber that is filled by two significant veins in teleosts, for example, hepatic veins and the left and right parts of the Cuvierian ducts that gathers the blood from the left and right matched lateral veins, the substandard throats, the anterior cardinals and the posterior cardinals. The blood from the sinus venous flows into the atrium, the largest chamber in the fish heart. The blood is pushed into the ventricle by a weak muscular contraction of the atrium. The ventricle is almost as large as the atrium which is the only muscular chamber that drives the blood throughout the fish body by its contraction. Bulbus arteriosus is the last chamber of the fish heart in teleosts (Fishman and Richards 1982). In elasmobranchs, it is cornus arteriosus. They are elastic and reduce the pressure of the blood that leaves the ventricles to give a constant even flow. The difference between bulbus arteriosus and cornus arteriosus is that the bulbus arteriosus of bony fish contains no valves, while the cornus arteriosus of rays and sharks contains valves (Fishman and Richards 1982).

9.8 The Digestive System of Fish

The fish digestive system includes the digestive tract and its associated glands that help for digestion. The process of nutrition in the fish is concerned with the ingestion, processing and retention of food and with the elimination of the undigested foods and wastes. The additional extra organs which include the oral organs, teeth, tongue, liver, pancreas and nerve bladder are additionally connected with this system. The gut is related to a nutritious waterway and involves three important parts, namely, the front gut, mid-gut and rear gut. Each of the parts goes through additional separation to incorporate a few fragments of the gut. Foregut separates to incorporate the parts that starts with mouth, followed by the buccal cavity, pharynx, throat, and stomach. The mid-gut separates into the small digestive tract. The hindgut separates into the internal organ and the terminal aperture.

9.8.1 The Digestive Channel

Mouth The mouth is a cut like structure in fishes. The mouth is surrounded by the lips.

Buccal Cavity and Pharynx The buccal cavity and pharynx are not clearly marked off from each other. The pharynx is largely utilized for respiration purpose than for digestive action. The buccal cavity consists of the tongue and teeth. The teeth of vertebrates, as a rule, might be isolated into two principal types, which include the epidermal teeth and the dermal teeth. The earlier types are formed from the stratum corneum of the epidermis and limit to only the buccal funnel and the tongue of agnathans. The second type is formed from the dermis and is a remnant of dermal armour. In chondrichthyes the teeth are scattered throughout the roof of the buccal cavity and it is found to attach with cartilaginous jaws with the assistance of the connective tissue. The osteichthyes fishes teeth might be separated into three kinds, based on their situation in the oral hole. The overall type of teeth changes as indicated by taking care of propensities for the fishes. These might be pointed, round, bent, knife formed.

Tongue The tongue begins as a folded structure from the base of buccal despondency. This comes up short on all muscles, yet is upheld by a hyoid curve that stretches out through it. Teeth, tactile receptors, and little papillae are openly noted on this organ known as the tongue.

Oesophagus The pharynx opens into the throat and the last it opens into the stomach. These three structures, the pharynx, throat and stomach are not obviously separated from each other with the exception of histologically. Throat ordinarily bears longitudinal overlay to allow a more noteworthy distensibility and its mucosal covering involves to a great extent the squamous cells.

Stomach The stomach is an important organ for storing and maceration process of food substances. The true stomach should consist of an internal lining that contains the gastric organs. The shape of the stomach varies as per the convenience of space in the body cavities of several species of the fishes. The stomach is normally separated into a board anterior portion, which is very near to the heart, hence also called the cardiovascular stomach. These back into a smaller portion known as the pyloric stomach.

Intestine The intestine is the major part of digestive system following the stomach. The intestine is subdivided into two major portions, the anterior portion is a long but the narrow portion of the intestine is called the small intestine, which is lying immediately following the stomach. It receives ducts from the pancreas as well as the liver and is known as the duodenum. The remaining parts are known as the ileum.

Pyloric Caeca Most of the fishes are having pyloric caeca, arising from the mid-gut region, which is found just after the pylorus. This may be visually impaired, sac-like or rounded diverticula of unsure capacities. These are varying in numbers in different fishes and it does not follow any specific arrangement among the organization.

Anus It is the last region in the gut. It has a minute opening present at the posterior region in the digestive tract.

9.9 The Reproductive System

The mode of reproduction in fish is sexual reproduction that involves egg and sperm. Large eggs are produced by the ovaries in small numbers whereas the testes produce small sperms in large numbers that evolve as motile spermatozoa. The endocrine organ that produces growth factors and sex steroid hormones in male fishes is the testes.

9.9.1 The General Structure of Teleost Testes

The fish testes consist of two bifurcations of tissues, the germinative or spermatogenic tissue and the intertubular tissue or interstitial tissue. There are two types of cells within the germinative compartment, germ cells and somatic Sertoli cells. These two cells together form the germinal epithelium. The development and survival of germ cells depend on constant intimate contact with Sertoli cells. The germ cells are established in various phases of spermatogenesis relying upon the season or the age of the males. The spermatogenic tissue forms the spermatozoa which are collected by the efferent duct. The efferent duct lies caudal to the testicles. Efferent duct participates in seminal fluid composition regulation and thereby determines the environment where the spermatozoa capacitating occurs and also is the site of sperm storage. At last, the sperm is transported through the duct system to the genital pore for fertilization (Grier 1992).

9.9.2 Anatomy of Testes, Efferent Duct and Seminal Vesicles

The testis in fish is paired, which extended organs that are connected by the fibrous connective tissue that is known as mesorchium to the dorsal body wall. It is located ventrally to the swim bladder, dorsal to the intestine inside the coelomic cavity. From the dorsal surface of each testis arises the efferent duct, which continues to the spermatic duct to form a short, unpaired division before opening towards urogenital papilla. The spermatic duct along with the efferent duct forms the testicular duct system. It is involved in nourishment, storage, secretion of the seminal fluid, reabsorption of spermatozoa, and transport of the sperm and also balances the composition of the ions present in the seminal fluid to facilitate sperm motility (Grier 1993).

9.9.3 Spermatogenesis

A very few numbers of diploid spermatogonial stem cells generate numerous highly differentiated, flagellated spermatozoa that carry a recombined haploid genome by a developmental process called spermatogenesis. This process can be divided into three stages. The first stage is the spermatogonial or the mitotic stage wherein the mitotic process of the spermatogonia produces many germ cells. Among different species, the number of spermatogonial generations varies. For example, 10–12 generations of spermatogonia were found in *Gambusia affinis*, 14 in the guppy (*Poecilia reticulata*) and 6 in *Oncorhynchus mykiss*. During the second stage which is the meiosis, the spermatogonia separate into spermatocyte which goes through two meiotic divisions.

This leads to the reshuffling of the maternal and paternal genetic information during the process of crossing over; it takes place in the first step of meiotic division and changed into a haploid genome through the second step of meiotic division. During the third stage which is the spermatogenic stage, the haploid spermatids that arose out of meiosis separate as flagellated spermatozoa. The changes in the morphological characters of the germ cells that happen during the process of spermiogenesis are almost similar among the different types of species (e.g. maximum DNA condensation, decrease in the volume of cytoplasm and its sub-cellular organelles, and also the separation of flagellum). Mostly the final structure of spermatozoa may be different in species and can give taxonomic insights. The period of meiosis and spermiogenesis differs between the ranges of 1–3 weeks in four exotic species, yet it could require 1–3 months in the survival of the species in cold waters or temperate (Matta et al. 2002).

9.9.4 Vitellogenesis in Fishes

Vitellogenesis or yolk deposition is a process when a female germ cell is engaged with the reproduction of lecithotrophic creatures during which yolk is formed when nutrients are being deposited in the oocyte. Oocyte development and yolk formation (vitellogenesis) is a hormonally controlled and directed procedure. In fish, the basically exogenous production of vitellogenin is started by gonadotropins and regulated by oestrogens. Vitellogenin is a species-specific protein secreted by hepatocytes, delivered into the circulation system and effectively utilized by developing oocytes (Babin et al. 2007).

Vitellogenesis [vitellus (Latin) means yolk and genesis (Greek) means creation) is the process of oocyte development portrayed by the accumulation and formation of the protein in egg yolk present in the ooplasm. The process of vitellogenesis is illustrated by five events: transport of vitellogenins to the developing oocyte by means of the bloodstream, hepatic production of the precursors of vitellogenin yolk that is induced by the action of oestrogen, selective uptake of vitellogenins by the oocyte through receptor-intervened endocytosis, storing the proteins in the yolk globules, platelet, or granules and transferring of vitellogenins into their yolk proteins derivatives.

The development of the ovarian follicle has been separated into previtellogenic (the primary development) and vitellogenic (the secondary development) stages; during this period the significant supplements of protein and lipid are needed for the development of larva and embryo, which are stored within the oocyte. During the process of previtellogenesis, oocytes start accumulating the neutral lipids that are stored as droplets of lipids in the ooplasm. During the process of vitellogenic development, oocytes additionally collect phospholipid-rich yolk protein precursors named vitellogenins.

Vitellogenins are secreted from the liver and transported to the ovary by the circulatory system and the same has been utilized by the oocytes and prepared into their yolk protein derivatives. At the time of completion of the vitellogenesis process, the ovaries are loaded completely with yoked oocytes, which eventually continue the maturation and ovulation process. The role of the vitellogenins-determined yolk may be significant for the oocyte development, involving up to 80–90 per cent of the dry parts of an ovulated egg in certain species variety (Cerda et al. 2007).

9.10 Fish Ecology

The ecology of the fish is the study of interactions of the fishes with the physical and living components of their surroundings. Fishes are noted to have a varied range of habitats, such as subterranean waters, temporary ponds in dry surroundings, lakes, rivers, estuaries, coral reefs and pelagic ocean waters. In the world, the highest number of living fishes occupies marine water, but the highest species densities tend to occur in freshwaters. The diversity in species densities may be associated with larger habitat difficulties and opportunities for the geographical segregation of populations in freshwater environments.

The ecology of the fish is significantly influenced by the properties of water, including the density of water, range of heat, viscosity, and miscibility as a solvent. The fishes do not have to spend large amounts of matter and energy for the development as well as the maintenance of an extensive skeleton to support the body since the water is more denser (approximately, 750 times) than the air. Mostly the fishes are having a gas-filled swim bladder which helps the fishes to maintain neutral flexibility more than a variety of water depths with a small energetic investment. The high density of water helps in the sound transmission much more capably than through air. The sound reduces gradually in the water; hence the sonic waves travel over extremely long distances. The inner ear and the acoustico-lateralis system help the fish in perceiving sound.

9.11 Growth in Fish

9.11.1 Factors Influencing Growth Rate

The growth rate is significantly variable and relies on various ecological factors like temperature, dissolved oxygen content, salinity, and ammonia content photoperiod, level of rivalry, nature of food consumed, age and condition of development of the fish. Temperature is quite possibly a main ecological variable alongside different component that impacts development rate. Consequently, ideal food consumption for good development is a temperature-dependent factor. Dissolved oxygen level relies upon temperature, and likewise is a significant factor influencing the development pace of fishes. Perhaps, the fishes are deprived of additional high-impact energy needed for the development and proliferation, if dissolved oxygen level reduces under a specific concentration. If the concentration of ammonia is high, it will hinder the development rate. Moreover, the rate of development relies upon the density of the population. Higher densities hinder the development and lower densities will in general raise it. These could be due to the dispute over the food accessible. Rivalry can be intra-specific and inter-specific.

The accessibility of the food may depend upon the influences of temperature rate and the development on basis of season. Development is quick during summers, when a lot of food is accessible, and delayed during the winters. Essentially photoperiod can influence seasonal development. Age and development are additionally significant elements. In the first few months of development until maturation fishes grow very fast in length. Afterwards, an enormous amount of energy is directed towards the development of gonads; subsequently, the pace of body development slows down. The growth rate of mature fishes is considerably lesser than the immature fishes. Mature fishes are mostly heavier per unit of length when compared to juvenile fishes, mainly due to the large size of gonads.

9.11.2 Growth Regulation

The development and growth of fishes are regulated by specific hormones, particularly the hormone secreted from the pituitary gland, and is known as growth hormone. Hypophysectomy brings about the suspension of development in different fishes, whereas administration of the mammalian growth hormone by injection resulted in a notable increase in the rate of growth. This might be because of improvement in the rate of food intake and conversion, possibly by activating the mobilization of fat stored in the body, insulin secretion and synthesis of protein. This is also accomplished by the thyroid hormone, in particular, triiodothyronine. An increase of triiodothyronine accelerates the rate of growth and the utilization of food in specific species like trout and salmon. The 11-ketotestosterone and testosterone are likewise reported to help achieve enhancement in the food effectiveness of regular carp while adding to its eating regimen.

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Chapter 10
The Use of Immunopotentiators in Aquaculture

Yue Zhao and Wen-Chao Liu

Abstract Immunopotentiator can activate the immune system, improve the specific and nonspecific immunity of aquatic animals, thereby enhancing the ability to resist infection by pathogenic microorganisms. In recent years, aquaculture diseases have occurred frequently. In order to maintain the health of fish and other aquatic animals, and increase the benefits of aquaculture production, adding growth-promoting immunopotentiator in feed can improve the immune response, promote growth, and reduce the occurrence of diseases. As a substance that effectively controls the occurrence of aquatic animal diseases, it has been widely used in aquaculture. Through its regulation and improvement, the immunity and growth performance of aquatic animals can not only reduce environmental pollution but also ensure human food safety. This article reviewed the research progress of some commonly used immunopotentiator and its biological activities, hoping to provide a valuable reference.

Keywords Immunopotentiators · Immunity · Aquatic animals

10.1 Introduction

As an important industry, aquaculture has received extensive attention from various countries all over the world. However, the emergence of aquatic animal diseases has caused serious economic losses to aquaculture production. Among them, the loss of aquaculture production caused by infectious diseases is as high as 10%. In order to avoid diseases and promote the rapid growth of aquatic animals, a large number of antibiotics or chemical drugs are used, but the disadvantages gradually become apparent. Antibiotics have been banned in many regions such as the European Union, the United States, and China. Therefore, the research on alternatives to growth-promoting antibiotics is becoming more and more popular. Among them,

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the immunopotentiator can not only effectively improve the immunity of animals, but also play a role in fighting diseases and promoting growth. β-Glucan, antibacterial peptides, plant extracts, Chinese herbal medicines, and probiotics are some of the commonly used immune enhancers. The research progress on the biological activity of the above five immune enhancers is reviewed.

10.2 β-Glucan

β-Glucan is a class of non-starch polysaccharides that are commonly found in the cell walls of bacteria, fungi, yeasts, and plants. β-Glucan can be used as an immunomodulator to participate in innate immunity and acquired immunity. β-Glucan binds to polysaccharide receptors on the surface of macrophages, neutrophils, and lymphocytes, thereby affecting cell transmission, ultimately affecting gene expression and lymphocyte function of these cells, and regulating the body's immune function, and the various functions of lymphocytes and the content and ratio of cAMP and cGMP in the cells change.

β-Glucan is different from other immune enhancers such as lipopolysaccharide and has no adverse effect on the appetite and growth of aquatic animals, and the addition of β-glucan to feed can promote the growth of aquatic animals. Adding 0.09% β-glucan to the feed of *Larimichthys crocea* can significantly improve its growth performance (Ai et al. 2007). β-Glucan is added at an amount of 1.0% –1.5%; it can significantly improve the tilapia growth performance and feed utilization (Chi et al. 2006). Dietary 250 mg/kg of β-glucan can improve digestive activity, promote nutrition metabolism, and thereby improve growth performance of *Litopenaeus vannamei* (Zhao et al. 2010).

As lower vertebrates, aquatic animals have an immature immune system and mainly rely on nonspecific immune responses to fight infections. The binding of β-glucan to the receptor activates innate immunity, and the use of β-glucan as an immunopotentiator for aquatic animals has attracted more and more attention. As an effective immunostimulant for aquatic animals, β-glucan has been extensively studied. Many research results show that β-glucan can improve the nonspecific immune function of aquatic animals. Adding 0.05% β-glucan to the feed to the fourth week can increase the phagocytic activity of blood cells of *Ietalurus punetaus*, and there are more active cells in the spleen and head kidney, indicating that β-glucan can improve the innate immune system (Sanchez et al. 2017). Other studies have shown that adding β-glucan to feed can increase the growth rate and serum antiprotease activity of gilthead sea bream, and the increase in phagocytic activity can be observed after 2 to 4 weeks of feeding (Guzman-Villanueva et al. 2014). Other studies have shown that the addition of different levels of β-glucan can observe a significant increase in the number of red blood cells, total white blood cells, lymphocytes, and monocytes of the *Trachinotus ovatus*, which indicates that its immune effect is enhanced (Do Huu et al. 2016). Ai et al. (2007) added 0.09% and 0.18% β-glucan extract into the diet of *Larimichthys crocea*, respectively, and observed that the activities of serum lysozyme, phagocytosis and respiratory burst in head kidney macrophages were increased to a certain extent in the low-dose group, and the mortality rate of *Larimichthys crocea* was decreased after being challenged by Vibrio harveyi. After adding a commercial immune enhancer whose main component is β-glucan to Scophthalmus maximus feed, it was found that the transcription level of major pro-inflammatory cytokines was reduced (Miest et al. 2016). The expressions of IL-1β and INF-γ were significantly upregulated in the gilthead sea bream fed with β-glucan for 4 weeks (Guzman-Villanueva et al. 2014). In addition, Jiang et al. (2016) demonstrated that β-glucan significantly increased the content of complement C3 and factor B in zebrafish eggs, and the embryos treated with β-glucan were more resistant to bacteria.

β-Glucan can also play an important role in intestinal epithelial defense mechanism, intestinal epithelial integrity, and intestinal flora of aquatic animals, so as to ensure their healthy growth. Schmitt et al. (2015) studied the intestinal immune mechanism of *Oncorhynchus mykiss* and found that β-glucan can increase the number of mucosal secretory cells in the epithelial barrier of the proximal small intestine, and the host defense tower is abundantly expressed in the epithelial layer. Indigestible β-glucan may induce a change in the composition of the intestinal flora, thereby indirectly affecting the immune system. Adding β-glucan to *Cyprinus carpio* feed can also regulate the microbial community in its intestinal tract (Kuhlwein et al. 2013).

Antioxidant enzymes are one of the important lines of defense for organisms to respond to oxidative stress. In addition to measuring the antioxidant capacity of the animal body, they are often closely related to the body's immune level. β-Glucan, as a polysaccharide with a wide range of immune activities, plays a certain role in improving the body's antioxidant capacity. Studies have confirmed that adding a certain amount of β-glucan to the feed of *Litopenaeus vannamei* can enhance the superoxide dismutase activity of the shrimp (Tan et al. 2013; Bai et al. 2010; Liu et al. 2005; Yang et al. 2005). In addition, research results show that adding a certain amount of β-glucan to feed can significantly increase the serum superoxide dismutase activity and total antioxidant capacity of *Lateolabrax japonicus* and can also enhance glutathione peroxidase activity and reduce serum Hydroxyl radical and malondialdehyde content (Cao et al. 2015). In summary, β-glucan has good biological activity on aquatic animals (Table 10.1).

10.3 Antimicrobial Peptides

Antibacterial peptides are mostly small peptides composed of 20–50 amino acid residues, with small relative molecular weight and strong thermal stability, and they are widely present in organisms. It has antibacterial, anti-infective, and anti-viral infection activity. Most antimicrobial peptides have cationic properties, and their positively charged residues will electrostatically attract the negative charges on the bacterial cell membrane. After the antimicrobial peptide is inserted into the bacterial

Table 10.1 Application of β-glucan in aquaculture

β-glucan	Aquatic animals	Effect	Reference
β-1,3 glucans	*Larimichthys crocea*	Growth-promoting, Improve immunity	Ai et al. (2007)
β-1,3 glucans	Tilapia	Growth-promoting, Feed efficiency	Chi et al. (2006)
β-1,3 glucans β-1,6 glucans	*Litopenaeus vannamei*	Growth-promoting, Anti-oxygen	Zhao et al. (2010) Liu et al. (2005) Yang et al. (2005) Bai et al. (2010) Tan et al. (2013)
β-1,3/1,6-glucans	*Ietalurus punetaus*	Improve immunity	Martínez et al. (2017)
β-1,3/1,6-glucans	*Sparus aurata*	Growth-promoting Improve immunity	Guzman-Villanueva et al. (2014)
β-Glucans	*Trachinotus ovatus*	Improve immunity	Do Huu et al. (2016)
β-1,3/1,6-glucans	*Scophthalmus maximus*	Improve immunity	Miest et al. (2016)
β-1,3 glucans	Zebrafish	Improve immunity	Jiang et al. (2016)
β-Glucans	*Oncorhynchus mykiss*	Improve immunity Regulate intestinal microbes	Schmitt et al. (2015)
β-1,3/1,6-D-glucans	*Cyprinus carpio*	Regulate intestinal microbes	Kuhlwein et al. (2013)
Brewer's yeast extract	*Lateolabrax japonicus*	Anti-oxygen	Cao et al. (2015)

outer membrane, hydrophobic residues will form a certain secondary structure, while the hydrophilic part will combine with the membrane lipids to form the perimeter wall of the channel and form a transmembrane ion channel, which will destroy the integrity of the bacterial membrane and lead to a large outflow of intracellular materials, and eventually lead to cell death (Wen and Gao 2013). Another method of sterilization is that the antibacterial peptides directly enter the bacterial cells, act on specific targets in the cells, and kill bacteria by interfering with the growth and metabolism of bacteria (Ge et al. 2018; Zhang et al. 2017; Dong et al. 2015a). Antimicrobial peptides can also enhance immunity by modulating the expression of certain growth factors and their receptors, activating body's signaling pathways and acting synergistically with nonspecific immunity in animals (Ting et al. 2018; Pan et al. 2012). Antimicrobial peptides can be obtained by direct extraction and purification from organisms, chemical synthesis and expression of antimicrobial peptide genes through prokaryotic or eukaryotic expression systems, and recombinant antimicrobial peptide genetic engineering products can be obtained on a large scale, because animals and plants contain fewer of antimicrobial peptides and chemical synthesis is more expensive, genetic engineering of recombinant antimicrobial peptides is currently the most commonly used method (Wu et al. 2015; Wang et al. 2010; Chiou et al. 2009).

A lot of research has been done on antimicrobial peptides in aquaculture. Studies have shown that antimicrobial peptides have inhibitory effects on a variety of common pathogens, can significantly reduce the number of pathogens, and maintain aquaculture water environment and intestines in a micro-ecological balance (Xue et al. 2017; Li et al. 2015). Adding antimicrobial peptides to the basic feed can increase the quality increase rate, feed coefficient, and growth rate of aquatic animals such as *Litopenaeus vannamei* (Hu et al. 2018; Qin et al. 2016; Chen et al. 2015; Chai et al. 2012), *Eriocheir sinensis* (Wang et al. 2011b), tilapia (Lin et al. 2015; Zhai et al. 2015; Wang and Li 2012; Huang et al. 2011; Jiang et al. 2011; Huang et al. 2009), *Cyprinidae* (Dong et al. 2017, 2015b, c; Jia et al. 2015; Lin et al. 2013), etc., and increase its fullness and improve meat quality, thereby improving its production performance. It can also improve the immune organ vitality of aquatic animals such as *Lateolabrax japonicus* (Xia et al. 2012), *Carassius auratus* (Wang et al. 2014), *Megalobrama amblycephala* (He et al. 2017), and *Epinephelus coioides* (Su et al. 2019), and improve the serum immune indicators to improve the immunity of animals by stimulating immune signal pathways, related receptor proteins and enhancing the expression of nonspecific immune factors and other nonspecific immune responses to eliminate pathogens, activate the body's immune system, and change the immune system (Pan et al. 2012).

Fish intestinal wall and intestinal contents contain a large number of bacteria-based microorganisms. The products of metabolism or synthesis of these flora under physiological conditions can be used as vitamins and other substances required for fish growth. The normal intestinal mucosal morphology and function in the intestine are very important. The destruction of the intestinal mucosal morphology and structure may cause disorders of the intestinal barrier function and imbalance of the intestinal immune system, which may lead to diseases. Antimicrobial peptides can maintain the healthy growth of fish by inhibiting the conditional pathogenic bacteria that grow abnormally in the fish intestine, and can also affect the morphological structure of the fish intestine to indirectly regulate nutrient requirements, digestion and absorption, and ultimately promote growth and development. The addition of 50 mg/kg of antimicrobial peptides can increase the number of beneficial bacteria and lactic acid bacteria in the intestine of Gifu tilapia and reduce the number of pathogenic bacteria (Zhao et al. 2016). However, when this amount is exceeded, the number of lactic acid bacteria will decrease, and the number of pathogenic *Escherichia coli* will increase. It shows that excessive antimicrobial peptides may destroy the homeostasis of the normal flora in the intestine and cause an imbalance of the intestinal flora. Certain strains in the intestine can secrete amylase, lipase, and protease. The level of digestive enzyme activity in the intestine can directly affect the digestion, absorption, and utilization of feed nutrients by fish.

10.4 Plant Extracts

Plant extracts refer to natural biologically active ingredients extracted from plants through physical, chemical, or biological methods and have certain types of natural substances. Plant extracts contain many active ingredients, which can regulate signal pathways to exert a variety of beneficial biological activities by stimulating target organs and other signal factors. Plant extracts have biological activities such as food inducement, growth promotion, immunity enhancement, anti-oxidative stress, and antibacterial activities. According to its main active ingredients, it can be divided into alkaloids, flavonoids, polysaccharides, glycosides, and volatile oils. Sanguinarine, curcumin, chlorogenic acid, and allicin have strong antibacterial effects on *Aeromonas hydrophila* (Yu et al. 2016; Wang et al. 2007, 2009). Plant extracts contain polysaccharides, alkaloids, glycosides, and terpenoids and other immunologically active substances, which can improve the lysozyme activity in the blood of fish, the phagocytic activity of white blood cells, complement activity, and other immune indicators (Alishahi et al. 2010; Ardó et al. 2008).

Studies have shown that some plant extracts contain phagotrophic active substances. For example, asafetida and dried tangerine peel have a significant effect on *Lateolabrax japonicus* (Zhang et al. 2008). Plant extracts are also rich in nutrients such as protein, amino acids, sugars, minerals, and vitamins, which can promote growth. Many studies have shown that adding plant extracts to the feed of aquatic animals can improve the growth performance. The seaweed polysaccharide extracted from seaweed plants can promote the immunity and intestinal function of banana shrimp and ultimately promote its growth (Liu et al. 2020). Adding 10 mg/kg of Chlorella polysaccharide to feed can increase the food intake, weight gain rate, and specific growth rate of mullet (Akbary and Aminikhoei 2017). The addition of 1 g/kg dandelion polysaccharide can increase the feed utilization rate of the *Trachinotus ovatus*, increase its food intake, and thus increase the final weight, weight gain rate, and specific growth rate (Tan et al. 2017). Zhou et al. (2015) showed that adding 40 mg/kg of Soy isoflavones to feed can improve the growth performance of *Trachinotus ovatus*, but when it reaches 80 mg/kg, its effect is reduced. It may be that the addition of soy isoflavones is drug-dependent. Adding allicin, *Panax quiquefolium* L., green tea, *Cinnamomum tamala* (Bauch. -Ham.) Nees et Eberm, Carum carvi L. and Saponin to feed can promote the growth of *Oreochromis niloticus* and improve feed conversion efficiency (Ahmad et al. 2011; Ahmad and Abdel-Tawwab 2011; Shalaby and Khattab 2006; Francis et al. 2005). Curcumin can significantly promote the growth of *Larimichthys crocea*, increase feed utilization, and improve its body color (Wang and Wu 2007). Chlorogenic acid can significantly increase the weight gain rate of *Trionyx sinensis, Cyprinus carpiovar Jian*, and *Ctenopharyngodon idella* (Li et al. 2014; Zhang and Wen 2012; Wen et al. 2010), rhubarb anthraquinone can improve the feed conversion efficiency of *Macrobrachium rosenbergii* (Liu et al. 2010), and caffeine can increase the feed efficiency of sea bream (Chatzifotis et al. 2008). Dandelion water extract can promote the activity of amylase and protease in the intestine of *Carassius auratus*, thereby promoting the growth (Peng and Shen 2010). Curcumin increases the activity of protease and amylase in the intestine of *Ctenopharyngodon idella* and increases its relative growth rate (Hu et al. 2003).

The immune system of aquatic animals includes nonspecific immunity and specific immunity. Nonspecific immunity is considered to be the first line of defense against invading pathogens and plays an important role in immune defense. Improving the immunity of aquatic animals is an effective way to prevent diseases. Sun et al. (2018) studies have shown that feeding pearl gentian grouper with *Panax notoginseng* saponins for 8 weeks can significantly increase its plasma complement 3(C3), complement 4 (C4), and immunoglobulin M (Ig M) content and increased the expression levels of head kidney immune-related genes interleukin 10 (IL-10), TOLL-like receptor protein (TLR3), and transforming growth factor-β (TGF-β). Feeding carp with 25 g/kg berberine root powder in the feed can significantly increase its head kidney lysozyme (LZM), C3 content, and phagocytic activity, and increase the immune-related genes interleukin 1β (IL-1β), tumor necrosis factor -α (TNF-α), LZM, and C3 mRNA expression levels (Zhou et al. 2016).

The active ingredients contained in plant extracts can scavenge free radicals in the body to reduce oxidative damage. Adding curcumin to feed can reduce the content of malondialdehyde in the serum of *Scophthalmus maximus* (Wang et al. 2016b). Chlorogenic acid can significantly increase the activity of lysozyme and superoxide dismutase (SOD) in the plasma of *Cyprinus carpiovar Jian* (Zhang and Wen 2012) and increase the activity of alkaline phosphatase in the serum of *Ctenopharyngodon idella* (Song et al. 2011). Adding 0.25% of astragalus polysaccharide to feed can significantly increase the SOD activity of *Micropterus salmoides* (Lin et al. 2017). Akbary and Aminikhoei (2017) found that the addition of *Chlorella* polysaccharides can significantly increase the total antioxidant capacity of the liver of *Mugil cephalus*, the activity of SOD, and reduce the content of malondialdehyde.

Studies have shown that plant extracts can also increase the tolerance of aquatic animals to some fungi (Baba et al. 2016). Cinnamon can increase the resistance of *Oreochromis niloticus* to *Aeromonas hydrophila* (Ahmad et al. 2011). Adding houttuynia cordata extract, lemon peel essential oil and other plant extracts to feed can increase the survival rate of aquatic animals after bacterial infection (Baba et al. 2016; Dhayanithi et al. 2015). These studies show that plant extracts have a good effect in resisting the invasion of pathogenic bacteria and are worthy of extensive promotion and research.

10.5 Chinese Herbal Medicine

Chinese herbal medicine includes Chinese medicine and herbal medicine. Chinese medicine is derived from herbal medicine, and herbal medicine is a supplement to Chinese medicine. The theory of traditional Chinese medicine believes that Chinese herbal medicines are plants, animals, minerals, and simple processed products that basically do not change their physical and chemical properties found in nature and directly used for medicinal purposes. It is characterized by low toxic and side effects, resistance to drugs, and immune promotion, which can significantly enhance the disease resistance of animals and improve the immune function of animals (Chen et al. 2014). Chinese herbal medicine also contains active ingredients such as glycosides, organic acids, polysaccharides, alkaloids, tannins, and flavonoids, which can inhibit the activity of pathogenic microorganisms in aquatic animals and promote immune function and production performance.

Some Chinese herbal medicines have a fragrance, which can improve the palatability of the feed after being mixed with the feed. It can also increase the palatability of the feed by stimulating the taste buds of fish, and has the effect of enhancing appetite. Adding 0.2% to the feed can significantly increase the carp's food intake (Guo et al. 2005). Studies have shown that adding traditional Chinese medicine containing berberine and kaempferol to feed can significantly promote the food intake of *Acipenser schrenckii* (Zhu et al. 2010). It has been reported that feed additives composed of 2% *Scutellaria baicalensis* Georgi and *Poria cocos* can increase the growth rate of *Sciaenops ocellatus* (Ma et al. 2009). Studies have shown that adding a compound preparation of *Houttuynia cordata*, honeysuckle, licorice, and *Poria cocos* to feed can improve the growth of *Ctenopharyngodon idella* (Tan et al. 2015).

Many Chinese herbal medicines, as natural antibacterial and fungicides, can significantly enhance the immunity and disease resistance of animals (Gong et al. 2014; Kong et al. 2006), and can also increase the specific and nonspecific immunity of aquatic animals, greatly reducing the prevalence of aquatic animals. Adding plantain and *Houttuynia cordata* can increase the nonspecific immune index of *Rachycentron canadum* (Wu et al. 2016), and adding berberine can significantly increase the acid phosphatase, lysozyme activity and C3, C4 content of *Megalobrama amblycephala* (Chen et al. 2016). Studies have found that using *Haloxylon ammodendron* as a feed additive can significantly increase the immune indexes of carp such as immunoglobulin, lysozyme, protease, and complement activity (Hoseinifar et al. 2016). Angelica polysaccharides can increase the number of white blood cells in the blood of *Epinephelus* spp. and enhance the phagocytosis of white blood cells (Wang et al. 2011a). Feeding tilapia with guava leaf extract can improve nonspecific cellular immunity and humoral immunity (Gobi et al. 2016). Some Chinese herbal medicines have a certain killing or inhibitory effect on pathogenic bacteria. Astragalus and honeysuckle added to the feed alone or together can significantly increase the phagocytic activity of *Oreochromis niloticus* blood cells. If two Chinese herbal additives are used alone or in combination, the infection can be reduced mortality after *Aeromonas hydrophila* (Ardó et al. 2008). The use of guava leaf extract can significantly reduce the mortality of tilapia infected with *Aeromonas hypophila* (Gobi et al. 2016). The use of a traditional Chinese medicine mixture consisting of astragalus, angelica, hawthorn, licorice root, and honeysuckle can reduce the mortality of *Oreochromis niloticus* infected with *Aeromonas hydrophila* (Tang et al. 2014). As an additive, Galla Chinensis can effectively prevent bacterial sepsis in carp (Jiang and Zheng 2006). *Centella asiatica* extract can significantly improve the antibacterial activity of *Oreochromis niloticus* against

Flavobacterium columnare (Rattanachaikunsopon and Phumkhachorn 2010). The essential oil or water extract of *Cinnamomum cinnamomea* leaves can significantly inhibit the Gram-positive and -negative bacteria of *Litopenaeus vannamei.* The water extract of *Cinnamomum cinnamomea* can reduce the sensitivity of *Litopenaeus vannamei* to *Vibrio alginolyticus* (Yeh et al. 2009). Injection of toona sinensis extract can significantly increase the phenoloxidase activity of *Litopenaeus vannamei* and effectively reduce the mortality rate after infection with *Vibrio alginolyticus* (Hsieh et al. 2008). Based on these effective biological activities, Chinese herbal medicine may become a good substitute for antibiotics.

10.6 Probiotics

Probiotics are a class of active microorganisms that are beneficial to the host. They are the general term for beneficial microorganisms that colonize the intestine and reproductive system, improve the host's microecological balance, and exert beneficial effects on the intestinal tract (Chen 2016). Probiotics can adhere to, colonize, and multiply on the intestinal mucosa, and compete with pathogenic bacteria for adhesion sites and nutrients, so as to achieve the effect of inhibiting pathogens (Ringø et al. 2007). Most probiotics can also produce acidic substances, thereby inhibiting the growth of pathogenic bacteria.

Probiotics can affect intestinal permeability and enteric nervous system, and the combination of probiotics and probiotics has many advantages to promote the healthy development of aquaculture, it can enhance the metabolism of carbohydrates, proteins, minerals, etc., and enhance aquatic products. Immunity of aquatic production ultimately improves (Luan et al. 2017; Ushakova et al. 2015). Studies have found that adding compound probiotic lactic acid spore M3 to conventional feed can increase the weight gain of *Ctenopharyngodon idella* (Wang et al. 2016a), and studies have found that adding spp. Bacillus to feed can effectively improve the survival rate and growth performance of *Ilyoplax deschampsi* (Talib et al. 2016). Bacillus probiotics added to plant-based feed can change the intestinal flora of European sea bass and ultimately improve its growth performance (Serra et al. 2019).

Probiotics have strong adaptability to the environment, can grow and reproduce rapidly, and become the dominant flora in a short period of time, thereby inhibiting the growth of harmful bacteria in the environment, and indirectly improving the body's immunity. Studies have found that the combined use of *Bacillus subtilis* and *Rhodopseudomonas palustris* has a strong inhibitory effect on *Escherichia coli*, *Staphylococcus aureus*, and *Yersinia* small intestine in the intestine of *Carassius auratus auratus* (Shao et al. 2018). Predecessors found through high-throughput sequencing that *Rhodobacter capsularis* and *Bacillus cereus* have stronger inhibitory ability against Vibrio and other harmful bacteria in the intestine of *Litopenaeus vannamei* and can increase the growth rate (Shang et al. 2018).

After entering the intestine, probiotics can interact with the immune system to increase the activity of lymphocytes and phagocytes, enhance the immunity of aquatic animals and the resistance to infectious diseases, so as to achieve the effect of disease resistance. Bacillus also has a positive effect on *Epinephelinae*. They can enhance the immune system of grouper by increasing serum phosphatase activity (Li et al. 2019). Adding the probiotic *Paenibacillus polymyxa* to the feed can promote the formation of mucus proteins by upregulating the related genes in the intestinal tissue of tilapia, thereby enhancing the ability of the gastrointestinal barrier to eliminate pathogens. In addition, studies have found that by regulating insulin-like growth factors (IGF) 1 and IGF 2 gene expression, the addition of probiotics can also affect the tilapia immune system (Midhun et al. 2019). Studies have shown that composite bacteria solid fermentation products and composite bacteria enzyme preparations can effectively promote the growth of *Litopenaeus vannamei* and improve serum immune activity (Liu et al. 2018). Based on the good biological activity of probiotics, it can be widely used with aquatic products.

With the development of intensive and large-scale breeding, there is an urgent need for aquatic animal breeding to prevent diseases. As a substance that effectively controls diseases, immune enhancers play an important role in aquatic animal breeding. On the one hand, immune enhancers have good effects such as growth promotion, disease resistance, and immunity enhancement. On the other hand, it also has good security. As a safe and effective active substance, immunostimulants will be used more widely and reasonably in the aquaculture industry. Therefore, more in-depth research may be required in this field to enable the aquaculture industry to achieve better development.

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Chapter 11
Immunostimulants and Their Uses in Aquaculture

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Abstract Aquaculture is, without a doubt, the most reliable source of food in the future, especially protein-rich meals for the world's rising population. On the other hand, poor practices and maintenance issues cause stress in the fish, which increases their susceptibility to disease, resulting in diseases and disease outbreaks. Notwithstanding these negative influences on aquaculture, the availability of relatively easy remedial treatments that can rescue a large number of fish and, of course, the aquaculture economy is a bonus. This chapter discusses how immunostimulants appear to be superior corrective measures. Immunostimulants are numerous, relatively affordable, biodegradable, and protect against many infections in a single host, which is currently impossible with existing methods.

11.1 Introduction

Aquaculture intensification has become a necessary method of maximizing earnings in recent years. Fish and fishery products are rich in essential micronutrients and proteins that help maintain a balanced diet and good health. Shellfish and finfish convert feed to protein significantly more than livestock such as pigs, chickens, and cattle. Microbial infections have established themselves as a substantial barrier to aquaculture development. Aquaculture is increasingly using antibiotics because the industry needs to expand in the fight against rising resistance to antibiotics. Examples of pathogenic microbes that cause several economic problems that emerge from intracellular pathogens. Environmental care may be further supported by introducing new technologies, including products that have more significant benefits for humans, which improve the health of the environment (Sakai 1999).

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Immunostimulants for fish have been the focus of extensive study since 1990. Immunostimulants are substances that boost an animal's resistance to infections in general. They may be used to treat both evident and subclinical diseases. For overt illness, they offer substantial safety benefits as medical agents. They have none of the harmful environmental consequences that antibacterial medications and live vaccines have, and they create no undesired residues since they are natural substances (Treves-Brown 2000). Immunostimulants may be fed to farmed fish for lengthy periods, giving them a significant advantage over vaccinations. The stress that fish experience during handling and stocking can weaken their immune systems. It is difficult to keep large groups of fish protected from disease because their immunity to certain diseases is short-lived. The benefits of using immunostimulants include the reduced incidence of subclinical infections, which increases animal growth and decreases feed costs.

Immunostimulants are dietary supplements that stimulate the body's natural (nonspecific) defensive systems and raise resistance to certain infections. It is difficult to eradicate all ailments affecting aquatic animals only via immunizations (Wang et al. 2017a). Immunostimulants may increase the baseline levels of the immune response, increase survival, growth, and pathogen resistance (Bondad-Reantaso et al. 2005). Immunostimulation is one technique developing prominence in aquaculture and is considered a potential development (Li et al. 2006). Immunostimulants were shown to be beneficial in improving nonspecific immunity and resistance to fish and crustacean illnesses. However, particular concerns about the use of immunostimulants relate to the short-term nature of immunological indices used to assess effectiveness, the possibility of long-term adverse effects, or self-damage caused by uncontrolled immune effector production. It has been advised that more testing in large-scale manufacturing units be conducted. This chapter discusses the many kinds and sources of immunostimulants that are routinely employed in aquaculture. Each immunostimulant has a unique impact based on the source, dosage, mode of administration, duration of exposure, and species to whom it is delivered. Thus, immunostimulation appears to be the most appropriate treatment option for aquaculture disease concerns at the moment, at least until more effective treatments become available (Bricknell and Dalmo 2005).

11.2 Definition of Immunostimulants

Immunity systems in animals are classified as either specific or nonspecific. Body systems have specific names due to their supporting resistance to an exotoxin or toxin that an invading pathogen may release. The size of such parasites might range from a virus to a helminth. Nonspecific mechanisms, once triggered, raise the body's tolerance to any external chemicals, not just the stimulant (Treves-Brown 2000). According to the definition, "an immunostimulant is a chemical, drug, stressor, or activity that enhances nonspecific defense mechanisms or the particular immune response when used in conjunction with vaccination or infection"(Anderson 1992).

In practice, immunostimulants are promising dietary supplements that can help control disease in various species found in aquatic animals and improve disease tolerance in upregulating host protective mechanisms against opportunistic pathogen microorganisms found in the ecosystem. While vaccines are by far the most widely used immunostimulants in aquaculture, it appears that many in the industry search for new immunostimulants because they are simple to administer and provide wide-ranging protection.

11.3 Concept of Immunostimulants

Aquaculture has exploded in popularity as a means of food processing over the past few years. However, livestock fish cultured in confined spaces such as tanks, ponds, or reservoirs experience many problems resulting from stressful environments and infectious diseases, both of which are widespread problems in commercial fish culture. Infectious disease outbreaks also emerged as a restriction on aquaculture production. The outbreaks spread due to the unregulated migration of live marine species, which resulted in the transmission of pathogenic species between countries. Antibiotics and chemotherapeutic agents have been used in aquaculture to avoid or manage bacterial infections for approximately two decades. Unfortunately an increased number of antibiotic bacteria, negative effects on the indigenous microflora of juvenile or adult peppers and antibiotic residual deposition in the fish tissue, and the ecosystem pose a risk to human or animal health, are ineffective or sustainable (Labh and Shakya 2014). Vaccination is a highly effective method of preventing the spread of infectious diseases among fish in a community, but it is also costly and time-consuming. A single vaccine is effective against a single pathogen, but its effectiveness is limited due to the complicated antigenic structure against various pathogens.

11.4 Immunostimulants in Fish Diets

A more production-grade diet is enhanced with various natural compounds that are supposed to enhance innate immunity. Promotions for the diets say that fish given improved diets have better health and survival than fish given standard production diets, but they do not present any evidence to back up their claims. Immersion, injection, intestinal or intra-gastric delivery of immunostimulants are still being studied in the lab, but these procedures are not practicable for large-scale manufacturing (Vallejos-Vidal et al. 2016). While laboratory studies have shown beneficial immunostimulatory effects at the molecular and cellular levels, fish farmers have reported inconsistent results. Under less homogeneous manufacturing settings, the effects determined in vivo or in vitro in controlled trials cannot be guaranteed. As the activities of these chemicals are poorly known, and the results of feeding experiments have not consistently demonstrated good benefits, the use of immunostimulants as food supplements is viewed with caution and suspicion. Some goods are of dubious origin and nature. There is no compelling explanation of their mechanism of action or evidence of effectiveness under real-world situations. The precise process through which immunostimulants are absorbed is unknown.

On the other hand, immunostimulants are pushed in aquaculture to combat the immunosuppressive effects that stressors have on typical aquaculture processes. A feed additive may have a beneficial influence on fish health by improving growth rates. Immunostimulants for aquaculture might come from chemical or biological sources. However, the latter prevails in research and the limited available feeding trials. Primitive organisms such as mycelial fungi and yeasts and a few bacterial preparations are used to make most of the immunostimulants that are put into commercial fish diets. In an environment where a wide range of these cells and their by-products are present, nonspecific immunity has evolved through evolution in higher species such as fish. Pathogens and other compounds found in water that cause the immune response continually drain fish and other aquatic species. Through selection, chemicals that stimulate the immune system, such as disease invaders, have resulted in varying degrees of immunological response in fish that inhabit drastically diverse habitats. Although various candidates from various sources were evaluated for their usefulness in activating nonspecific immune responses in fish, only those that effectively protected fish against experimental infection are described in depth.

11.5 Immunostimulants Have the Following Characteristics

- High effectiveness.
- Broad range of action.
- Assist phagocytic cells in their function.
- Boost their bactericidal activity.
- Natural killer cells activate the complement system.
- Increase the activity of lysozyme.
- Activate antibody responses.
- There are no toxic side effects.
- Toxic residues are not retained.
- No influence from the environment primarily improve nonspecific immune response.
- It is simple to extend to fish and shellfish larvae.

11.6 Immunostimulants Advantages

1. Immunostimulants are successfully and widely used to improve fish health, production, and welfare.
2. This enhances lysozyme activity and antibody response, phagocytic cell's bactericidal abilities, increases the activity of natural killer cells, and stimulates complement systems in aquatic organisms.
3. Nonspecific immune responses are boosted, and aquatic environmental quality is improved.
4. Immunostimulants are beneficial for controlling fish diseases by increasing both specific and nonspecific defense mechanisms.
5. Many immune-stimulating substances have been developed to boost the animal's immunity.
6. Levamisole increased phagocytic activity in neutrophils, while myeloperoxidase activity in neutrophils increased leucocytic and serum lysozyme levels.
7. Immunostimulants are frequently required to improve vaccine efficacy.
8. Immunostimulants rely heavily on target cell receptors to detect them and, as a result, activate defense mechanisms.
9. Myeloid Differentiation Factor 88 upregulates the immunostimulant Toll-like receptor signaling pathways.
10. Inflammatory cytokines such as IL-1, IL-6, and TNF-∞ are stimulated by immunostimulants. C-reactive protein, complement components, metallothionine, and ceruloplasmin are all released when this happens. When more cytokines present, endothelial cell adhesion molecule expression is increased, which then helps promote leukocyte diapedesis.
11. Immunostimulants can help fish survive after being exposed to a pathogen by strengthening their innate defense mechanisms. ssRNA, CPG, MDP, ß-glucan, and LPS all increased viral resistance. Parasite resistance was aided by ß-glucans, MDP, and Levamisole.
12. Immunostimulants are treatments that are used instead of or in addition to vaccination. It also promotes growth and increases stress survival rates.
13. Immunostimulants added to food as food additives induced blood leukocyte phagocytosis and secretion of extracellular bursts.

11.7 Immunostimulants Disadvantages

1. High expense.
2. Limited efficiency when parentally administered.
3. Are ineffective against all illnesses.
4. Over-doses of immunostimulants result in immunosuppression.
5. Immunostimulants occasionally fail to provide better protection or increased immunity.

6. On the other hand, immunostimulants are widely used in aquaculture; however, intrinsic resistance to columnaris disease has not been investigated.

11.8 The Classification of Immunostimulants

Immunostimulants are agents that improve the immune system's efficiency. Various immunostimulants, such as vaccinations, induce an immune reaction to specific antigens. Nonspecific immunostimulants lack antigenic sensitivity and are commonly used to treat persistent infections, immunodeficiency, autoimmunity, and neoplasms. Vaccinations against viruses, colony-stimulating agents, interferons, interleukins, preventive vaccines, antigen mixes, and virus vaccines are also examples of immunostimulants. Other immunostimulants are those that do not fall under either of these categories. Immunostimulants are a relatively new addition to aquaculture, but they are rapidly evolving, with more variations and applications being affordable. The most commonly used immunostimulants in aquaculture are as follows.

11.9 Sources

Immunostimulants are substances (nutrients) that trigger or augment the function of some of the components of the immune system. Improving the tolerance of infectious diseases by nonspecific defense mechanisms can improve immunostimulants. The most convenient way of providing immunostimulants is by infusion or ingestion (Yin et al. 2006). The efficacy of the vaccine would improve and the dosage needed for the same benefit would decrease. The strategy to enhance larval survival by increasing innate responses was proposed as immunomodulation in larval fish before the adaptive immune response was developed to defend pathogenesis successfully. It is suggested that feeding the larval fish with immunostimulants as a dietary aid may be helpful to improve the intrinsic defenses of the animal without harming the developing animal (Bricknell and Dalmo 2005). In vitro screening should be used to clarify immunostimulation processes; in vitro screening methods should be used in order to determine whether there are benefits to live fish. Fish feeding the diets with the supplements of immunostimulants were significantly higher than fish feeding non-supplemental diets with the total IgM serum amounts. Many food additives like antioxidants, carotenoids, and herbal therapies were studied in aquaculture with the generally positive results of tension reduction, an improvement in the function of intrinsic parameters, and strengthening of disease tolerance (Yin et al. 2009). In vivo and in vitro reactions are the two different measurement mechanisms. Many experiments demonstrated that several compounds, like synthetic ones (Rao et al. 2006), animal and plant materials (Ardó et al. 2008; Rao et al. 2006), and bacterial (Goetz et al. 2004), can be used as immunostimulants to improve the nonspecific immune response of cultured fish organisms.

Saponin (Ninomiya et al. 1995), aloe (Kim et al. 1999), glycyrrhizin (Jang et al. 1995), and azadirachtin (Logambal et al. 2000) have all been shown to improve fish innate immunity. Certain plants contain abundant volatile oils, saponins, phenolics, tannins, alkaloids, polysaccharides, and polypeptides. These natural plant extracts have anti-stress, antimicrobial, and immunostimulant properties (Citarasu et al. 2002). Immunostimulants are gaining popularity as an alternative to the medications, pesticides, and antibiotics commonly used to combat fish diseases, partly because, unlike vaccinations, immunostimulants improve the intrinsic (or nonspecific) immune response (Sakai 1996). For the last decade, researchers have examined the genomic organization and characterization of immune-related genes and their expression in response to various immunostimulants (zymosan, peptidoglycan, and b-glucan) in order to gain a better understanding of the molecular mechanisms of crustacean immunity

11.10 Synthetic Derived Immunostimulants

11.10.1 Levamisole

The US Food and Drug Administration (FDA) has authorized Levamisole, a synthetic phenylimidathiazole, to treat ruminant helminths. Levamisole's immunostimulatory action against bacterial infection and parasite infestation are significant in aquaculture. It enhances nonspecific defense mechanisms by increasing cytotoxicity, lymphokine synthesis, cell function suppression, and macrophage and neutrophil phagocytic activity. It has been shown that it may enhance the nonspecific immune response of carp, gilthead sea bream, and rainbow trout (Ispir and Dorucu 2005). Increased disease resistance is also shown by Levamisole's immunostimulatory properties when taken orally or through immersion (Kajita et al. 1990)

11.10.2 Immunoactive Peptide (FK-565)

FK-565 is an immunoreactive lactoyltetrapeptide (FK-156) identified from *Streptomyces olivaceogriseus* cultures. FK-565 is synthetic immunostimulants that is reported to develop resistance to *Aeromonas salmonicida* in rainbow trout by activating phagocytes when *Yersinia ruckeri* or *A. salmonicida* antigen preparations are combined with FK-565; in vitro vaccination results in increased humoral antibody (Kitao et al. 2009)

11.10.3 Muramyl Dipeptide

Stimulation with different bacterial components or products activates macrophages. In fish, activated phagocytes have been demonstrated to play a crucial role in bacterial infection defense. However, the mechanisms of fish phagocyte activation and the link between cell activation and host defense against bacterial infection remain unknown. Muramyl dipeptide is an bactrial derivatives, derived from Mycobacterium. It activates macrophages, B lymphocytes, and the alternative complement pathway increase the ability of renal leukocytes to phagocytose, burst into action. It has been shown to increase antibody activity, stimulate polyclonal lymphocyte activation, and activate macrophages (Kodama et al. 1993).

11.10.4 Chicken Egg Fermentation Products (EF203)

When supplied orally to *Oncorhynchus mykiss* (rainbow trout), fermented chicken egg products (EF203) include immunoactive peptides that have an immunomodulatory effect (Wang and Chen 2005). After being treated with EF203, the chemiluminescent responses of renal phagocytes increased significantly. Fish treated with EF203 demonstrated increased phagocytic activity, and the immunomodulatory effect was dose-dependent. In both natural and experimental beta-hemolytic streptococcal infections, fish treated with EF203 showed more excellent resistance

11.10.5 Biological Derivatives

Numerous biological components have been uncovered in recent years in a variety of animals. While mammals have extensively studied the use of biological factors as immunostimulants, very little research has exclusively focused on the potential use of biologic genetic factors as immunostimulants in fish. The majority of these chemicals are currently missing information on their immune functions. Thus, until we understand the immunological processes regulated by the factors, their use will be a matter of trial and error. Several efforts, however, have been undertaken to investigate their possibilities in a variety of fish species.

11.10.6 Peptidoglycans

Peptidoglycans are polymers made of a chitosan chain, a peptide bridge, and peptide subunits found in bacteria's cell walls. Peptidoglycans have a unique composition to the cell walls of many prokaryotes, and their activity increases with hydrolysis.

Peptidoglycans stimulate development and boost aquatic animals' resistance to infections and immunology, according to research. Peptidoglycans are vital immunostimulants that help aquatic animals manage their immune systems.

11.10.7 Lipopolysaccharide

Lipopolysaccharides, also known as lipoglycans or endotoxins, are enormous molecules consisting of a lipid and a polysaccharide covalently linked. Lipopolysaccharide is a polysaccharide in gram-negative bacteria's cell wall. In sea bream *Pagrus major*, this chemical can stimulate B cell proliferation and increase the macrophage phagocytic activity (Salati et al. 1987). Lipopolysaccharide helps prevent *A. hydrophila* infections and activates the rainbow trout's natural immune response (Nya and Austin 2010). These chemicals are powerful even at deficient levels and may be contaminants in bacterin preparations and fish immunization programs. It may also stimulate the synthesis of a macrophage-activating factor in goldfish lymphocytes (Neumann et al. 1995).

11.10.8 Whole Bacteria Cells

Vibrio anguillarum bacterin (inactivated whole-cell vaccine), supplied with immersion, injection, and oral vaccination, is the most effective vaccination for salmonid fish (Sakai 1999). Fish and shellfish were shown to be immune to the *V. anguillarum* bacterin. Norqvist et al. (1989) discovered that immunization of rainbow trout with attenuated *V. anguillarum* increases resistance to *Aeromonas salmonicida* challenge.

11.10.9 Probiotics

Probiotics, beneficial microorganisms that improve the host's use of food and its disease tolerance, optimize the environment of aquatic animals by colonizing microflora where aquatic animals live (Verschuere et al. 2000). Several probiotic strains, including Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Carnobacterium, Shewanella, Bacillus, Aeromonas, Vibrio, Enterobacter, Clostridium, and Saccharomyces spp., have been used in the aquaculture sector. The following characteristics can be found in probiotics: (Farzanfar 2006): (1) antagonistic toward pathogens, as probiotic bacteria are; (2) beneficial to the host animal; and (3) capable of surviving in or colonizing the gut of an aquaculture animal (Farzanfar 2006). Probiotics have been studied extensively in aquaculture and are usually added directly to the feed or water. In addition, probiotics balance the use of intestinal flora and feed, promote the production of aquatic animals, increase immune function and disease tolerance; and improve water quality by absorbing or reducing species and harmful substances.

11.10.10 Prebiotics

Prebiotics are indigestible fibers that promote beneficial commensal bacteria in the gut, thereby benefiting the host's health. Fructooligosaccharides, glucan, mannan oligosaccharide, and inulin are all examples of immunosaccharides. They directly enhance innate immune responses, such as phagocytic activation, neutrophil activation, alternative complement activation, and lysozyme activity. Furthermore, immunosaccharides directly activate the innate immune system by interacting with pattern recognition receptors (PRR) found on innate immune cells. Additionally, they may stimulate innate immune cells when combined with microbe-associated molecular patterns (MAMPs). Indeed, probiotics stimulate the innate immune system in two ways: (1) by activating it directly and (2) by promoting the development of commensal microbiota. (Song et al. 2014). Numerous studies have shown that feeding prebiotics and immunosaccharides to aquacultures of various vertebrates and invertebrates is favorable. Additional study is required to describe the ligand-receptor interactions, signal transduction pathways involved, and the kinds of cytokines produced to increase the efficiency of prebiotics and probiotics used in aquaculture and to search for new immunostimulatory prebiotics (Gómez and Balcázar 2008). Studies on the impact of prebiotics and probiotics on the immune systems of finfish and crustaceans would gain more credibility if innate immune responses were physiologically connected to overall gut health (Pérez et al. 2010). Additionally, Bacillus S11 can increase the survival rate, fitness, and disease tolerance of *P. monodon* during the breeding phase. Bacillus is a genus of gram-positive, aerobic, or facultatively anaerobic, endospore-forming, rod-shaped bacteria that are often present in the soil, water, and associated with plants. *Bacillus subtilis* is currently used in aquaculture, terrestrial livestock, and human consumption to treat and prevent gastrointestinal disorders through oral bacteriotherapy and bacterioprophylaxis.

11.10.11 Vibrio Bacterin

The most effective salmonid fish vaccination is *Vibrio anguillarum* bacterin. In fish and shellfish, immunostimulation of the *V. anguillarum* bacterin was observed. The migration of hemocytes treated with vibrio bacterin can be enhanced in black tiger shrimp. By activating macrophages, *V. anguillarum* lipopolysaccharides may behave as immunostimulants.

11.10.12 Clostridium butyricum Cells

By activating leucocytes, including phagocytosis and superoxide anion generation, oral injection of *C. butyricum* bacterin can increase rainbow trout resistance to vibriosis.

11.10.13 **Achromobacter stenohalis** *Cells*

Inactivated *A. stenohalis* may improve chemical luminescent reactions in renal cells, complement activity and *A. salmonicida* challenge resistance.

11.10.14 Glucan

Numerous glucan types have been investigated in fish, including yeast glucan, peptide-glucan-1,3, and glucan. Yeast glucan (1–3 and 1–6-linked glucan) and 1,3glucan are derived from *Saccharomyces cerevisiae* and *Schizophyllum commune* cell walls. Inactivity is increased through cellular and non-cellular defense systems such as lysozyme activity, phagocyte activity, complementary activity, and bactericidal macrophage.

11.10.15 β-Glucans

Among the several immunostimulants used in aquaculture, b-glucan is a potential immunostimulant. Since it is a glycoside-linked homopolysaccharide, it is vital to the cell wall of some plant, fungal, bacterial, champagne, yeast, and seaweeds. It is a composition made up of glucose molecules. Glucans containing a helical or spiral backbone due to the complex intramolecular connectivity of yeasts and bacteria are produced. Glucans are recognized as an external molecular pattern by the immune systems of aquatic animals. B-glucans in aquatic animals have been investigated extensively, and specific aquatic species' development has been shown to be supporting. The primary emphasis on b-glucan was captured by improved knowledge of its receivers and action mechanism. The receptor in the animal's body identifies and binds to b-glucan, providing a high level of resistance and an improved animal immunological response (Sirimanapong et al. 2015).

11.10.16 Freund's Complete Adjuvant

Freund's complete adjuvant is a mineral oil adjuvant with *Mycobacterium butyricum* that has been killed or inactivated. Respiratory burst, phagocytic, and NK cell activity were enhanced when Freund's total adjuvant was used. Injection of Freund's complete adjuvant with *A. salmonicida*, *A. hydrophila*, *V. ordalli*, Furunculosis, Red mouth disease, and vibriosis has shown better disease resistance in freshwater fish.

11.10.17 Lectin

Lectins are a wide family of proteins binding on glycoproteins and/or carbohydrates. In pathogen recognition and host defense, lectins were found. For instance, lectins are raised in fish cells in response to microorganism/viral infection and induce antiviral conditions. Lectins have been used in animal vaccines as an adjuvant because they attract additional cells to the site of inflammation and regulate the immunological activities of the recruited cells. Lectins inhibits infections by activating immunocytes, such as phagen cells, and lymphocytes despite their modest defensive properties. As a result, lectins can be used to prevent diseases as immunostimulants in aquatic species.

11.10.18 Antibacterial Peptides (ABPs)

ABPs represent small molecular polypeptides with a wide range of species of antibacterial activity. ABPs consist of between 20 and 60 amino acid residues. ABPs depolarize bacterium membrane and result in cell content loss or peptide interactions with DNA, RNA, and other targets, ultimately leading to bacterial death. ABPs are tiny proteins generated from animals quickly degraded by proteases after completing their bactericidal function. ABPs have been shown to have a low rate of rejection in vivo. Additionally, ABPs are non-toxic and ecologically friendly compounds that may be used to safeguard aquaculture animals. Due to their low concentrations and expensive equipment costs which restrict manufacturing scale, extracting ABPs from animals is difficult. At the moment, the only practical approach for producing ABPs is genetic engineering. Numerous ABPs have been cloned from aquatic organisms, including catfish and zebrafish. ABPs' mechanism of action from aquatic animals and target protein structure on the surface of pathogens are linked, as are pathogens and their aquatic animal targets. Suppose the immune function of an animal is impaired. In that case, biological factors such as immunological molecules from aquatic animals are not able to adequately target proteins on the surface of pathogens since impaired immune function interferes with pathogen detection, which leaves the animal susceptible to various diseases.

Biological factors used as immunostimulants may therefore contribute to strengthening immune defenses and help prevent disease.

11.11 Polysaccharides Derivates

Polysaccharides are essential biopolymers and are found in microbes, animals, and plants. For decades, polysaccharides have been acknowledged as immunostimulants. Many marine species, including microalgae, marine fungi, and corals, have been established as effective sources of polysaccharides, due to the wide range of growth promotions, antioxidants, immunostimulants, anti-tumor, anti-inflammatory, anti-stress, and anti-toxicant properties. Applied research on polysaccharides in aquaculture can generally be classified into three distribution methods: Additive for the Pond, Injection into the Individual, and Feed Additive. The immunological impact is studied using delivery mechanisms. Polysaccharides are commonly used as feed additives in aquatic animal breeding facilities due to their convenient application techniques. Digestion and consumption of supplemented polysaccharides are more accessible, owing to its ability to promote digestive enzyme secretion (amylase, protease, and lipase, among other things), which increases food consumption and digestion, as well as improvement of the health and development of aquatic animals (Choi et al. 2014; Wang et al. 2016).

11.11.1 Chitin and Chitosan

Chitin and chitosan are also crucial in aquaculture. Chitin is a kind of nonspecific immunostimulator with a short-term impact. The chitin polymer consists mainly of crustaceans and insects exoskeletons and few fungi's cell walls (Cheung et al. 2015). The effects of chitin on the immune system of shellfish are largely unknown in comparison to finfish. The shellfish defensive mechanism is thought to be dependent on innate immunity. Hemocyte-mediated reactions like phagocytosis, respiratory burst, and prophenoloxidase activity can be used to analyze the activities of mussels treated with immunostimulants' innate immune components. Chitin supplementation increases the resistance to a variety of diseases associated with aquaculture outbreaks. Rainbow trout were more resistant to *Vibrio anguillarum* infection after receiving chitin injections.

Chitosan, which is produced by alkaline deacetylation of chitin, is of great interest in the aquaculture industry due to its biodegradability, safety, nontoxicity, and biocompatibility (Chandy and Sharma 1990; Kumar et al. 2004). In addition to encouraging fish growth, preventing aquatic infections, and water treatment, this biopolymer substance has shown that many fish species activate and enhance their immunological status, primarily through the activation of innate immune components. Chitosan has been used in aquaculture as an immunostimulant to boost fish defenses against bacterial disease, a controlled-release vaccine, and a dietary supplement (Wang et al. 2017b). Research has noticed that Brooktrout with de-N-acetylated chitin has shown increased tolerance for *A. salmonicida*. Rainbow trout exposure to *A. salmonicida*, white shrimp to *Vibrio harveyi*, koi to *A. veronii*, Asiatic seabass, *Vibrio anguillarum*, kelp grouper to *P. dicentrarchi*, Nile tilapia to *A. hydrophila* (Lin et al. 2011; Ranjan et al. 2014; Wang and Chen 2005). Because chitin or chitosan is not a natural part of the fish cells, the innate immune system of fish can be recognized after exposure. The immunostimulatory properties of chitin and chitosan have been thoroughly proven in fish, with phagocyte activation and direct phagocytosis of chitin and chitosan particles being seen (Sangma and Kamilya 2015). This demonstrates the critical significance of particular receptors on immune cells in recognizing and activating them. Chitosan's method of action in modifying immunological responses is also unknown. The amount of amino or imino residues in chitosan that leucocyte mannose/fucose receptors can recognize is believed to be critical for immunological detection and subsequent immunological responses.

11.11.2 Lentinan, Schizophyllan, and Oligosaccharide

The cell and non-cellular mechanisms in fish can be improved by lentinan, schizophyllan, and oligosaccharides like producing lysozymes, phagocytes, and supplementing activity.

11.11.3 Quil A

In vitro bactericidal activity of rainbow trout increased by combining Quil A saponin with *Y. ruckeri* vaccinations. Quil A saponin can also help in the movement of leukocytes of yellowtail.

11.12 Animal-Derived Immunostimulants

11.12.1 Haliotis discus hannai *Hde (Abalone)*

Animal extracts derived from a variety of invertebrates act as immunostimulants. Hde (*Haliotis discus hannai*) is a glycoprotein fraction of an abalone water extract. It has the potential to improve the killing of tumor cells. It inhibits tumor development in vitro and in vivo (Davis and Hayasaka 1984).

11.12.2 Ecteinascidia turbinata

Ecteinascidia turbinata is a marine tunicate extract. Eel phagocytosis is enhanced, and survivability is boosted when *A. hydrophila* was injected. Furthermore, when a rainbow trout exposed to *E. turbinata,* it has immunostimulatory effects by showing phagocytic activity, anti-tumor activity, and NK cells (Jensen et al. 2002).

11.12.3 Firefly Squid

Watasenia scintillans, a firefly squid, may activate the Rainbow trout's immune system, such as the creation of superoxide anion, possible macrophage killing actions, and in vitro lymphoblastic transformation of lymphocytes.

11.13 Plant-Derived Immunostimulants

Plants contain natural immunostimulants, which humans have used for thousands of years (Tan and Vanitha 2004). Many herbs in traditional medicine have been found to have immunomodulatory properties. Specific agents stimulate both humoral and cell-mediated immunity while others stimulate the cell component of the immune system alone without affecting humoral or cell-mediated immunity, specifically phagocytical activity. Aquaculture is potential by the use of medicinal plants as natural and non-toxic compounds as an alternative to antibiotics and immunostimulants. The interest in these plants has grown globally, due to their easy preparation, low cost, and lack of harmful effects on animals and the environment. Numerous medicinal plants, including herbs, spices, algae, herbal medicines, herbal compounds, Chinese medicines, and commercial herbal products, are studied on diverse aquatic animals. Numerous medicinal and non-medicinal plants have proven immunostimulative and disease-protective properties in fish in the laboratory and several have been further investigated in detail at this time. Plant crude extracts have been shown to increase a range of innate immune elements, including complementary parameters, lysozyme, anti-protease, reactive oxygen, phagocytoses, breathing bursts, total nitric oxides, and glutathione peroxidase, and further protection against bacterial, fungal, viral, and parasitic diseases have been found (Van Hai 2015).

Numerous therapeutic plants have been evaluated and attempted to manage various fish and shellfish illnesses with positive results. *Acalypha indica, Phyllanthus niruri, Ocimum sanctum, Phyllanthus emblica*, Aloe vera, *Azadirachta indica, Emblica officinalis*, *Cynodon dactylon*, *Adathoda vasica Curcuma longa, Crossandra infundibuliformis,* Piper betle, *Solanum trilobatum, Murraya koenigi*, turmeric and *Mentha piperita*; Chinese herbs *Astragalus membranaceus*, *Isatis* *tinctoria*, *Polygonum multiflorum*, *Glycyrrhiza glabra*, *Massa medicata*, *Lonicera japonica, Scutelaria baicalensis, Crataegi fructus, Artemisia capillaries,* and *Cnidium officinale,* etc., have all been researched in recent years for their immunostimulatory reponse in fishes.

Chinese people have employed herbs as natural treatments and immunity boosters for thousands of years. Recently, there has been a surge of interest in the immune-stimulating characteristics of several herbs employed in aquaculture. Combinations of Chinese herbs may be used in aquaculture to boost nonspecific immunological qualities such as bacteriolytic activity and leucocyte function. Chinese herbs are organic and include a diverse array of active components. Numerous studies on the immunostimulatory properties of various components have been conducted in rat, chicken, and human cell lines. The active ingredients in Chinese herbs were identified following a thorough screening method in humans and other animals. These chemicals are not addictive or toxic to humans or other animals, and they can be used indefinitely without developing drug tolerance or accumulating prescription metabolites. Chinese herbs contain various complex constituents, including proteins, polysaccharides, flavonoids, alkaloids and vitamin E, fatty acid, and minerals, which may provide nutrition, antiviral and bactericidal activity immunological protection. Due to their availability and efficacy against a wide variety of diseases, herbal extracts may be used as immunostimulants in fish culture. The majority of herbs and plant extracts should be taken orally to most effectively activate the immune system. However, the effect is dose-dependent, and there is still a risk of overdosage. Additionally, they may be used to treat some viruses, parasites, and fungi-related disorders. Immunostimulant-incorporated diets provided to shrimps demonstrated superior performance on immunological, hematological, and biochemical parameters. Herbal compounds are capable of inhibiting the formation of oxygen anions and scavenging free radicals. Herbal extracts have antioxidant activity comparable to metal ion chelators, superoxide dismutase, and xanthine oxidase inhibitors. Herbal extracts have antioxidant activity comparable to metal ion chelators, superoxide dismutase, and xanthine oxidase inhibitors.

11.13.1 Ocimum sanctum

Ocimum sanctum is referred to as the “queen of herbs.” The best approach for the management of aquaculture systems is to develop an efficient immune system. *Ocimum sanctum* plants are an essential source of medicines because of their abundance of secondary metabolites (Wagner et al. 1994). The antibacterial activity of leaves-derived *O. basilicum* essential oil, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus,* and *Staphylococcus aureus*, was investigated in gram-negative and gram-positive bacteria. Additionally, this plant contains vitamins A and C, which increase antibody synthesis by up to 20%, thereby protecting against infection.

O. sanctum has shown immunomodulatory properties in various animal species (Singh et al. 1996). Feed with extract of *O. sanctum* resulted in a substantial decrease in *Epinephelus tauvina* against infection with *Vibrio harveyi* (Sivaram et al. 2004). According to Logambal et al. (2000), dietary consumption of *O. sanctum* also boosted antimicrobial peptide response and disease resistance against the infection of *Oreochromis mossambicus* with *A. hydrophila*. In addition, *L. rohita* showed *O. sanctum* elicits a nonspecific immune response to A. *hydrophilia*, increased total immunoglobulins and lysosomal activity and beneficial effect on hematological and biochemical markers. In addition, *L. rohita* showed *O. sanctum* elicits a nonspecific immune response to *A. hydrophilia*, increased total immunoglobulins and lysosomal activity and beneficial effect on hematological and biochemical markers. (Das et al. 2015).

11.13.2 Phyllanthus emblica

Phyllanthus emblica or amla has antioxidant, antifungal, antibacterial, and anti-inflammatory properties (Bhattacharya et al. 2000). In addition, Amla fruit pulp includes a high concentration of vitamin C, which has been shown to have immunostimulatory properties. Tilapia's anti-SRBC response was improved by *P. emblica* acetone extract, but both crude extract and a water-soluble fraction of *P. emblica* fruit were stimulating tilapia immune response.

11.13.3 Azadirachta indica *(Neem)*

It is a well-known Indian "wonder" tree. Biomedical research has established neem's anti-tumor, anti-HIV, and antimicrobial properties. Azadirachtin, a triterpene *of Azadirachta indica*, increased leucocytes, respiratory explosion activity, and SRBC primary and secondary antibody responses in tilapia. Logambal and Michael (2001) report that in Tilapia *Oreochromis mossambicus*, *A. indica* enhances primary and secondary immunological responses. Neem leaf water extract is a powerful immunostimulant, as evidenced by humoral and cellular responses. Additionally, it has been demonstrated that neem oil has immunostimulant properties, activating cell-mediated immune systems selectively in order to elicit a more robust response to a future mitogenic or antigenic assault.

11.13.4 Solanum trilobatum *(Purple Fruited Pea Eggplant)*

Solanum trilobatum is an antibiotic for various anticancer and antibacterial activities. The herbal extract of *Solanum trilobatum* includes soba, b-solamarine, solaine, solasodine, glycoalkaloid, diosogenin, and tomatidine (Shahjahan et al. 2004). The effects of *S. trilobatum* water and hexane-soluble fractions on nonspecific immune systems of Tilapia and disease resistance were investigated. After a challenge with *Aeromonas hydrophila*, all water-soluble fraction doses significantly increased the production of reactive oxygen and lowered mortality percentages (Divyagnaneswari et al. 2007).

11.13.5 Eclipta alba *(Bhringraj)*

Eclipta alba is an Asteraceae plant that is abundantly accessible and distributed throughout India. Numerous medical benefits have been attributed to this plant. For example, oral administration of an aqueous extract of *Eclipta alba* leaf helps to improve nonspecific immune responses and disease resistance in *Oreochromis mossambicus* against *A. hydrophila* (Citarasu et al. 2006).

11.13.6 Zingiber officinale *(Ginger)*

Ginger contains a beneficial natural organic compound that improves specific immune responses and enhances resistance to infectious diseases. Roots and extracts include polyphenol chemicals (6-gingerol and its derivatives) with significant antioxidant potential. Ginger rhizome has been shown to effectively control various viral, bacterial, fungal, and parasite diseases in aquaculture due to its antioxidant, growth promoter, and immunostimulant characteristics (Shakya 2015).

11.13.7 Echinacea purpurea *with* Allium sativum *(Garlic)*

Echinacea is a well-known plant that has been linked to immunological regulation in humans and animals (See et al. 1997). There is substantial evidence that the polysaccharide fraction of Echinacea has an immune system protective effect, macrophages and other immune cells are activated. *Allium sativum* increases body weight growth, survival rate, and resistance to *Aeromonas hydrophila* (Aly and Mohamed 2010). Both compounds had longer effects and increased resilience to cold stress during the winter season.

11.13.8 Camellia sinensis

Green Tea (GT) extracts contain a wide range of catechins that display biological activity in antioxidants, anti-angiogenesis, and antiproliferative tests, implying that they could help prevent and treat various types of cancer. Green tea supplementation increased the protein content of the fish body while maintaining the lowest lipid content. Hematological and biochemical indices increased in fish given green diet, while control fish had the lowest levels. Furthermore, the survival of fish with *A. hydrophila* has increased. These findings suggest that GT supplementation is a promising immunostimulant that can enhance fish performance and health (Yoshida et al. 1999).

11.13.9 Aloe vera

Aloe vera is one of the most valuable members of the tropical Liliaceae family. *A. vera* contains over 200 active ingredients, 75 nutrients, 20 minerals, 18 amino acids, 12 aloin, famodin, antrokinon, and barbaloin vitamins, and 12 plant chemicals (Mandrioli et al. 2011). *Aloe vera* may enhance both specific and nonspecific immune responses in common carp when administered orally. This appears to be achieved primarily by an increase in lysozyme activity, serum bactericidal activity, total protein, and IgM levels. Supplementing feed with *Aloe vera* (0.5%) has increased resistance to *Aeromonas hydrophila* and Aeromonas septicemia. In addition, the relative percent survival (RPS) of fish treated with *Aloe vera* was increased (Alishahi and Abdy 2013).

11.13.10 **Cynodon dactylon** *(Bermuda Grass)*

Immunostimulants like Bermuda grass (*Cynodon dactylon*) boost immunological responses in farmed shrimp by raising bactericidal activity, phagocytosis, and respiratory bursts, as improving pathogen resistance. Therefore, the in vivo antiviral effects of a *Cynodón dactylon* plant extract were evaluated after oral infection of *Penaeus monodon* with the white spot syndrome virus (WSSV). Results showed *C. dactylon* extract to be highly effective in preventing WSSV infection in *P. monodon* without mortality and no evidence of WSD in 2% and 40% death in 1%, respectively WSD was shown (Balasubramanian et al. 2008).

11.13.11 Glycyrrhizin

Glycyrrhizin is a glycosylated saponin containing one glycyrrhetinic acid molecule. The immunomodulatory properties of glycyrrhizin are anti-inflammatory and antitumor. Furthermore, Glycyrrhizin therapy improved macrophages' respiratory burst activity and rainbow trout lymphocytes proliferating in vitro (Wada et al. 1987).

11.13.12 Essential Oil as Immunostimulant

Essential oils are used to suppress pathogenic microorganisms in aquaculture and also function as immunostimulants. Anti-allergic properties have been discovered in the essential oil of *Ocimum sanctum*. Cinnamaldehyde's carbonyl group is assumed to be necessary for antimicrobial activity since it binds to cellular proteins and prevents them from working correctly (Rattanachaikunsopon and Phumkhachorn 2010). Cinnamon oil can protect Nile tilapia against *Streptococcus iniae* infection. It has the most inhibitory action. So, there was no evident variation in the growth metrics. However, fish cannot show their potential immunity at low temperatures, the diet of Zataria multiflora essential oil somewhat improved common carp immunity (Vaseeharan et al. 2013).

11.14 Nutritional Factors as Immunostimulants

Fish health and immunological reactions may be affected by diets. Nutritional components are dietary components necessary for the average growth and development of fish. Many of them undoubtedly play an important role in the immunological response. A, B6, C, E, and fluoride and iron minerals are micronutrients that can affect diseases' resistance. While there is disagreement, vitamins and minerals are not formally defined as immunostimulants because they improve the immune system by providing substrates and acting as co-factors necessary to function effectively in the immune system. Essential or non-essential nutrients may, alone or in combination, directly or indirectly alter immunological and fish health processes. Amino acids play a vital role in defense mechanisms since their different proteins, including antibodies, are produced, and essential immune control pathways are regulated. Several studies on several fish species showed increased resistance to parasites, bacteria, and viruses by supplementing the dietary nucleotide. Several studies have shown that the health of warm-blooded animals needs dietary fat. Vitamin C deficiency was associated with immunological suppression and susceptibility to infectious diseases. In addition, lack of dietary vitamin E was linked to cell-degenerative, nonspecific diseases.

11.14.1 Vitamins

For animal growth and development, vitamins are needed. Vitamins are usually derived by diet because they are seldom synthesized in vivo. For animal growth and development, vitamins are needed. Vitamins are usually derived by diet because they are seldom synthesized in vivo. If long-term consumption of vitamin is insufficient, growth delays, health loss resistance and death can occur. Vitamin C and vitamin E have recently become increasingly popular in marine animals as vitamin immunostimulants.

11.14.2 Vitamin C

Vitamin C, also called ascorbic acid, have been used in animals as an accepter or hydrogen donor. High doses of vitamin C for aquatic animals improved immunity and disease resistance (Mazik et al. 1987). Vitamin C is required for various physiological functions, including development, infection resistance, stress response, wound healing, and likely lipid metabolism, through its effect on carnitine synthesis when administered with feed. Vitamin C is required for different biological processes, including cell function, neuromodulation-related collagen synthesis, hormone production, and the immune system. Tewary and Patra (2008) discovered that increasing dietary vitamin C levels greatly improved resistance against *A. hydrophila*. Vitamin C, 1000 mg/kg diet, has enhanced the nonspecific immune response through increased oxidative burst, pinocytosis, and rainbow trout activity. Vitamin C boosts hemolytic activity when administered at significant levels, which means complement activity is enhanced.

11.14.3 Vitamin E

Vitamin E, also known as tocopherol, is a group of phenolic compounds with biological activity. Vitamin E was shown to enhance both cell-mediated and specific immunity from infection. Vitamin E deficiency in trout results in diminished resistance to *Y. ruckeri* (Blazer and Wolke 1984). Tocopherols, commonly classified as VE, are a class of biologically active phenolic compounds. A sufficient dose of vitamin E can enhance antibody development, complement antibody activity, promote lymphocyte proliferation and differentiation and cytokine production, and boost cytotoxicity and phagocytosis (Blazer and Wolke 1984).

11.14.4 Carotenoids

Carotenoids are the basic source of yellow, orange, and red plant pigments, are widely distributed in nature.

In recent years, carotenoids or vitamin A precursors have received more attention due to their shown health benefits in humans and other animal models (Krinsky 1992). While certain pure carotenoids can be produced, natural carotenoids are abundant and cheap, making them an appealing source of pigments for food supplementation. Carotenoids are needed for phagocytosis, serum complement activity, serum lysozyme activity, nonspecific cytotoxicity, and disease resistance in fish.

11.14.5 Fatty Acids

It has been proposed that omega-3 polyunsaturated fatty acids can improve fish illness resistance. This is because fatty acid ratios are critical for disease resistance.

11.14.6 Trace Elements

Deficiencies in antioxidant trace elements (co-factors for several enzymes) such as copper (Cu), zinc (Zn), selenium (se), and manganese (mn) have been proven to affect animal immunity. Deficiency in Cu, Mn, and Zn lowered natural leukocyte killer activity and antibody production of fish (Watanabe et al. 1997). Zinc and selenium supplementation protected fish from infection with *Edwardsiella ictaluri* (Paripatananont and Lovell 1995; Wang and Lovell 1997). When the rainbow trout treated with amino acid-chelated trace elements the lysozyme activity, total immunoglobulin levels, antibody production and survial rate were reported to be higher (Apines et al. 2003).

11.15 Derivatives in Algae

In aquaculture, microalgae are widely employed for nutritional purposes to a broad spectrum of aquatic beasts. Microalgae are high in antioxidants, high in nutrition, and non-toxic. They can therefore be examined for people or animals as a therapeutical carrier. Examples of naturally occurring wild-type microalgae can be utilized as an immunostimulant to improve nonspecific immune response and growth performance (Ma et al. 2020). Algae are a valuable source of agarose, agarose, and carrageenan, including carbohydrate polymers. They also make oil and pigments that no other organism can produce (Mohan et al. 2019). Almost all algae have received little attention in spite of being a rich source of bioactive compounds. For example, in Nile tilapia, they lived longer following challenges with *A. hydrophila* when a dried cell suspension was given for Spirulina (Mahmoud et al. 2018). The N-oxide-quaternary alkaloid fraction of Channa striata, a marine chlorophycean macroalga, was reported to have immunostimulatory and disease resistance potential against *A. hydrophila* (Balasubramanian and Michael 2016). In an experiment, however, the marine macroalgae *Tetraselmis chuii* and *Phaeodactylum tricornutum* could not protect fish (Sparusaurata L.) from infection with *Photobacterium damselae* subsp. Piscicida (Cerezuela et al. 2012). Laminaran is a 1, 6-branched 1, 3-D-glucan abundant in sublittoral brown algae like Phaeophyceae. Because almost all B-(1, 3) D-glucans are insoluble in water, they are more challenging to treat than aqueous soluble laminaria. Laminaran, derived from *Laminaria hyperborea*, also has immunomodulatory properties.

11.16 Nucleotides

Nucleotides are the basic building blocks of DNA, consisting of a nitrogenous base, a five-carbon sugar, and a phosphate group. Dietary nucleotides are considered conditionally essential nutrients under certain physiological situations. They are needed for a variety of biological processes. They serve as co-factors in cell signaling and metabolism, as well as DNA replication precursors (Peng et al. 2013). They are the basic building blocks of nucleic acids, and they play an essential role in cell creation and repair (Trichet 2010). They have been shown to stimulate fish innate and adaptive immune responses and boost resistance to viral, bacterial, and parasitic diseases (Burrells et al. 2001).

11.17 Cytokines

Cytokines are glycoproteins or low molecular weight polypeptides (less than 30 kDa) that have been found in humans to date. Cytokines are key regulators for the immune system, and the discovery of these genes in fish may contribute to the development of aquaculture vaccines and immunostimulants. Fish were reported to contain cytokines comparable to human cytokines. Cytokines can be utilized as markers to evaluate fish's innate immune response (Sakai et al. 2021). It functions as an "informant," interconnects and separates diverse immune system components to assemble and stimulate immune responses. Cytokines can affect host defense in several target cells in a paracrine or autocrine approach. Cytokines include interleukins, chemokines, interferons, stimulating colony factors, and tumor necrosis factors (Savan and Sakai 2006).

11.18 Hormones

11.18.1 Growth Hormone (GH)

Growth hormone has various positive effects, including the stimulation of lipolysis, gluconeogenesis, protein synthesis, and immunological responses. Exogenous growth hormone (GH) is mitogenous in fish, promoting NK cells and superoxide leukocyte anions (Sakai et al. 1996). In addition, GH impacts immunocompetent cells directly, for example, macrophages, lymphocytes, and NK cells (Sakai 1999).

11.18.2 Lactoferrin

Lactoferrin (LF)—previously known as lactotransferrin (LTF)—is a glycoprotein involved in the innate immune system. Lactoferrin affects the development and multiplication of various pathogens, including bacteria, viruses, protozoa, and fungi. LF seems to impact innate immune cell function in gilthead sea bream, notably respiratory burst and natural cytotoxic activity (Esteban et al. 2005). Dietary feeding of bovine lactoferrin improved immunological indices and resistance to *Aeromonas hydrophila* infection in *Macrobrachium rosenbergii* (Chand et al. 2006).

11.18.3 Thyroxine

Thyroxine hormones are essential in the control of fish development and nutrition intake. All cells in the body need thyroid hormone to increase enzyme production necessary for cellular metabolism, particularly anabolic activities. A growing amount of research indicates that thyroid hormones are critical in controlling fish development, growth, and reproduction.

11.19 Immunostimulants: Their Use

To ensure that immunostimulants are used appropriately, it is critical to understand the animal's pace, dosage, type of administration, and health condition.

11.19.1 Immunostimulants at the Appropriate Time

Immunostimulants may be used in combination with or in instead of vaccination. Fish might be treated in advance of known illness episodes, such as seasonal exposure to a pathogen. The most convenient moment to provide immunostimulants and vaccines is concurrent with the fish's exposure to a particular antigen. Both chemicals are included in feed or supplied by immersion. Immunostimulants can be used before the spread of illness to minimize disease-related mortality. However, the amount and timing of each drug should be established. Immunostimulants may be used to reactivate the immune system after a period of immunosuppression triggered by stress.

11.19.2 Administration Route

Generally, the method/route of administration of an immunostimulant determines the effect. Immunostimulants may be delivered intravenously, subcutaneously, or orally. For giant fish/shrimp, an injection can be the most cost-effective process. In smaller shrimp/fish, the immersion approach could be the most cost-effective. However, these approaches are time-consuming, tedious, and frustrating. Oral immunostimulant administration is a non-stressful procedure that can be used on fish of any size but involves a large dose of the immunostimulant. Although oral and immersion approaches have been shown to enhance/stimulate the innate immune system against pathogens successfully, oral administration remains the most practical process.

11.19.3 Dosage

The correct dose largely dictates the immunostimulant's influence. In certain situations, increasing the dosage does not provide further defense or activation of the immune system. For example, levamisole and glucan at higher doses can inhibit the immune response, whereas low doses may be ineffective. The immunostimulant's role is not dosage dependent; higher doses do not stimulate but may suppress the immune system.

11.19.4 The Mechanism of Action of Immunostimulants

Immunostimulants are being investigated as a possible solution to antibiotics and chemotherapeutics. Immunostimulants, alternatively referred to as immunostimulators, activate the immune system by stimulating or increasing the activity of one or more of its components. Immunostimulants are substances of natural or manufactured origin with various chemical properties and action mechanisms. Immunostimulants are compounds that stimulate an animal's immune system, increasing its resistance to microbial infections. Immunostimulants confer tolerance to illness by supporting the nonspecific immune response. They stimulate/activate/enhance the function of immune components such as phagocytic cells, natural killer cells, complement, lysozyme, T and B cell activity, inflammatory agents, and macrophage activity. In shrimps, it increases phagocyte production, phenoloxidase activity, superoxide dismutase activity, complete hemocyte count, and respiratory burst activity, among other things.

Fish survival systems for infection control are based on biochemical mechanisms known as immunity. Immunity may be innate (nonspecific/natural) or acquired (specific/acquired). Innate immunity is present at birth and is not organism-specific. Since formation, exposure to microorganisms yields adaptive immunity. It is species-specific because it has immunological memories. The epithelial surface (gill, skin, and gut) acts as a mechanical shield to fish from pathogen attack. The epithelium's surfaces are covered with a mucous layer, which prevents bacteria, fungi, and parasites from attaching; additionally, it contains reactive substances such as iron-binding proteins (which inhibit bacteria's growth by limiting the amount of free iron available), lectins (a natural agglutinin that immobilizes bacteria by neutralizing endotoxins and facilitating phagocytosis), and C-reactive proteins. Additionally, the nonspecific protection mechanism includes chemical factors such as lysozyme (an antimicrobial substance), some antimicrobial proteins such as transferrin (which protects the cell from viral infection), complement (which facilitates phagocytosis and leads to inflammation), and natural killer (NK) cells (which destroy a broad range of microbes and produce tumor cells). Nonspecific immune cells such as leukocytes (macrophages, monocytic cells, neutrophils, and monocytic cells) contain microbial substances used to destroy bacteria intra- and extracellularly. Acquired or pathogen-specific immunity is critical for defenses against specific pathogens. Acquired immunity comprises humoral (immunoglobulin and T cell receptor production) and cellular components (graft rejection and delayed hypersensitivity). Immunostimulants are nutritional additives that boost the body's natural defensive mechanisms and improve tolerance to particular pathogens. Immunostimulants promote disease tolerance by enhancing nonspecific immune responses; thus, no memory portion is created, and the length of the immune response is relatively brief. In addition, immunostimulants promote phagocytosis by increasing the activity of phagocytic cells such as granulocytes, phagocytes, and macrophages and supplement activity in mucous and lymphocytes.

11.20 Conclusion

Aquaculture is certainly the most reliable food source in the future, particularly protein-rich meals for the increasing global human population. Poor practices and care issues, on the other hand, cause fish stress and contribute to susceptibility to disease and, as a result, to infections and disease outbreaks. Despite the harmful impact of these dissuasive pressures on aquaculture, there are elementary correctional measures available to protect a significant number of fish and, by extension, the aquaculture business. The manufacture of successful immunostimulants has been made with techniques of genetic engineering, which enhance the immunogenicity of the desired immune response. These immunostimulants may activate specific immunological processes without provoking a generalized immune response that has severely deleterious effects. The benefits of gene expression of biological factors employed as immunostimulants inside an animal are as follows: (1) the factors are stable and effective immune stimulants; (2) they have a particular antibacterial spectrum; (3) they are non-toxic to the host; and (4) drug resistance is unlikely to emerge. Thus, novel immunostimulants can become alternative bioactive agents, perhaps displacing conventional antibiotics, and future research may uncover new immunostimulant sources and applications.

Immunostimulants have the potential to be a viable replacement for vaccinations and antibiotics. Many of the immunostimulants used in finfish research have been microbial compounds like glucan and algal polysaccharides. These are not, however, pure homogeneous compounds. Immunostimulation caused by these compounds may be attributed to endotoxin and other impurities unless purified to homogeneity. Purification of plant or microbial products is thus required. This purification may indirectly or even directly benefit the environment by eliminating overexploitation of natural resources. Once a purified compound with its structural features is available, large-scale microbial or chemical production may reduce or even eliminate natural plant material requirements. The optimal method for giving immunostimulants was discovered to be intraperitoneal. Extracts provided to fish as a feed supplement may or may not have the same efficiency as those given parenterally. Farmers should augment their feed with immunostimulants with relative simplicity and at a lower cost if the extraction technique is not too expensive and repetitive. Finally, before drawing any conclusions, laboratory findings should be confirmed or repeated in large-scale agricultural environments. Furthermore, research professionals should be familiar with the appropriate indicators (e.g., ROS generation, ceruloplasmin) linked to disease resistance in fish species.

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Chapter 12

Production, Maintenance and Benefits of Seaweeds in Tropical Regions

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Abstract The mariculture, a type of aquaculture, is the process in which the seaweeds, a type of macroalgae, are cultivated. The seaweed involves various medicinal, biological, and socio-economical applications. The seaweeds are autotrophic and can produce numerous compounds with many important aspects. It has been used for a long time as a nutrient, which is rich in protein, dietary fibres and lipids. Along with the bio-molecules, it has numerous bioactive compounds with multi-potential uses in various industries. The products of the seaweed cultivation majorly involve the consumption by human beings, whereas the other major impact of seaweeds has been observed in the maintenance of marine food chain and marine ecosystem. The seaweed cultivation has been seen majorly around the coastal regions but it does not denote that coastal areas are necessary for seaweed cultivation. This chapter provides information about different types of seaweeds, their characteristics, lifecycle, production, application and cultivation techniques of the seaweeds, particularly in tropical regions. The discussion continues to know about their nutritional values, followed by the details about the different regions and countries that fall under the tropical category, and the three major production methods of seaweed cultivation along with its advantages and disadvantages and the factors that can harm the seaweed cultivation have also been discussed in this chapter. The chapter also covers the threats and harm to the seaweeds as well as the factors that increase the growth of the seaweeds.

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12.1 Introduction

Seaweeds are also known as marine algae which are distributed along the coasts from tropical to the polar region. It has been receiving much interest in the present era due to its potentialities. It is very nutritive as well as with numerous medicinal properties. This chapter provides information about different types of seaweeds, their characteristics, lifecycle, production, application and cultivation techniques, particularly in tropical regions.

12.2 Aquaculture

Aquaculture, which is also referred to as fish farming, is being practised all over the globe in the naturally occurring freshwater lakes to artificially built tanks (Food and Agricultural Organisations of the United Nations 2021). The aquaculture products are mainly used for consumption by human beings in the day-to-day diet. The aquaculture involves different stages beginning with hatchery, in which the eggs of respective fishes hatch artificially in a hood or cabinet. The hatchery stage is followed by feeding the hatched ones with appropriate nutrients and food till it attains the matured form. The matured or adult fish is taken for farming purposes, which is the third stage. The fishes, once matured and attain the stage to harvest in the farm, is then packaged and transported for various purposes (Crespi and Coche 2008).

Aquaculture may be regarded as one of the most prominent and efficient culturing since the benefits are considerably higher. Approximately around 50% of the global fish is consumed for dietary purposes (Food and Agricultural Organisations of the United Nations 2021). India ranks second in aquaculture among the worldwide nations and Andhra Pradesh ranks first among the states of India. Aquaculture falls into various categories like mariculture that uses seawater, fish farming that is performed with either seawater or freshwater, the algal culture that cultivates the algae, integrated multi-trophic aquaculture involving the combination of different tropical levels, inland pond culturing and open-net pen and cage systems that use the artificially constructed ponds and cages, re-circulating system culturing that involves the recirculation of water constantly, and flow through methods, in which the fish is stocked in a lengthier unit (Mirzoyan et al. 2010).

12.2.1 Seaweed

Seaweed is a macroalga, which may be red, brown, black or green in colour and are grown in the seawater and seashores (Townsend and David 2012). Seaweed is also referred to as sea vegetables and is a rich source of nutrients. The red algae fall under Rhodophyta division and it includes species like *Palmaria palmata, Gelidium amansii, Chondrus crispus, Porphyra* species, etc., the brown algae include Phaeophyceae class and the most common brown algae includes kelps and *Fucus* (Sheath and Robert 1984). The sea lettuce, which is *Ulva* spp., is being grouped under the green algae or Chlorophyta seaweeds. The marine environment is being enriched with macroalgae, which approximately involves 1800 different species of brown algae, 6200 species of red algae and 1800 green algal species. Among these vast species, brown algae are the larger ones (Cribb 1953).

12.2.2 Red Seaweeds

The red algae have the pigments phycoerythrin and phycocyanin, which may be responsible for the red colour (Kadam et al. 2013). In addition to these pigments, chlorophyll is also present in the red algae. Among these three pigments, phycocyanin is the most important (Cian et al. 2014). These compounds are widely used as a natural dye in milk products, soft drinks and also in cosmetic materials including eyeliner, lipstick, etc. In addition to this, the compounds also have potential health benefits with antioxidant, antimicrobial, anti-inflammatory, hepato- and neuroprotective properties (Sekar and Chandramohan 2008).

The red algae also contain polyunsaturated fatty acid, particularly eicosapentaenoic acid and arachidonic acid (Kumari et al. 2013). It is also having rich amounts of phenolic compounds, which is having more potent antioxidant properties. This seaweed produces polysaccharides including agar and carrageenans (Usov 2011). The agar is an important component of the cell wall. The unique property of the agar is the capability to form reversible gels; hence, it has wide uses in food additives (Imeson 2009; Liu et al. 2020). It also has many biological activities including antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, anticancer and hepatoprotective properties (Jin et al. 2017; Mazumder et al. 2002; Bhattarai and Kashyap 2016; Coura et al. 2015; Higashimura et al. 2013; Liu et al. 2017). The agar is used as stabilizing as well as a gelling agent in the food industry, cosmetics industry and pharmaceutical industry (Freile-Pelegrín and Murano 2005; Marinho-Soriano and Bourret 2005; Villanueva et al. 2010; Ganesan et al. 2020a, b).

Another important compound isolated from the red seaweeds is carrageenan. Much red seaweed produces more concentrations of carrageenan and is called carrageenan producers (carrageenophytes, Pereira and Mesquita 2003). It has been widely used in the pharmaceutical industry because of its antioxidant, antimicrobial, anticancer, anti-inflammatory, immunomodulatory and neuroprotective activity (Chattopadhyay et al. 2008; Souza et al. 2018 Vanderlei et al. 2011; Talarico et al. 2004; Yuan et al. 2006; Liu et al. 2013; Poupard et al. 2017; Sattanathan et al. 2020a, b). In the plant, the carrageenans stimulate defence mechanisms particularly against various viruses, increase the growth of the plant and also maintain abiotic stresses (Mercier et al. 2001; Sangha et al. 2011; Ghannam et al. 2013; Shukla et al. 2016).

12.2.3 Brown Seaweeds

The brown algae have the pigment fucoxanthin, which may be responsible for the brown colour, and the chlorophylls are also present (Chandini et al. 2008). The concentration of fucoxanthin may differ in different species of brown seaweed. Several studies suggest that fucoxanthin have antioxidant, anticancer and anti-obesity activities (Yan et al. 1999; Maeda et al. 2005; Pigmen et al. 2014). Myristic acid and palmitic acid are the saturated fatty acids present in large quantities in brown seaweed. In polyunsaturated fatty acids, the brown seaweeds contain arachidonic acid, linolenic acid and eicosapentaenoic acid (Khotimchenko 1998; Graeve et al. 2002). Plastoquinones and chromanols and chromenes are meroditerpenoids (phenolic compounds) present in the brown seaweeds (Reddy and Urban 2009). These phenolic compounds have potent antiviral (particularly HIV), antibacterial, anti-diabetic, anti-obesity and neuroprotective activity (Artan et al. 2008; Kim and Kong 2010; Murray et al. 2018).

Alginate is the most important polysaccharide in brown seaweeds and it has been found in the cell walls of the brown seaweed (Andrade et al. 2004). It has been used widely in the food industry, textile industry, cosmetic industry and also in the pharmaceutical industry (Wiltshire et al. 2015). It also controls glucose and cholesterol in the blood due to its dietary fibre character (Wolf et al. 2002). Laminarin is another polysaccharide present in brown seaweeds (Kadam et al. 2015). The concentration varies depending on the temperature and salinity of the water (Rioux et al. 2010). It can be used to activate macrophages leading to immunostimulatory, wound healing and anticancer activities (Kadam et al. 2015) and it also influences the metabolism in the intestine (Déville et al. 2007). Because of its fibre nature, it helps to reduce the cholesterol and triglycerides in the liver; hence it also controls blood pressure. The cell walls of the brown seaweed contain fucose-containing sulphated polysaccharides called fucoidans (Imbs et al. 2014). *Undaria pinnatifida* is rich in fucoidan. This can be used as a good anticoagulant agent; it also exhibits antiviral and antioxidant properties (Mandal et al. 2007; Chandía and Matsuhiro 2008). It is used to moisturize the skin; hence it has anti-ageing properties (Wijesinghe et al. 2012).

12.2.4 Green Seaweeds

The green seaweeds contain only chlorophylls and carotenoids. It contains polyunsaturated fatty acids including linoleic acid, palmitolinolenic and palmitidonic polyunsaturated fatty acids (Khotimchenko 1993; Khotimchenko et al. 2002; Kumari et al. 2010). The green seaweeds are rich in sulphated polysaccharides called ulvans. It is water-soluble and its concentration ranges between 18–29% (85–84). Ulvans are not digestible by humans, but they can act as dietary fibre. It is used in biomedical fields particularly in tissue engineering and drug delivery (Alves et al. 2013; Wijesekara et al. 2011; Venkatesan et al. 2015; Cunha and Grenha 2016). These compounds have potent antioxidant, antimicrobial, anti-tumour and hypolipidemic activity (Alves et al. 2013).

12.3 Physical Characteristics of Seaweed

The seaweeds contain various parts such as conceptacles which contain the reproductive structures of the algae with a receptacle, a swollen tip of the fertile frond. The algae are attached firmly to the substratum or any surface with the help of a hapteron, which is also referred to as holdfast (Dayton 1985; Graham 2004). The hapteron extends to a stripe that is tough and flexible and elevates the algae above the surface and along with lamina (also named as a blade) it helps to withstand the wavy action. Apart from these parts, seaweeds also possess mid-rid, which is the central vein of the lamina and air bladders that are capable of keeping the lamina buoyant and near the surface of the water for the purpose of photosynthesis in an efficient manner. The lamina contains the midrib medulla at the centre and the cortex cells as an extension from the midrib medulla. It is then covered by the epidermis layer.

12.4 Lifecycle of Seaweed

The life span of seaweed varies, some are annual and some of them have a longer life span of 6–10 years. Seaweed reproduction happens via its spores. The male and female reproductive cells, i.e. zoospore matures into male gametophyte and antheridia followed by female gametophyte respectively. The male gametophyte matures to sperm and the female gametophyte matures to egg followed by the fusion of egg and sperm forming the zygote. The zygote grows into sporophytes and matures as seaweed (Fig. 12.1).

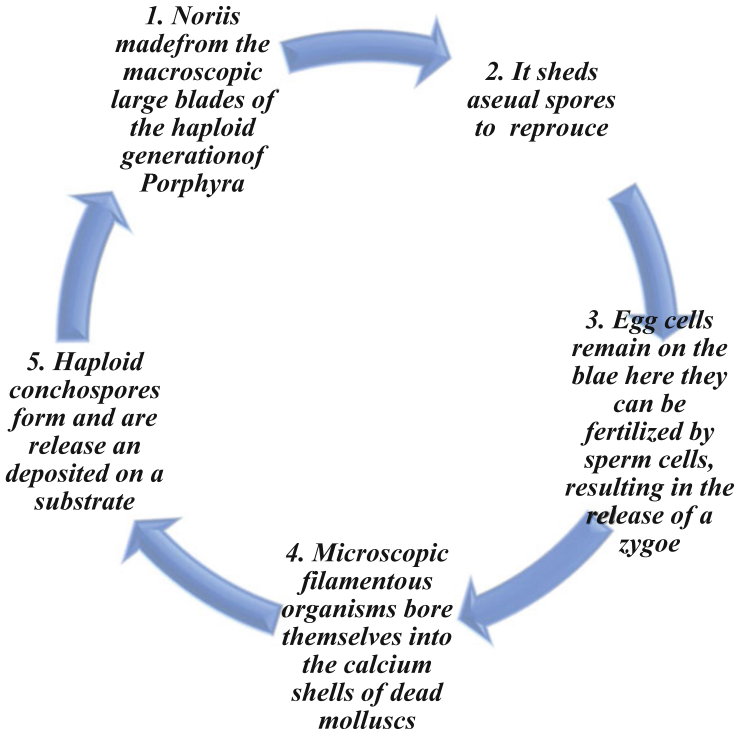

Fig. 12.1 Reproduction in seaweed

12.5 Seaweeds: Production

The production of seaweed is being elevated by about 5.7% approximately every year globally, according to Fisheries and Aquaculture (FAO). According to FAO, Asian countries are predominant in seaweed culturing accounting for about 99.05% in quantity and 99.36% in quality (Fisheries and Aquaculture 2014). Around 98% of the seaweed harvested worldwide, and five genera of seaweed were found to be predominantly harvested, namely *Saccharina*, *Undaria*, *Porphyra*, *Eucheuma*/*Kappaphycus* and *Gracilaria* (Suo and Wang 1992; Pereira and Yarish 2008).

12.6 Nutritional Content in Seaweed

Seaweed has been found to have various beneficial properties for humankind, with a wide range of nutrients like minerals, vitamins, proteins, carbohydrates and fibres (Paul et al. 2007). Since ancient times, seaweeds have been cultivated and consumed by humans and as feed for live stocks. It has been reported that *Pyropia* spp., (Nori) has 1.5 times higher vitamin C content than oranges. All the seaweeds categorized under the Phaeophyceae family have a sufficient amount of vitamin B (Salehi et al. 2019). Depending upon the species, the carbohydrate content might vary from 20% to 76% (dry weight). Seaweeds have a higher amount of carbohydrate which is stored in the form of functional and structural polysaccharides (Holdt and Kraan 2011). The composition of fat varies remarkably between different types of seaweeds. The polyunsaturated fatty acids and lipid content of seaweeds are reported to increase during the cold and summer season, respectively (Rajapakse and Kim 2011). Seaweeds are also a vital source of iron.

The iron content of *Sargassum* spp. seaweeds is 156.9 mg/100 g of dry weight and it also aids in the absorption of iron from a rice meal. The iron content of *Sargassum* spp. may vary from 81 to 290 mg/100 g of dry weight and the maximum content is recorded during the month of July (Cherry et al. 2019). Genus *Gracilaria* (red algae) is the most cultivated seaweed which is a great source of both insoluble and soluble dietary fibres. The insoluble dietary fibre helps in improving gastrointestinal and cardiovascular function. Soluble fibre aids in the reduction of cholesterol and glycaemia (Rosemary et al. 2019).

The brown algae rank in the topmost as a good source of iodine. The fat content of the seaweed has been found to be lower when compared to the other nutrients, whereas the protein and calcium contents range from higher to lower and are species-specific in nature. The brown algae have lower protein content, which accounts for only up to 15%, whereas the red algae and green algae have higher protein content ranging up to 30% (Kolanjinathan et al. 2014). Another *Ulva* species, called *Ulva lactuca,* green algae has been found to have a high protein and carbohydrate content but a lesser fat content (Rasyid 2017).

Fucoidan derived from *Undaria pinnatifida* were reported to have greater anti-diabetic and anticancer effects. Koh et al. (2020) reported that fucoidan had prominent activity on amyloglucosidase, α- amylase and α-glucosidase. Lu et al. (2018) demonstrated that fucoidan obtained from *U. pinnatifida* significantly exhibited proliferation inhibition in breast cancer cells. Traditionally, brown seaweeds are used in thyroid goitres treatment (Salehi et al. 2019). Fucoxanthin derived from *Sargassum horneri* was found to be effective in reversing the cognitive impairment caused by scopolamine in mice (Lin et al. 2016). Ulvan, fucoidan and S-heterogalactan have displayed strong antioxidant activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) (De Jesus Raposo et al. 2015; Le et al. 2019; Zhao et al. 2018). Sulphated polysaccharide (fucoidan) derived from *Turbinaria decurrens* and *Dictyota bartayesiana* displayed maximum antiretroviral activity (Sanniyasi et al. 2019).

Almost all the seaweeds contain nutrients such as vitamins (A, B1, B2, C, E and K), calcium, folate, potassium, iron, manganese and copper. *Himanthalia elongate,* a brown seaweed, which is commonly referred to as rockweed, has been ranked top among the seaweeds with higher fibre content. It has a total fibre content of about 9.8 g/100 g net weight, whereas other brown algae, *Ascophyllum nodosum* contains 8.8 g/100 g net weight fibres ranking it into the second position. The various mineral composition of seaweeds has been given in Table 12.1 (Mac Artain et al. 2007).

Table 12.1 Mineral composition of some economically important seaweed

Seaweed	Ca	K	Mg	Na	Cu	Fe	Iodine	Zn
Ascophyllum nodosum	575.0	765.0	225.0	1173.8	0.8	14.9	18.2	–
Laminaria digitata	364.7	2013.2	403.5	624.6	0.3	45.6	70.0	1.6
Himanthalia elongata	30.0	1351.4	90.1	600.6	0.1	5.0	10.7	1.7
Undaria pinnatifida	112.3	62.4	78.7	448.7	0.2	3.9	3.9	0.3
Porphyra umbilicalis	34.2	302.2	108.3	119.7	0.1	5.2	1.3	0.7
Palmari apalmata	148.8	1169.6	97.6	255.2	0.4	12.8	10.2	0.3
Chondrus crispus	373.8	827.5	573.8	1572.5	0.1	6.6	6.1	–
Ulva spp.	325.0	245.0	465.0	340.0	0.3	15.3	1.6	0.9
Enteromorpha spp.	104.0	351.1	455.1	52.0	0.1	22.2	97.9	1.2

Ca calcium, *K* potassium, *Mg* magnesium, *Na* sodium, *Cu* copper, *Fe* iron, *Zn* zinc.

12.7 Applications of Seaweed

The seaweed varieties are involved in various applications environmentally and biologically (Fig. 12.2). The environmental applications include the protection of coastlines against soil erosion (Dayton 1985) as well as the significant contribution towards the aquatic carbon cycle (Thiel et al. 2007; Ugarte and Sharp 2011). The marine food chain is being maintained and balanced mainly by seaweeds. The nutritional contents and energy are directly provided to the marine animals by eating the seaweeds directly or indirectly after being decomposed into small particles. The coastal animals have their shelter and habitat in the beds of seaweed for their whole life or for the part of their life. Seaweeds serve as nurseries for the species that are commercial in nature like rock lobster, abalone, etc.

Seaweed has been widely used in the treatment and management of human diseases as they were found to have some medicinal properties. The seaweed has the capacity of reducing the cholesterol levels of plasma that paves the way for using the seaweed in the management of cardiovascular disease, which is mainly caused due to high cholesterol levels (Imenez-Escrig and Sanchez-Muniz 2000). The seaweeds were also found to have antimicrobial, antifungal and anti-inflammatory properties. Erythema, a disease caused mainly due to increased flow in superficial capillary blood, may be suppressed by seaweeds, *Undaria pinnatifida*, a brown seaweed, and *Ulva linza,* green seaweed. Another clinical condition that may be controlled by these two seaweeds is oedema (Mohammed et al. 2008).

The hyperglycaemic condition may be effectively suppressed by a type of brown algae, *Pelvetia babingtonii,* as it has the potential to inhibit α-glucosidase activity (Ohta et al. 2002; Smit Albertus 2004). Seaweeds can also be used against cancer, diabetes and also against certain viral infections. The antagonistic compounds present in algae have made them capable of functioning as antibiotics, which can be used against infections by bacteria, viruses and fungi (Hoppe 1979). Extracts of red seaweeds like *Solieria robusta*, brown algae like *Lyengaria stellata*, *Colpomenia sinuosa*, *Spatoglossum asperum* and green algae like *Caulerpa racemose* have exhibited hypolipidemic activities. Serum total cholesterol levels, triglyceride ranges

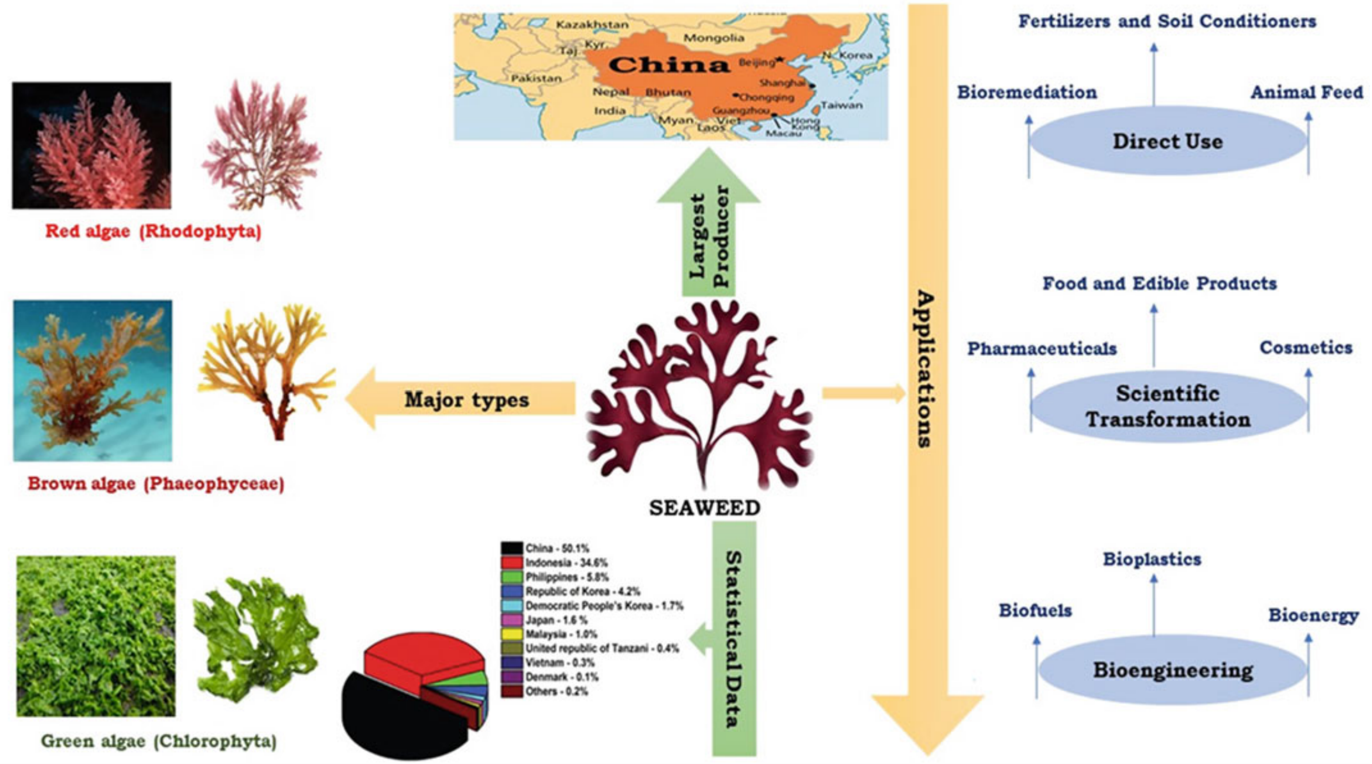

Fig. 12.2 Facts about the seaweeds

and low-density lipoprotein levels decline in the animal models after the treatment with these algal extracts (Ara et al. 2002; Smit Albertus 2004).

Seaweed has an important role in improving thyroid function as they are the rich source of iodine (Lazarus and Smyth 2008; Smyth et al. 2016; Smyth 2021). Gut health can also be improved by the seaweed intake as they possess carrageenan, agars, fucoidans, which act as prebiotics, non-digestible fibres and also the sulphated polysaccharides increase the beneficial bacteria growth in the gut as well as the short-term fatty acids that paves a way for healthy gut lining (Cherry et al. 2019; Zaporozhets et al. 2014). Fucoxanthin is present in the brown algae that have a role in managing the blood glucose level (Peng et al. 2011; Airanthi et al. 2011; Mise et al. 2011; Heo et al. 2010).

12.8 Tropical Regions in the World

Geographically, the Equator that is considered as the line through the centre of our planet falls at zero degree latitude, dividing the earth into Northern and Southern hemispheres. The regions that are found around the Equator are referred to as tropical regions. Approximately around 40% of the total surface area on Earth and 36% of the landmass together comprises tropical regions in the world (National Geographic Encyclopaedia 2017). India, an Asian country in the southern part of the continent, falls almost in the tropical region, whereas all the other countries in Southeast Asia fall in the tropical regions totally (Morgan 2011). The list of tropical countries in the world has been given in Table 12.2.

12.9 Tropical Marine Life

Ocean majorly influences the tropical marine climate. The tropical marine climate is commonly observed in the islands and the regions around coastal areas that are located 10°–20° either north or south to the Equator. The main seasons in the tropical marine climate are wet season and dry season, with the temperature ranging between 68 ° F and 95 ° F. The rainfall measures over 39–59 in. annually. The wind blows and passes through the warm seas and retains its moisture throughout the year. The countries like Brazil, Madagascar and Queensland, etc., are majorly in the tropical waters and exhibit this kind of moisture winds throughout (Filho and Walter Leal 2017; Wilson and Mark 2016).

Table 12.2 List of tropical countries across the world

Continent	Country
North America	Mexico
Central America	All of Central America
North and Central America	All of the Caribbean islands
South America	Colombia
South America	Ecuador
South America	Peru
South America	Bolivia
South America	Colombia
South America	Venezuela
South America	Guyana
South America	Suriname
South America	French Guiana
North America	Chile
North America	Argentina
North America	Paraguay
North America	Brazil
Western Asia	Yemen
Western Asia	Parts of Saudi Arabia
Asia	Oman
Asia	United Arab Emirates
Australia	Australia
Oceania	Micronesia
Oceania	The Marshall Islands
Oceania	Kiribati

12.10 Seaweed Cultivation in Tropical Regions

The artificial method of cultivating the seaweed is called as mariculture method. Seaweed cultivation has reached 27% of the total marine aquaculture production with the production of about 27.3 million tons annually in the year 2014 and the growth rate has been estimated to be 8% per year from 2014. The favourable climatic conditions for seaweed cultivation have been listed in the following paragraph.

The temperature of the water, in which the seaweed has to be cultivated, should be optimum between 25 °C and 30 °C, in order to have the best growth. The salt content in the water, which is denoted by the term "salinity", should be ideally more than or equal to 28 ppt (parts per thousand). The movement in the water that indicates the speed and direction of the water is known as water current and the moderate range is considered for better seaweed growth as the strong water current can wash away the seaweeds. There are various methods available for cultivating seaweeds and three of them are majorly used. Those methods are discussed as follows.

12.10.1 Off-Bottom Method

The off-bottom method is also referred to as the fixed bottom method. This method involves the placement of wood stakes at 20–25 cm apart from each other, into the bottom of the sea in the straight line in different rows. The preferred diameter of the stakes is between 5 and 10 cm and the ideal length is 1–1.5 m. Approximately about 500 centimetres of the stake is buried in the soil or sand in order to stand strong against the water currents safely. The following Fig. 12.3 shows the diagrammatic representation of the off-bottom method of seaweed cultivation.

A polypropylene rope of about 5 m in length has been stretched strongly in between the two stakes. The preferred thickness of the polypropylene rope is about 3 mm. Thirty pieces of polypropylene strings, known as raffia, are attached to the rope. The seaweed, which is to be planted, should be approximately about 150 g in weight to be tied to the raffia or polypropylene strings. The polypropylene rope should be suspended at the height of 20–30 cm above the sea bottom in order to protect the seaweed that grows from being blended into the sand and at the same time, 20–30 cm underneath the surface of the water in order to avoid the direct exposure of seaweeds to the sunlight as the seaweed would be killed when exposed directly to the sunlight. The stakes are preferred to be sharper at the ends to penetrate the sand or soil in which they are placed. The most preferred stakes are mangrove or bush timber that can withstand the seawater for a longer period without rotting.

The major advantages of the off-bottom method of seaweed cultivation are that it is simpler to construct as well as easier to manage. It is economically low and the farm may be easier to reach the low tide areas. Although there are advantages, it also has few disadvantages like locating a good area for farming is being difficult and the rough weather can damage the seaweeds as the farm cannot be moved when it is in unfavourable conditions. The grazer fishes, which can damage the seaweeds, are easier to be found around the crops and a drying rack needs to be constructed separately.

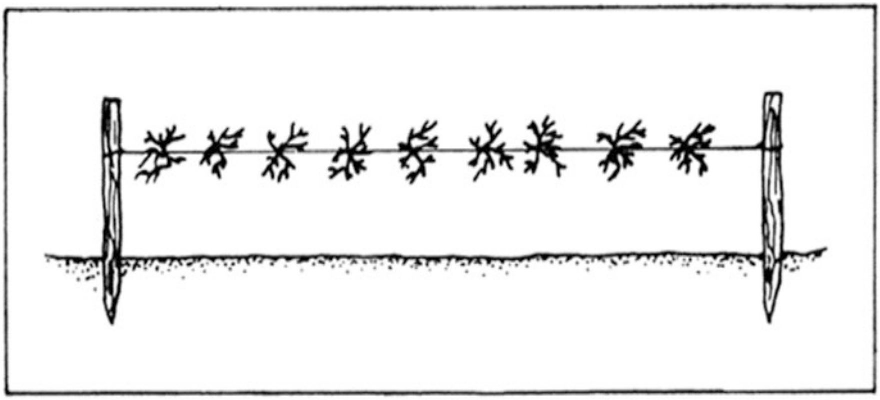

Fig. 12.3 Off-bottom method

12.10.2 Raft Method

The raft method is also named the floating method. As the name implies, seaweed cultivation by using this method involves the floating of seaweed by attaching them to a floating device that can move along the tides of the seawater accordingly. The floating device may be the simple frame that is made up of wood (mangrove), bamboos or bush timber which are readily resistible to the seawater for a longer period of time. The method involves the seaweed placement attached to the floating device to be underneath the surface of the water at least 50 cm. The preferred length of the floating device is 2.5 m that is made into a square and the ropes to be tied into the square frame in definite rows. The ropes of ideal size are 3 mm and these ropes can make up to 15 rows with 10–15 cm distance in between each of them. As with the off-bottom method, raffia is tied to the ropes and about 15 pieces of the crops are tied to the single row. Figure 12.4 shows the model of the floating method of cultivation of seaweed.

Like the off-bottom method, the floating method also has its own advantages and disadvantages. The advantages include the convenient utilization of the shallow and deep waters for the cultivation and the sandy sea bottom is not necessary for seaweed growth. Moreover, the floating rafts are easier to move to the various places and the planting can be done on the shore and rafts can be moved after that into the seawater conveniently. No drying rack is needed to be constructed separately and the seaweed growth has been observed to grow faster in the floating or raft method of cultivation. Even though, it has these advantages; it is difficult to find bamboo or other floating materials to construct the frames that are resistant to seawater for a longer duration.

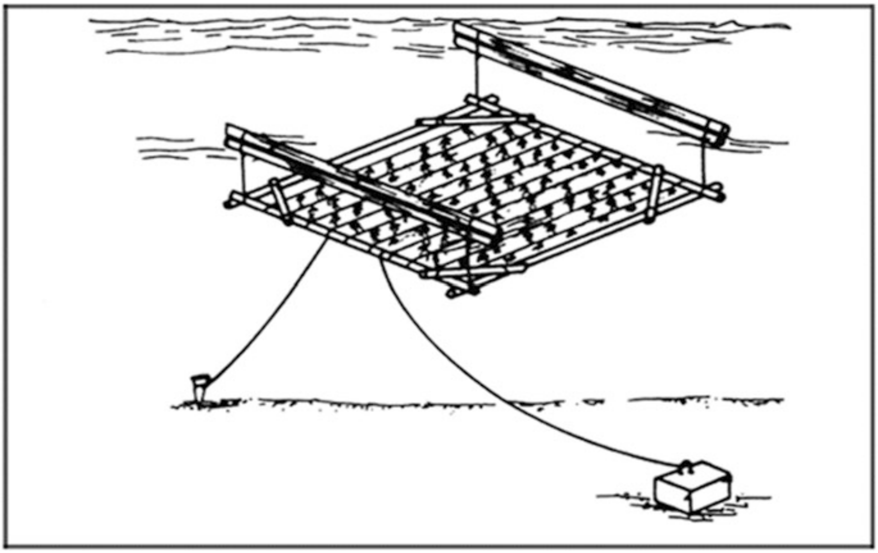

Fig. 12.4 Raft method

Rough weather may not favour the framework and the frames may break or sink into the seawater during the rough weather. The sea carriage like boats may damage the rafts and the frames cannot be handled single-handed.

12.10.3 Long Line Method

The long line method is another way of cultivating the seaweed and it shares a lot of similarities with the floating method. The long line method involves the hanging of seaweeds to be cultivated to the rope that is suspended by the floating materials. The rope diameter is preferably 10–15 mm, which is tied to the floating materials that are about 4–5 m in length. The raffia holds the seaweed like that of the other two methods. The seaweed attachment to the raffia can be done in two ways.

The first method involves the attachment of 0.5–1 m rope to the mainline and the raffia are to be attached to the additional ropes. The second way involves the attachment of raffia directly to the main lines. Figure 12.5 shows the long line method of seaweed cultivation. The seaweeds should be hanged to the mainlines in a way such that they are capable of receiving sufficient sunlight. The better distance is that the suspension should be made 0.5 m underneath the water surface.

As with all the other methods, the long line method also has its own advantages and disadvantages. The advantages include the convenience of setting up the long line almost anywhere compatible and the growth of seaweeds are faster. The long lines can be moved to other areas if necessary, during unfavourable climatic conditions. Even though it has these positive sides, the negative side involves the economy, since the ropes for long lines are costlier. The harvesting and plantation on the shore are not easier and the floaters are costlier than the other methods. Like the floating method, the long line method can also be damaged by the sea carriage like motor-driven boats that disturbs the water current.

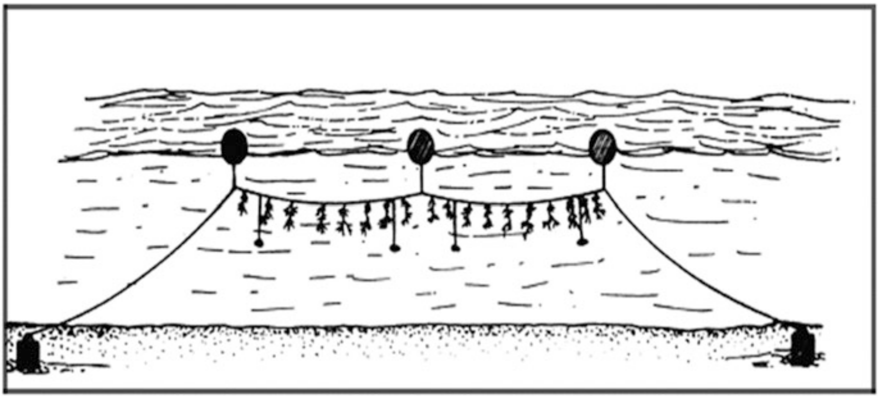

Fig. 12.5 Long line method

12.11 Harm to Seaweed

Although the seaweed can be cultivated and maintained by various methods, they have certain harm created naturally as well as artificially. Those harmful factors are discussed as follows.

12.11.1 Natural Predators

The natural predators are none other than the grazers of the seaweed. The seaweed grazers majorly involve the rabbit fish and puffer fish. The other aquatic animals like sea urchins and sea turtles also damage the seaweed plants and the rabbit fishes are also the major destructors of seaweeds. But the rabbit fish lives around the coral heads majorly and it is better to cultivate the seaweed farms away from the coral head areas (Fig. 12.6a, b).

The rabbit fish is not a major problem during the summer season or during the warmer climatic conditions. The unused stakes of wood or other rubbish materials are the home of rabbit fish and the other predators, and hence it is better to keep the seaweed cultivation region neat and clean.

12.11.2 Diseases

There are no specific diseases that affect seaweeds and one of the conditions is whitening of the branches, which may be a fungal infection. The discolouration of the branches makes them appear white or pink in colour.

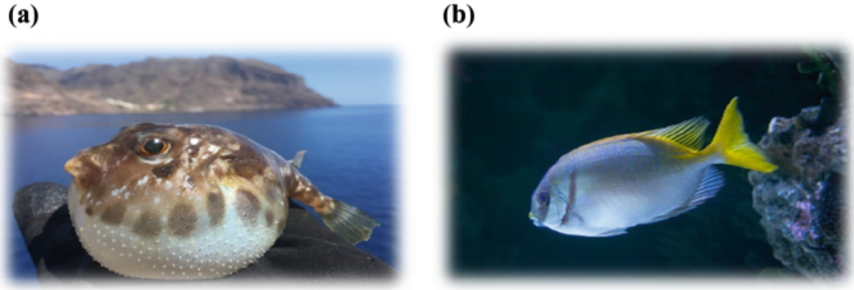

Fig. 12.6 (**a**) Puffer fish, (**b**) rabbit fish

12.11.3 Weather

The bad weather or climatic condition includes the rough water currents and heavy rainfall, as well as the cyclones that occur during the summer season are more dangerous. Since natural disasters like cyclones and heavy rainfall cannot be avoided or prevented, safety measures may be taken in order to reduce the destruction or loss. Harvesting the seaweeds before the period of the expected cyclones and can be protected from wind and rain by placing them on the shore. In the seaweed cultivation of *Kappaphycus alvarezii* in Saugi Island during the rainy season and dry season, the rainy season was found to favour its cultivation and enhance its production (Ali et al. 2017).

In Tanzania for the marine algae environment, the temperature of seawater at its surface is about 24.3–29.7 °C. The nutrients of seawater are found to vary during different seasons. In most temperate areas, the NO_3^- level of seawater in its surface undergoes a strong seasonal cycle because of seasonal thermoclines and it is found that in winter NO_3^- levels are maximum about 5–20 micromolar based on the geographical location, whereas in spring the rise in temperature results in the minimal level of NO_3^- due to the thermocline forms (Roleda and Hurd 2019). The damaged seaweeds must be replaced by the new seaweeds and the tangled lines may be entangled and secured further.

12.11.4 Factors That Increase the Seaweed Growth

The fertilizers with phosphate content can be preferred while the urea and KCl can be avoided. Maximization of the depth of water in the pond and minimizing the water exchange percentage can help in the seaweed production efficiency. Increasing the pH of the soil and lowering the Fe level in the water can influence the positive growth of seaweed. Reducing the epiphyte population by improvising the density of milkfish also aids in the development of seaweed (Mustafa and Sammut 2010). The nitrate concentration in the rainy season of seaweed cultivation was about 1.12–2.17 ppm whereas during the dry season it was about 1.24–1.96 ppm. The pH of the water during the rainy season was about 7.68–8.14 and during the dry season 8.25–8.39 (Ali et al. 2017). In common the factors like change in temperature, dissolved oxygen and phytoplankton production contribute to the alteration in pH of the water (Chen and Durbin 1994). The physical-chemical and biological factors are assumed to affect the absorption rate of nutrients (Harrison and Hurd 2001). Knowledge about the nutrients like nitrogen, phosphorous and carbon requirements for the seaweeds are a must for its cultivation (Hurd et al. 2014). When there is a higher requirement of nutrients more than its supply, then the nutrient starts to limit the growth of the seaweed, which is known as limiting (Harrison and Hurd 2001). *Fucus vesiculosus* Linnaeus during the limitation in nitrogen content it was to increase the uptake of phosphate (Perini and Bracken 2014).

12.12 Conclusion

The technologies for cultivating seaweeds have developed an interest for the past decades because of their nutritional and medicinal importance. Recent research urged to increase the production of more seaweed due to its high demands in the market. Hence, it is highly essential to optimize the new cultivation techniques with eco-friendly by multi-disciplinary approach with healthy and cost-efficient.

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Chapter 13
Pharmacological Importance of Seaweeds

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Abstract The seaweeds are the advantageous organism present in water bodies. Marine algae belong to thallophyta in plant kingdom and are classified into three groups, namely Phaeophyceae, Rhodophyceae and Chlorophyceae. The seaweeds contain expansive bioactive compounds which make them therapeutically important. Marine algae are also employed in production of functional foods, pharmaceutical products, cosmetics industries, nutraceuticals and fertilizers which makes them economically valuable. The secondary metabolites like fucoidan, phlorotannin, meroterpenoids, laminarin, fucoxanthin, bisindole, carrageenan, ulvan, lectins and fucosterol present in seaweeds are found to be effective against various diseases like diabetes, depression, cancer, cardiovascular disease, neurodegenerative disease, inflammation and obesity. Various pivotal pharmacological activities of the seaweed are discussed in this chapter.

Keywords Seaweeds · Marine algae · Pharmacological activity · Bioactive compounds · Seagrasses · Phytoplankton

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13.1 Introduction

Marine flora is the reservoir of novel bioactive compounds which are significant in health promotion and drug development. Seaweeds (Marine algae) are beneficial eukaryotic organisms that dwell in salty water and are regularly called sea vegetables. Marine algae are unicellular, non-flowering, photosynthetic organisms and 1 and 50 μm in diameter. Marine algae do not possess true stem, leaf and roots but they have holdfast that appears like a root in terrestrial plants, strips that act as stem and blades that are similar to leaves (Romero et al. 2021). Three kinds of plants are present in seas; they are classified as sea grasses, phytoplankton and marine algae. In the plant kingdom marine algae belong to Thallophyta. Marine algae are categorized into three types, namely Rhodophyceae (red algae), Chlorophyceae (green algae) and Phaeophyceae (brown algae) based on their morphological character, pigments and reproductive structures (Kolanjinathan et al. 2014).

Red algae are thin and delicate, abundantly found in intertidal to the sub-tidal area in sandy shores that comprise nearly 7000 species. In red seaweeds, chlorophyll (green pigment) is masked by phycoerythrin (Red pigment) which is employed in photosynthesis. Carrageenan, allophycocyanin, phycoerythrin, phycocyanin, mycosporine-like amino acids and α, β-carotenoids are common bioactive compounds in red marine algae. Red seaweeds are mostly used in food industries as binding or gelling agent (puddings and ice creams) and preparing pharmaceutical products (Sabarianandh et al. 2020). Brown algae are huge, yellow or golden-brown colour varying from small filamentous to complex form which is found in shallow rocky. They are known as dusky plants which belong to the Phaeophyta phylum and 1500–2000 species are available. Brown algae contain a unique secondary metabolite called phlorotannin. Brown seaweeds are widely employed in functional foods as an emulsifying agent, pharmaceuticals and cosmetics industries because of the bioactive compounds like fucoidan, alginates and laminarin present in the cell wall of brown seaweeds (Kandale et al. 2011; Ganesan et al. 2020a, b; Hakim and Patel 2020). Green algae are eukaryotes found in less salinity area on rocky as well as sandy beaches. Due to the presence of chlorophyll (green pigment) which is essential for photosynthesis, they appear green in colour. They are present in three forms like colonial, unicellular and multicellular (Kılınç et al. 2013). The present chapter is aimed at compiling the importance of secondary metabolites and pharmacological activities of seaweeds.

13.2 Anti-diabetic Activity

Methanolic extract of *Ecklonia cava* was tested for anti-diabetic activity in mice induced with streptozotocin. *Ecklonia cava* extract was potential in reducing the glucose concentration in serum and maintained glucose homeostasis. It also reduced the total cholesterol and triglycerides levels in the liver (Kim and Kim 2012).

Ethanolic extract of *Sargassum polycystum* with various concentrations (150 and 300 mg/kg) was tested for anti-diabetic activity in diabetic rat injected with streptozotocin. After 22 days of treatment with *Sargassum polycystum* extract, the rats displayed anti-diabetic activity by decreasing the glycosylated haemoglobin and blood glucose levels and also reduced the atherogenic index, total cholesterol and triglyceride levels (Motshakeri et al. 2013). Aqueous extract of Malaysian seaweed species *Sargassum binderi*, *Halimeda macroloba*, *Turbinaria conoides* and *Padina sulcata* were investigated for anti-diabetic activity. Among them, *Halimeda macroloba* (green seaweed) has shown 70.58% maximum inhibition against the α-glucosidase (Chin et al. 2015).

Unnikrishnan et al. (2015) studied two edible seaweeds of *Sargassum* sp. (*Sargassum wightii* and *Sargassum polycystum*) for their antihyperglycaemic capacity using different extracts. Ethyl acetate and petroleum ether extracts of *Sargassum wightii* displayed higher inhibitory activity against α-glucosidase and followed by this methanolic extract displayed an inhibition effect on dipeptidyl peptidase-IV. Whereas *Sargassum polycystum* (methanolic extract) displayed maximum inhibitory action against dipeptidyl peptidase-IV and α-glucosidase. Three seaweeds, namely *Padina tetrastomatica*, *Caulerpa scalpelliformis* and *Acanthophora spicifera,* were tested for their antihyperglycaemic activity against alloxan-induced rats. After 28 days of the experiment, the results displayed that ethanolic extract of *Acanthophora spicifera* had a significant effect against diabetes-induced rats (Radhika and Priya 2015).

Unnikrishnan and Jayasri (2017) investigated the anti-diabetic capacity of *Chaetomorpha antennina* green algae using different solvents including acetone, benzene, methanol, ethyl acetate and petroleum ether in both in vivo and in vitro models. The finding proved that in in vitro studies the methanolic extract was successful in inhibiting the dipeptidyl peptidase IV (DPPIV), α-amylase and α-glucosidase, and in in vivo condition, blood glucose levels were found to be reduced in streptozotocin-injected rats upon treatment with methanolic extract for 28 days. The acetone extract of *Undaria pinnatifida* and *Laminaria digitata* had shown 92.04% and 64.15% significant inhibition against α-glucosidase, respectively, when compared with other brown seaweeds like *Sarcothalia crispate* and *Sargassum polycystum*. The maximum inhibition was displayed by *Undaria pinnatifida* which is due to the presence of bioactive compound fucoxanthin (Zaharudin et al. 2019). Compounds sulphated polygalactans and dieckol extracted from red and brown seaweeds have also displayed anti-diabetic activity (Sun et al. 2018).

Nasirian et al. (2019) studied the antihyperglycaemic effect of *Nannochloropsis oculata* microalgae in Wistar rats induced with Streptozotocin. The results presented administration of *Nannochloropsis oculata* has significantly reduced serum glucose concentration in the diabetic group. Nguyen et al. (2019) found that ethyl acetate extract of *Laurencia dendroidea* was capable of inhibiting α-glucosidase in gliclazide-induced diabetic mice. Three hours after administration with *Laurencia dendroidea*, diabetic mice were diagnosed with reduced levels of glucose in the blood. Yuan et al. (2019) examined the antihyperglycaemic effect of polyphenol obtained from *Lessonia trabeculate*. The results demonstrated that after 4 weeks of administration, polyphenols were effective in lowering the fasting glucose levels in the blood and also efficient in managing oxidative stress and lipid profile. Upon treatment with fucoidan, glucose uptake was found to be stimulated in normal adipocytes (Sim et al. 2019).

Ismail et al. (2019) tested six seaweeds *Ulva lactuca*, *Padina pavonica*, *Pterocladia capillacea*, *Turbinaria decurrens*, *Sargassum muticum* and *Sargassum acinarium* for anti-diabetic activity using five different extracts. The results displayed that ethanolic and acetone extracts were found to be effective. *Turbinaria decurrens* (acetone extract) presented higher inhibition of 96.1% followed by the *Sargassum muticum* (ethanolic extract) with 94.3% inhibition and *Sargassum acinarium* (ethanolic extract) displayed 91.8% of inhibition on the α-amylase enzyme. On α-glucosidase enzyme, maximum inhibition of 97.4% was exhibited by *Turbinaria decurrens* acetone extract at 90 mg/ml concentration. Aroyehun et al. (2020) investigated the *Caulerpa racemosa* for its anti-diabetic activity in rats with diabetes-induced through streptozotocin administration. The *Caulerpa racemosa* (ethyl acetate extract) displayed a considerable anti-diabetic effect in rats by reducing glucose levels. Streptozotocin-administrated diabetic rats were examined for anti-diabetic activity; rats were treated with polysaccharides isolated from *Undaria Pinnatifida*, *Ascophyllum nodosum* and *Fucus vesiculosus* algae. The finding indicated that *Fucus vesiculosus* was potential in managing kidney and liver damage produced by hyperglycaemia (Jia et al. 2020).

Fucoidan belongs to the sulphated polysaccharide group which is found in various brown seaweeds. Fucoidan isolated from the *Undaria pinnatifida* was investigated against starch-hydrolysing enzymes and the results displayed that fucoidan had remarkable activity on α-glucosidase, α-amylase and amyloglucosidase (Koh et al. 2020). Zhong et al. (2021) studied the anti-diabetic mechanism of polysaccharides in *Undaria pinnatifida* using in vivo (streptozotocin injected mice) and in vitro (HepG2 cells) methods. In vivo studies suggested that Up-4 and Up-3 are potential inhibitors of α-glucosidase. In vitro condition exhibited that Up-4 and Up-3 showed a noticeable decrease in the postprandial glucose level. Daub et al. (2020) found that fucoidan, an active compound present in the aqueous extract of *Ecklonia maxima* seaweed, has a strong effect on α-glucosidase and α-amylase. Priatni et al. (2021) cultivated and screened five different sp. of marine microalgae for α-glucosidase inhibition. *Porphyridium* sp. presented maximum inhibition (12.63%) of the α-glucosidase enzyme after 8 days (Fig. 13.1).

13.3 Anti-obesity Activity

Awang et al. (2014) studied the anti-obesity effect of *Sargassum polycystum* in rats induced with a high-fed diet. The rats were treated with different ranges of dosage of *Sargassum polycystum* (2.5, 5.0,10%). The results demonstrated that at 10% dosage exhibited higher degree suppression of weight gain and also reduced the

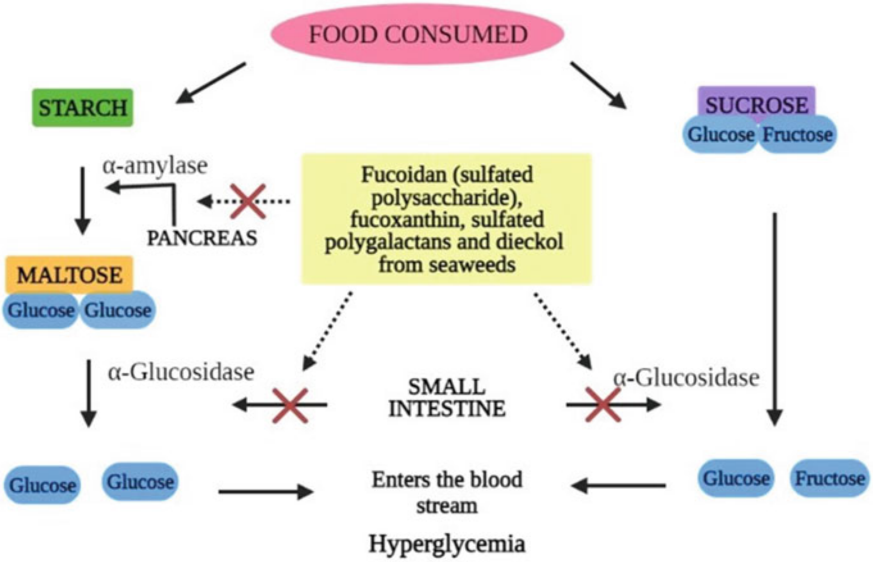

Fig. 13.1 Anti-diabetic activities of seaweeds

triglycerides and total cholesterol levels in plasma. Kwon et al. (2014) examined various compounds (dieckol, 8,8′-bieckol, 6,8′-bieckol, 6,6′-bieckol and phlorofucofuroeckol A) obtained from *Eisenia bicyclis* against anti-adipogenesis in 3 T3-L1 cells. The results depicted that phlorotannins have inhibited the adipocytes differentiation and 6,6′ -bieckol has significantly reduced the accumulation of lipids and the expression of adipogenic proteins.

Kang et al. (2017) studied six indole derivatives isolated from *Sargassum thunbergii*. The results suggested that indole-2-carboxaldehyde and indole-2-carboxaldehyde had an inhibitory effect on the accumulation of lipids, differentiation of adipocytes and expression of adipogenic proteins. Nakayama et al. (2018) found that *Palmaria mollis* could upregulate the PPARA pathway and inhibit the PPARG pathway in mice and zebrafish administrated with a high-fat diet. The lipid-lowering capacity of *Palmaria mollis* might be caused by lipogenesis inhibition and β-oxidation activation.

Yang et al. (2019) studied aqueous extract of *Gelidium amansii* for their anti-obesity effect in high-fat diet hamsters. The finding suggested that *Gelidium amansii* reversed obesity induced through a high-fat diet by stimulating lipolysis rate and induced expression of UCP-2 and PPARα. Reduction in total cholesterol and triglyceride levels was noticed in the liver of obese hamsters. Balasubramaniam et al. (2020) investigated the *Eucheuma denticulatum* for its anti-obesity activity in high-fat diet rats. The rats were fed with three different concentrations (10, 15 and 20%) of *Eucheuma denticulatum* for 7 weeks. The findings demonstrated that *Eucheuma denticulatum* fed animals displayed decreased weight gain, reduced liver enzymes and triglycerides levels. Lu et al. (2020) investigated *Plocamium*

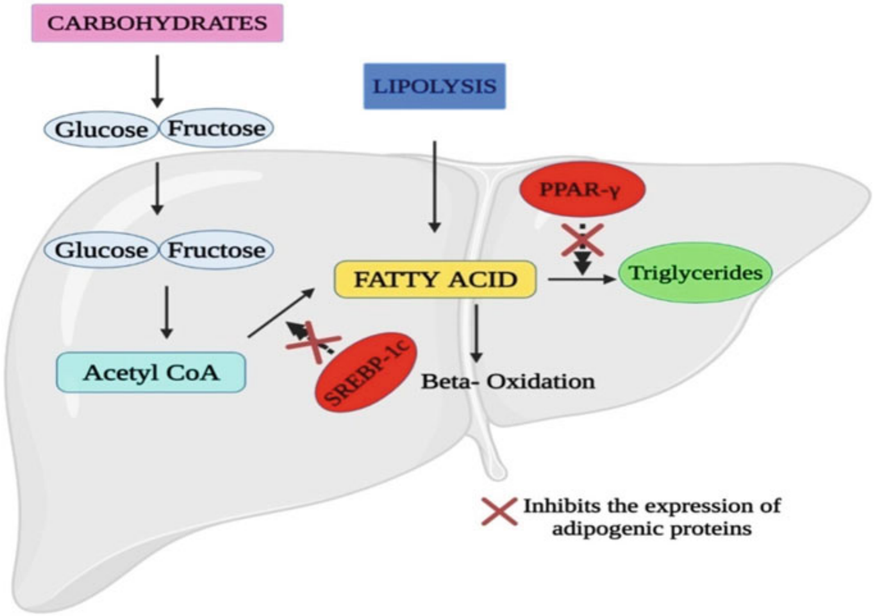

Fig. 13.2 Anti-obesity activities of seaweeds

*telfairiae'*s anti-obesity activity using various concentrations of ethanol ranging from 0 to 100% in 3 T3-L1 cells and obese mice. The findings demonstrated that 40% ethanol extract has potential in suppressing the expression of adipogenesis factors and reducing the accumulation of fat. In obese mice, 40% ethanol extract has remarkably decreased the white adipose tissue weight and also reduced the serum levels of total cholesterol and triglycerides. Lee et al. (2020) found that *Grateloupia elliptica* (60% ethanolic extract) has an anti-obesity effect on high-fat diet administrated mice and 3 T3-L1 cells. Under in vivo condition, administration of *Grateloupia elliptica* has notably decreased the white adipose tissue and body weight, and also decrease in the parameters including total cholesterol, triglycerides, leptin, and fatty liver and down-regulation of the expression of PPAR-γ and SREBP-1 (adipogenic proteins) were observed. In in vitro studies, *Grateloupia elliptica* inhibited the expression of adipogenic proteins and the accumulation of lipids (Fig. 13.2).

13.4 Neuroprotective Property

Eight seaweeds, namely *Padina gymnospora*, *Turbinaria conoides*, *Dictyota dichotoma*, *Gracilaria edulis*, *Hypnea valentiae*, *Enteromorpha intestinalis*, *Turbinaria ornate* and *Ulva reticulate,* were screened for neuroprotective activity.

The results revealed that *Padina gymnospora*, *Gracilaria edulis*, *Hypnea valentiae* and *Ulva reticulate* inhibited acetylcholinesterase activity with IC_{50} value 3.5, 3, 2.6 and 10 mg/ml, respectively (Suganthy et al. 2010). The Benzene extract of *Gelidiella acerosa* reversed the increased intensity of aggregation of amyloid-β and exhibited anti-aggregation activity (Syad and Devi 2015).

Methanolic extract of *Chondrus crispus* was tested for neuroprotective activity in *Caenorhabditis elegans* with 6-hydroxydopamine to induce Parkinson's symptom. The results displayed that *Chondrus crispus* (methanolic extract) has reduced the accumulation of α-synuclein. The *Chondrus crispus* was also efficient in preventing the loss of dopaminergic neuron (Liu et al. 2015). Meenakshi et al. (2016) studied the neuroprotective activity of fucoidan isolated from aqueous extract of *Turbinaria decurrens* in mice induced with Parkinson's through injection with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. The results demonstrated that fucoidan-administrated mice displayed an increase in 3,4-dihydroxy phenyl acetic acid, dopamine and homovanillic acid levels and improvement in behavioural tests of mice. Lin et al. (2016) tested Fucoxanthin isolated from *Sargassum horneri* against mice with cognitive dysfunction induced by scopolamine. The findings demonstrated that fucoxanthin is remarkable in reversing cognitive dysfunction. In addition, they also decreased the expression of brain-derived neurotrophic factor and choline acetyltransferase and increased the activity of acetylcholinesterase.

Silva et al. (2018) studied the neuroprotective activity of 5 various algae-like *Saccorhiza polyschides*, *Ulva compressa*, *Sargassum muticum*, *Codium tomentosum* and *Padina pavonica* in SH-SY5Y neuroblastoma cell line injected with neurotoxin (6-hydroxydopamine). The findings suggested that methanolic and dichloromethane extract of all algae has inhibited the effect of 6-hydroxydopamine in 24 h, except *Sargassum muticum* (dichloromethane extract). Depolarization of mitochondrial membrane potential caused by 6-hydroxydopamine was prevented by methanolic extracts of *Codium tomentosum*, *Ulva compressa* and *Sargassum muticum*. The caspase-3 stimulation was also inhibited by the methanolic extracts. Various extracts of *Turbinaria ornate* like aqueous, methanol and ethanol were tested against cognitive impairment in drosophila administrated with rotenone. Myricetin was isolated from methanolic extract of *Turbinaria ornate* with higher bioactive compounds. The findings demonstrated that flies induced with myricetin had exhibited potential improvement in behavioural tests. Myricetin had decreased the dopaminergic neuron degeneration and also significantly increased dopamine level (Dhanraj et al. 2018).

Oh et al. (2018) evaluated the neuroprotective activity of fucosterol obtained from *Ecklonia stolonifera* in the ageing rat. The results showed that fucosterol downregulated the expression of GRP78 and reduced cognitive dysfunction. Hydroethanolic extracts of *Gelidium pristoides*, *Ulva lactuca* and *Ecklonia maxima* at higher concentration potentially inhibited the butyrylcholinesterase and acetylcholinesterase activity (Olasehinde et al. 2019). Silva et al. (2019) studied the neuroprotective capacity of seven fractions (F1-F7) isolated from *Bifurcaria bifurcate* (dichloromethane extract) in SH-SY5Y cell line (neuroblastoma cell line) administered with 6-hydroxydopamine (neurotoxin). The findings revealed that F4 and F5 fractions displayed antioxidant and neuroprotective effect. The F4 fraction was also found to be significant in stimulating the reduction of H_2O_2 level and inhibiting the changes in mitochondrial membrane potential.

Bogie et al. (2019) demonstrated that 24(S)-Saringosterol isolated from *Sargassum fusiforme* displayed a neuroprotective effect by preventing cognitive dysfunction and improved (amyloid-β) Aβ phagocytosis. Shanmuganathan et al. (2019) found that *Padina gymnospora* and its bioactive compound α-bisabolol has a neuroprotective effect in *Caenorhabditis elegans* and N2a cells (mouse neuroblastoma). In vitro condition results displayed that acetone extract of *Padina gymnospora* restored the damage in lipid peroxidation and protein oxidation caused by Aβ. They also inhibited β-secretase and cholinesterase enzyme. In vivo condition revealed that *Padina gymnospora* inhibited the Aβ synthesis by downregulating the hsp-4, Aβ and ace-1 expression.

Glucuronomannan oligosaccharides were studied for their neuroprotective property. Adult mice were administrated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine with the dosage of 20 mg/kg for 7 days. Upon treatment with glucuronomannan oligosaccharides, the mice exhibited improvement in the behavioural tests. The results displayed that significant enhancement of the tyrosine hydroxylase had down-regulated the apoptotic pathway in dopaminergic neurons (Liu et al. 2020). Silva et al. (2020a, b) investigated the neuroprotective property of *Saccorhiza polyschides* in a neuroblastoma cell line (SH-SY5Y) induced with dopamine. The findings suggested that methanolic extract of *Saccorhiza polyschides* had a higher inhibitory effect on dopamine. Silva et al. (2020a, b) studied the neuroprotective property of dichloromethane and methanol *Codium tomentosum* in neuroblastoma (SH-SY5Y) cell line injected with 6-hydroxydopamine. The findings suggested that *Codium tomentosum* displayed neuroprotective activity by potentially decreasing the caspase-3 activity, depolarization of mitochondrial membrane potential and generation of reactive oxygen species. Martens et al. (2021) demonstrated 24(S)-saringosterol from *Sargassum fusiforme* seaweeds was efficient in improving cognitive impairment in Alzheimer's disease. The results displayed that 24(S)-saringosterol compound was found to be potential against the inflammatory and also inhibited the deposition of amyloid-β and decline in memory.

13.5 Anticancer Activity

The antitumor activity of aqueous extract of *Sargassum oligocystum* was studied in Burkitt Lymphoma cells (Human Daudi) and chronic myelogenous leukaemia cells (K562). The findings suggested that *Sargassum oligocystum* exhibited significant anticancer activity at 400 μg/ml and 500 μg/ml concentrations in K562 and Daudi, respectively (Zandi et al. 2010). Mhadhebi et al. (2014) investigated the aqueous extract of *Cystoseira sedoides*, *Cystoseira crinite* and *Cystoseira compressa* for their anticancer activity in MCF7 and HCT15 cell lines. The results exhibited that all the aqueous extracts had an anti-proliferative effect but *Cystoseira crinite* displayed a higher anti-proliferative effect.

Marudhupandi et al. (2015) studied the anticancer activity of fucoidan derived from ethanolic extract of *Turbinaria conoides* in the A549 cell line. The findings demonstrated that fucoidan had an inhibitory effect on the growth of the cells. Gomes et al. (2015) evaluated 13 species from Phaeophyta, Chlorophyta and Rhodophytas for anticancer activity in HeLa Cells (human cervical cancer cells). The results suggested that methanolic extract of *Dictyota menstrualis* and *Dictyota cilliolata* exhibited inhibition of cell proliferation. They exhibited different sized apoptotic bodies, condensed chromatin, stimulated the caspase-9 and caspase-3 activation and reduced the cell viability.

Sakthivel et al. (2016) investigated the anti-proliferative activity of various extracts of *Gracilaria edulis* in HepG2 (hepatocellular carcinoma), A549 (lung adenocarcinoma) and PC3 (prostate cancer) cell lines. After 48 h, the ethyl acetate extract of *Gracilaria edulis* had a significant effect on all the cell lines. But the ethyl acetate extract exhibited greater inhibition of growth against A549 cell lines. Alves et al. (2016) screened 12 marine algae for their anticancer activity in HepG-2 cells. The findings suggested that dichloromethane and methanolic extracts of *Asparagopsis armata* had strong cytotoxicity in HepG-2 cells followed by both the extracts of *Sphaerococcus coronopifolius* displayed cytotoxicity. The dichloromethane extract of *Plocamium cartilagineum*, *Sphaerococcus coronopifolius* and *Asparagopsis armata* also exhibited anti-proliferative activity.

Palanisamy et al. (2017a, b) studied fucoidan extracted from *Sargassum polycystum* to investigate the anticancer activity. The fucoidan displayed 90.4% of inhibition and also displayed maximum antioxidant capacity in the MCF-7 cell line. Costa et al. (2017) studied the anti-proliferative activity of lipid extracted from *Gracilaria* sp. in human urinary bladder and breast cancer cell lines. In both cell lines, lipid extract reduced the growth in a dosage-dependent manner. Akbari et al. (2018) studied the anticancer activity of *Sargassum boveanum* in HeLa cells through various extracts like chloroform, hexane, butanol and trichloroethane. The findings suggested that cell viability was decreased by chloroform extract followed by hexane in HeLa cells. Lopes-Costa et al. (2017) evaluated the anticancer activity of phloroglucinol (polyphenol) and fucoxanthin (carotenoid) from brown seaweeds alone or in combination with 5-fluorouracil (standard drug) in HT29 and HCT116 (colon cancer cell lines). Cell viability was reduced in both cell lines by fucoxanthin alone. In co-incubation fucoxanthin along with the 5-fluorouracil displayed a significant decrease in cell viability.

Vaikundamoorthy et al. (2018) studied the anticancer activity of *Sargassum wightii* in breast cancer cells. The SWP1 and SWP2 polysaccharide fractions were derived from crude polysaccharides. The results displayed remarkably reduced proliferation in breast cancer cells. In breast cancer cells apoptosis was stimulated by an increased generation of reactive oxygen species, nuclei damage and mitochondrial membrane cleavage and also increased the caspase 3/9 activity. Martins et al. (2018) tested anticancer of four macroalgae (*Cystosphaera jacquinotii*, *Pyropia endiviifolia*, *Himantothallus grandifolius* and *Iridaea cordata*) extracted with different solvents (ethanol, ethyl acetate, hexane and chloroform) in NIH/3 T3 (human epidermoid carcinoma) and A-431 (human epidermoid carcinoma) cell lines. The findings suggested that in the A-431 cell line, *Iridaea cordata* (ethyl acetate) exhibited maximum inhibitory activity. But in NIH/3 T3 cell line, all the extracted showed no cytotoxicity effect. Kumari et al. (2018) evaluated the anti-proliferative effect of endophytic fungus extracted from *Talaromyces purpureogenus* with various extracts in HeLa cells. After 28 days, the ethyl acetate fraction (potato dextrose broth) had the greater inhibition in HeLa cells. Lu et al. (2018) compared the anticancer effect of fucoidan isolated from *Undaria pinnatifida* with fucoidan commercially available in breast cancer cell lines. The findings indicated that fucoidan from *Undaria pinnatifida* significantly exhibited proliferation inhibition in breast cancer cells.

Narayani et al. (2019) tested fucoidan derived from *Sargassum cinereum* for anticancer activity in colon cancer cell line. In Caco-2 cells, fucoidan inhibited the growth with increasing dosage and induced apoptosis. Fucoidan also stimulated the generation of reactive oxygen species. The anticancer activity of *Padina australis* extracts were assessed in HeLa cell lines using different extracts, including dichloromethane, aqueous, butanol and hexane. The results revealed that extracts of *Padina australis* had significant anticancer activity by reducing the cell viability (Vaseghi et al. 2019). Arumugam et al. (2019) found that fucoidan polymers extracted from *Turbinaria conoides* have anticancer activity against hepatoblastoma-derived cell line. The results displayed that fucoidan presented cell viability in HepG2 cells. They also displayed a significant inhibitory effect on cell migration, proliferation and colony formation and induced apoptosis. Antitumor activity of *Chaetomorpha* sp. (aqueous and ethanolic extract) was evaluated in MDA-MB-231 and MCF-7 (breast cancer cell lines). The findings demonstrated that ethanolic extract had a higher growth inhibitory effect on MDA-MB-231 but aqueous extract does not show any significant inhibition on breast cancer cell lines (Haq et al. 2019).

Smyrniotopoulos et al. (2020) demonstrated the anticancer activity of active compound acyclic diterpene extracted from *Bifurcaria bifurcate* in breast cancer cells. The results showed that only 3 compounds 7, 8 and 11 had inhibited the growth moderately. The anti-proliferative activity of five seaweeds *Grateloupia indica*, *Caulerpa scalpelliformis*, *Sargassum linearifolium*, *Spatoglossum asperum* and *Caulerpa racemosa* were evaluated in HeLa cells (human cervical cancer cells) and Huh-7 cells (human hepatoma cancer). The results revealed that in Huh-7 and HeLa cells, hydromethanolic extract of *Caulerpa racemosa* displayed maximum inhibitory effect at lower dosage followed by *Caulerpa scalpelliformis* (Tanna et al. 2020). Zbakh et al. (2020a, b) evaluated the anticancer activity of 8 meroterpenoids extracted from *Cystoseira usneoides* seaweed in colon cancer cell line. The findings revealed that 6-cis-amentadione-10 -methyl ether and usneoidone Z exhibited significant growth inhibition in colon cancer cell line. The anticancer activity of methanolic and aqueous extract of *Cystoseira crinite* and *Sargassum linearifolium* was evaluated in MIA PaCa-2 (pancreatic cancer), MCF-7 (breast adenocarcinoma), THLE-2 cells (an immortalized liver cell line) and HepG2 (liver hepatocellular). The findings suggested that methanolic extract of *Cystoseira crinite* displayed higher anticancer activity followed by aqueous extract of *Sargassum linearifolium* in

MCF-7 cells. They also exhibited an increase in the expression of Beclin-1 and Bax protein and Bcl-2 expression was suppressed; thus they are capable of inducing autophagy and apoptosis in MCF-7 cells (Abu-Khudir et al. 2020).

13.6 Antiviral Activity

Koishi et al. (2012) examined dichloromethane/methanol extracts of *Padina gymnospora*, *Caulerpa racemosa*, *Canistrocarpus cervicornis* and *Palisada perforate* in Huh7.5 cell line invaded with dengue virus. The results revealed that all the seaweeds had antiviral activity against the dengue virus, except for DENV-4. Pujol et al. (2012) screened the various classes of sulphated polysaccharides extracted from six seaweeds (*Scinaia hatei*, *Cystoseira indica*, *Grateloupia indica*, *Stoechospermum margina*, *Gracilaria corticata* and *Caulerpa racemosa*) belonging to Rhodophyta, Chlorophyta and Phaeophyta against the dengue virus-2 in Vero cells. The results displayed that sulphated galactans isolated from *Grateloupia indica*, sulphated xylomannans extracted from *Grateloupia indica* and sulphated polysaccharides isolated from *Caulerpa racemosa* were found to have strong activity against the dengue virus-2. Soares et al. (2012) screened 36 species of marine algae for their antiviral activity against herpes simplex virus. The findings revealed that *Codium decorticatum* and *Ulva fasciata* both had maximum activity against herpes simplex virus-1 followed by *Laurencia dendroidea* which also had antiviral activity. The herpes simplex virus-2 was significantly inhibited by *Penicillus capitatus* followed by *Stypopodium zonale*.

Plouguerné et al. (2013) tested the antiviral property of sulphoquinovosyl diacylglycerols derived from *Sargassum vulgare*. The findings revealed that fractions F4II90 and F4I86 were potential against the herpes simplex virus. Kulshreshtha et al. (2015) studied the antiviral property of *Chondrus crispus* and *Codium fragile* in Vero cells against herpes simplex virus-1. The results suggested that enzymatic hydrolysates displayed potential antiviral activity against the herpes simplex virus. *Codium fragile* (enzymatic extract) has a maximum inhibitory effect against the viral activity. Aguilar-Briseño et al. (2015) examined the combined antiviral activity of fucoidan derived from *Cladosiphon okamuranus* and ulvan extracted from *Ulva clathrata* against Newcastle disease virus. The results displayed that ulvan exhibited cell-cell fusion inhibition. The combination of ulvan and fucoidan had a higher anti-spread effect.

Morán-Santibañez et al. (2016) tested sulphated polysaccharides derived from *Ulva intestinalis*, *Eisenia arborea*, *Solieria filiformis*, *Macrocystis pyrifera* and *Pelvetia compressa* for their antiviral property against measles virus in Vero cells. The results displayed that *Solieria filiformis* and *Eisenia arborea* exhibited higher viral inhibition. In addition, the combined action of *Solieria filiformis* and *Eisenia arborea* was studied which displayed far higher activity. Polysaccharides isolated from *Rhodymenia pseudopalmata*, *Sargassum fluitans*, *Solieria filiformis* and *Hydropuntia cornea* were screened for antiviral activity against herpes simplex virus-1 in Vero cells. The results displayed that *Solieria filiformis* and *Sargassum fluitans* had significant inhibitory activity against the herpes simplex virus-1 (Bedoux et al. 2017).

Lopes et al. (2017) studied seven polysaccharides derived from *Enteromorpha compressa* against herpes simplex virus in larynx epithelial cells carcinoma (HEp-2 cells). The results presented that SU1F1 showed significant antiviral activity. Garrido et al. (2017) examined the dolabelladienetriol extracted from *Dictyota pfaffii* in rats against the herpes simplex virus. The results showed that dolabelladienetriol had antiviral activity similar to acyclovir (standard). Cirne-Santos et al. (2018) screened ethanolic extract of *Osmundaria obtusiloba* against the zika virus in Vero cells. The findings revealed that *Osmundaria obtusiloba* exhibited antiviral activity by inhibiting the zika virus replication and also significantly induced the virucidal effect. Morán-Santibañez et al. (2018) screened *Silvetia compressa*, *Macrocystis pyrifera*, *Ulva intestinalis*, *Ecklonia arborea* and *Solieria filiformis* for antiviral activity against Measles virus in Vero cells. The finding displayed that polyphenol-rich extracts of *Solieria filiformis* and *Ecklonia arborea* were significant in inactivating viral particles (virucidal activity).

Sanniyasi et al. (2019) tested the antiviral property of fucoidan extracted from *Turbinaria decurrens* and *Dictyota bartayesiana* against human immunodeficiency virus HIV-1. The findings demonstrated that fucoidan inhibited the propagation of HIV. The 90.5% higher inhibition was exhibited by the aqueous extract of *Dictyota bartayesiana*. Cirne-Santos et al. (2019a, b) screened *Caulerpa racemosa*, *Osmundaria obtusiloba*, *Kappaphycus alvarezii*, *Bryothamnion triquetrum*, *Ulva fasciata* and *Laurencia dendroidea* for antiviral effect against chikungunya virus in Vero cells. The results exhibited that *Kappaphycus alvarezii*, *Osmundaria obtusiloba* and *Caulerpa racemosa* inhibited the replication of chikungunya. In addition, *Osmundaria obtusiloba* extract had maximum viral inhibition activity. Cirne-Santos et al. (2019a, b) examined *Dictyota menstrualis* against zika virus in Vero cells. The results suggested that diterpenes fractions (FAc-2 and F-6) displayed virucidal activity. F-6 suppressed the viral adsorption and FAc-2 had significant virucidal potential. FAc-2 along with Ribavirin had a remarkable synergistic effect which completely prohibited the viral replication.

Klongklaew et al. (2020) evaluated the antiviral activity of *Caulerpa lentillifera, Ulva intestinalis* and *Ulva rigida* in Pacific white shrimps infected with yellow head virus and white spot syndrome virus. The results displayed that aqueous extract of *Ulva intestinalis* was remarkable in inhibiting the yellow head virus and white spot syndrome virus infection. Cirne-Santos et al. (2020) studied the antiviral property of *Canistrocarpus cervicornis* and dolastane extracted from *Canistrocarpus cervicornis* in Vero cells against the chikungunya and zika viruses. The results displayed that *Canistrocarpus cervicornis* (crude extract) has antiviral activity against the chikungunya and zika viruses. Dolastane was found to be more efficient in inhibitory effect. They also found that dolastane and *Canistrocarpus cervicornis* (crude extract) were effective in inhibiting the replication of chikungunya and zika viruses. Yim et al. (2021) tested the polysaccharides and sulphated fucoidan isolated from *Hizikia fusiforme*, *Porphyra tenera*, *Laminaria japonica*, *Codium fragile*, *Sargassum horneri* and *Undaria pinnatifida* sporophyll for the antiviral property against entry of SARS-CoV-2 virus. The findings suggested that polysaccharide isolated from *Sargassum horneri* displayed maximum activity and followed by that *Hizikia fusiforme* exhibited antiviral activity against the entry of COVID-19.

13.7 Anti-inflammatory Activity

Souza et al. (2009) studied the anti-inflammatory effect of bisindole alkaloid derived from *Caulerpa racemosa* in mice with ear oedema induced by capsaicin. The results presented that bisindole alkaloid exhibited an anti-inflammatory effect in the formalin test. Rodrigues et al. (2012) studied the three sulphated polysaccharide fractions derived from *Caulerpa curpressoides* in association with anti-inflammatory activity in mice. *Caulerpa curpressoides* was found to be effective in reducing the migration of neutrophils and also significantly decreased paw oedema. Anti-inflammatory effect of aqueous extract of *Cystoseira sedoides*, *Cystoseira crinite* and *Cystoseira compressa* was studied in paw oedema induced by carrageenan in rats. All the extracts had an anti-inflammatory effect with increasing dosage in comparison to dexamethasone (Mhadhebi et al. 2014). Coura et al. (2012) examined the anti-inflammatory effect of the sulphated polysaccharides derived from *Gracilaria cornea* in mice injected with carrageenan. After 3 h of treatment with *Gracilaria cornea,* mice displayed inhibition in paw oedema; this was confirmed using myeloperoxidase activity.

Sulphated polysaccharides isolated from *Halophila ovalis* and *Sargassum wightii* were studied for their anti-inflammatory effect in Wistar rats administrated with Carrageenan. At a concentration of 10 mg/kg, both *Halophila ovalis* and *Sargassum wightii* exhibited reduction paw oedema and also reduced the migration of neutrophil by 53% (Yuvaraj et al. 2013). Bhatia et al. (2015) tested methanolic extract of *Porphyra vietnamensis* and Porphyran extracted from aqueous extracts of *Porphyra vietnamensis* in rats with oedema to study the anti-inflammatory effect. The anti-inflammatory effects of extracts were measured by the percentage of reduction in paw volume. *Porphyra vietnamensis* exhibited an inhibitory effect approximately close to standard (indomethacin). 5β-hydroxypalisadin B extracted from *Laurencia snackeyi* was tested for anti-inflammatory effect in zebrafish embryo administrated with lipopolysaccharide. The results suggested that 5β-hydroxypalisadin B normalized the oedema size of the yolk sac and heartbeat rate in dose-dependent manner (Wijesinghe et al. 2014). Ribeiro et al. (2014) examined the sulphated polysaccharide isolated from *Caulerpa racemosa* in rats with dextran and carrageenan. *Caulerpa racemosa* presented its anti-inflammatory effect by reducing the number of leukocytes and also decreasing paw oedema. Monoacylglycerol and monogalactosyl diacylglycerols extracted from *Fucus spiralis* were evaluated for their anti-inflammatory activity in RAW 264.7 macrophage cells. The monogalactosyl diacylglycerols were successful in suppressing the production of nitric oxide (Lopes et al. 2014).

Robertson et al. (2015) studied the lipid derived from the *Pavlova lutheri*, *Porphyra dioica*, *Palmaria palmate* and *Chondrus crispus* in THP-1 macrophages injected with lipopolysaccharide. *Palmaria palmate* and *Pavlova lutheri* (lipid extract) inhibited the pro-inflammatory cytokine production interleukin-6 and interleukin-8 (IL-6 and IL-8) and reduced the chemokine, TLR, cytokine-linked signalling pathway. Sanjeewa et al. (2017) studied the anti-inflammatory effect of crude polysaccharides isolated from *Sargassum horneri* in RAW 264.7 cells administrated with lipopolysaccharide. The results revealed that crude polysaccharides exhibited potential inhibitory action against the production of nitric oxide. In addition, down-regulated expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) proteins and interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α; an inflammatory cytokines) was observed. Costa et al. (2017) studied the anti-inflammatory activity of lipid extracted from *Gracilaria* sp. in RAW 264.7 cells. The lipid extract reduced nitric oxide production.

Kim et al. (2018a, b) studied the anti-inflammatory activity of Apo-9-′-fucoxanthinone isolated from *Sargassum muticum* in RAW264.7 cells and zebrafish embryos injected with lipopolysaccharide. The findings revealed that in in vivo studies Apo-9′-fucoxanthinone has the potential to reduce cell death, nitric oxide and reactive oxygen species. They also inhibited inflammatory cytokines and COX-2. Under in vitro condition, Apo-9′-fucoxanthinone decreased the prostaglandin E2 (PGE2), pro-inflammatory cytokines and COX-2. In addition, they suppressed the phosphorylation of mitogen-activated protein kinases (MAPK) and nuclear factor kappa B (NF-kB) pathway. Phlorotannin-rich fractions extracted from *Cladostephus spongeosis*, *Padina pavonica* and *Cystoseira sedoides* were screened for their anti-inflammatory activity in paw and ear oedema in mice. *Cystoseira sedoides* displayed an anti-inflammatory effect by reducing the percentage of paw and ear oedema in mice (Abdelhamid et al. 2018). Lectin was extracted from *Caulerpa cupressoides* was tested in rats with temporomandibular disorders induced by bormalin. The rats pretreated with lectins exhibited COX2, cytokines and intercellular adhesion molecule (Rivanor et al. 2018). Kang et al. (2018) tested aqueous extracts of *Hizikia fusiforme* and *Laminaria japonica* for anti-inflammatory activity in C2C12 myotubes incubated with lipopolysaccharide. The extracts of *Hizikia fusiforme* and *Laminaria japonica* were significant in reducing the TNF-α. Celluclast extracts of ten different seaweeds were screened for their anti-inflammatory effect on zebrafish and RAW 264.7 macrophages induced with lipopolysaccharide. *Chnoospora minima* (Celluclast extract) exhibited a potential anti-inflammatory effect by inhibiting the production of nitric oxide and suppressing the PGE2, iNOS, TNF-α and COX2 induced by lipopolysaccharide (Fernando et al. 2018).

Anti-inflammatory effect of CLGP1, CLGP2, CLGP3 and CLGP4 (purified polysaccharides) derived from *Caulerpa lentillifera* was tested in HT29 cells injected with lipopolysaccharide. The findings revealed that CLGP4 was effective in inhibiting the TNF-α and IL-1β protein expression and decreasing the production of mucin2 and SIgA. The anti-inflammatory effect of CLGP4 may be due to the sulphate groups present in them (Sun et al. 2020). Crude polysaccharides isolated from *Gelidium pacificum* was studied for their anti-inflammatory activity in THP-1 cells incubated with lipopolysaccharide. The results suggested that polysaccharides have a significant decrease in the production of nitric oxide and also reduced the expression of TRAF-6, TLR-4 and MyD88 (Cui et al. 2019). Different fractions (spiro-compounds and 2H-chromenyl derivatives) extracted from *Gracilaria salicornia*, diterpenoid extracted from *Sargassum ilicifolium* and 2H-pyrenoids extracted from *Turbinaria conoides* had an anti-inflammatory effect (Chakraborty and Antony 2020). LJSF4 (fucoidan) extracted from *Saccharina japonica* was tested for anti-inflammatory activity against zebrafish and macrophage cells administrated with lipopolysaccharide. In vitro, LJSF4 exhibited potential anti-inflammatory activity by reducing nitric oxide production and down-regulated cytokines (IL-6, TNF-α and IL-1β). In in vivo studies, LJSF4 decreased the rate of cell death and reduced the reactive oxygen species and nitric oxide production in zebrafish (Ni et al. 2020). Jin et al. (2020) tested fucoxanthinol for anti-inflammatory activity in macrophages with lipopolysaccharide. Fucoxanthinol inhibited the N-acylethanolamine acid amidase by binding in the active site. They also suppressed the palmitoylethanolamide levels, TNF-α, production of nitric oxide and inflammatory factors.

Zbakh et al. (2020a, b) screened the eight algal meroterpenoids (AMTs) extracted from *Cystoseira usneoides* to study the anti-inflammatory activity in THP-1 human macrophages activated with lipopolysaccharide. AMTs exhibited a reduction in the expression of iNOS and COX-2 and also suppressed the production of IL-1β, TNF-α and IL-6. Saraswati et al. (2020) examined the crude lipid extract and fractions of *Sargassum cristaefolium* (dichloromethane, methanol and acetone) for anti-inflammatory activity in co-incubated and pre-incubated RAW 264.7 cells. The results showed that acetone fraction had a strong inhibitory effect on nitric oxide production. Three salicornolides A-C extracted from *Gracilaria salicornia* were evaluated for anti-inflammatory effect. Salicornolide B exhibited strong inhibition against COX2 than the other two salicornolides (Chakraborty and Antony 2020). Fuente et al. (2021) examined the ethanol and dimethylsulphoxide extracts of both *Cystoseira amentacea* in L929 fibroblasts and RAW 264.7 macrophages. Both extracts were the potential to completely suppressing the production of nitric oxide. In addition, they also were effective in inhibiting inflammatory mediators like iNOS, IL-6, IL-1α and COX-2 induced by lipopolysaccharide and partially suppressed the overexpression of TNF-α.

13.8 Antibacterial Activity

Patra et al. (2015a, b) evaluated the essential oil of *Laminaria japonica* for their bactericidal effect against three food-borne bacteria. The results presented that *Laminaria japonica* exhibited a strong antibacterial effect against *Staphylococcus aureus* with the zone of inhibition of 11.5 ± 0.58 mm followed by *Bacillus cereus* with the zone of inhibition of 10.5 ± 0.57 mm at 25 ml/disc concentration. But they had no activity against *Escherichia coli*. Patra et al. (2015a, b) studied the essential oil of *Enteromorpha linza* for their antibacterial activity against *Salmonella typhimurium* and *Escherichia coli*. The bactericidal ability of essential oil of *Enteromorpha linza* against the *Salmonella typhimurium* and *Escherichia coli* may be due to the enzymatic degradation in the cytosol which leads to cellular lysis. The antibacterial activity of red pigment extracted from ethanolic extract of *Halolactibacillus alkaliphilus* was tested against *Klebsiella pneumonia*, *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus* and *Escherichia coli*. The red pigment was potential in inhibiting the *Salmonella typhi* and *Staphylococcus aureus* with 14 and 16 mm zone of inhibition, respectively (Suresh et al. 2015).

Ulva lactuca was examined for their bactericidal activity against ten different strains of *Staphylococcus aureus* with standards ampicillin and ancomycin disc. Except for Staphylococcus epidermidis and MSSA SH1000 strains, methanolic extract of *Ulva lactuca* showed a maximum inhibitory effect in lunar phase III (75 μg dics-1) (Deveau et al. 2016). Patra and Baek (2016) studied the essential oil extracted from *Enteromorpha linza* was investigated for its antibacterial effect against food-borne bacterial strains (*Staphylococcus aureus* and *Bacillus cereus*). The maximum zone of inhibition against *Bacillus cereus* was found to be 12.3–12.7 mm and maximum inhibition against *Staphylococcus aureus* was found to be 12.7–13.3 mm. Fucoidan isolated from *Spatoglossum asperum* was evaluated for their bactericidal activity against *Aeromonos hydrophila* using a well diffusion method. The results demonstrated that fucoidan displayed maximum bactericidal activity at 150 μg/ml concentration with a 33 mm zone of inhibition (Palanisamy et al. 2017a, b).

Capillo et al. (2018) screened various extracts (chloroform, methanol, diethyl ether, acetone and ethanol) of *Gracilaria gracilis* against gram-negative and gram-positive bacteria. Among all the extracts, ethanolic extract (200 μg) displayed a bacterial effect against *Bacillus subtilis*. Laminaran (polysaccharides) derived from *Cystoseira barbata* was studied for their antibacterial effect against the 8 gram-negative and gram-positive bacteria. Laminaran was tested in two different concentrations 20 and 50 mg/ml. The results demonstrated that laminaran was found to be effective against *Salmonella typhimurium* and *Escherichia coli* (Sellimi et al. 2018). Martins et al. (2018) studied the bactericidal effect of different extracts of *Iridaea cordata*, *Pyropia endiviifolia*, *Cystosphaera jacquinotii* and *Himantothallus grandifolius* against *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Ethanolic extract of *Cystosphaera jacquinotii* had the highest bactericidal effect against bacterial strains. Phlorotannins derived from the *Eisenia bicyclis* was investigated against the *Listeria monocytogenes* (food borne bacteria) for a bactericidal effect. Phlorotannins showed a strong anti-listerial effect against the *Listeria monocytogenes* with the minimum inhibitory concentration range of 16–32 lg/ml (Kim et al. 2018a, b).

Antibacterial activity of *Caulerpa lentillifera* and *Caulerpa racemosa* was studied using various solvents like aqueous, chloroform and methanol against *Escherichia coli* and *Staphylococcus aureus* (Methicillin-resistant). The results exhibited that *Caulerpa racemosa* (chloroform extract) had maximum bacterial inhibition and this activity may be due to the presence of monounsaturated and polyunsaturated fatty acids (Yap et al. 2019). Abu-Khudir et al. (2020) investigated *Cystoseira crinit* and *Sargassum linearifolium* for their antibacterial effect against gram-negative and gram-positive bacteria. Methanolic extract of *Cystoseira crinit* and *Sargassum linearifolium* was found to inhibit bacterial strains. Klongklaew et al. (2020) examined the aqueous extract of ulvan from *Ulva armoricana*, *Caulopa lentillifera*, *Ulva intestinalis* and *Ulva rigida* in shrimp against white spot syndrome virus. All the extracts had a bactericidal effect against *Vibrio* spp.

13.9 Anti-nociceptive Activity

De Souze et al. (2009) studied the anti-nociceptive effect of Bisindole alkaloid derived from *Caulerpa racemosa* in Swiss albino mice. The results exhibited that bisindole alkaloid decreased the nociception induced by acetic acid and inhibited nociception at 100 μmol/kg dosage in hot plate test. Rodrigues et al. (2012) studied the three sulphated polysaccharide fractions derived from *Caulerpa curpressoides* against anti-nociceptive activity in mice. 1% formalin was injected into mice in paw and acetic acid was injected to study the total number of writhes. *Caulerpa curpressoides* was found to be effective in decreasing the licking time and writhes induced by the acetic acid. Coura et al. (2012) examined the anti-nociceptive effect of the sulphated polysaccharides derived from *Gracilaria cornea* in mice. *Gracilaria cornea* induced mice showed a reduction in the licking time and slightly decreased the migration of neutrophil. In the hot plate test, *Gracilaria cornea* displayed activity at a concentration of 27 mg/kg.

Sulphated polysaccharides isolated from *Halophila ovalis* and *Sargassum wightii* were studied for their anti-nociceptive activity in Wistar rats. Both the *Halophila ovalis* and *Sargassum wightii* had potentially decreased the licking time in the formalin test at a dosage of 10 mg/kg in hot plate test; animal administrated with 5, 10 mg/kg of *Halophila ovalis* and 10 mg/kg of *Sargassum wightii* exhibited an anti-nociceptive activity (Yuvaraj et al. 2013). Ribeiro et al. (2014) examined the sulphated polysaccharide isolated from *Caulerpa racemosa* in Swiss mice. *Caulerpa racemosa* was found to be potential in decreasing the paw licking duration and total number of writhing after the second phase of treatment. Bhatia et al. (2015) tested methanolic extract of *Porphyra vietnamensis* and Porphyran extracted from aqueous extracts of *Porphyra vietnamensis* in mice to study the anti-nociceptive effect. *Porphyra vietnamensis* was found to be potential in decreasing abdominal writhes and also displayed a better analgesic effect.

Phlorotannin-rich (PHT) fractions extracted from *Cladostephus spongeosis*, *Padina pavonica* and *Cystoseira sedoides* were screened for their anti-nociceptive activity in Swiss mice. Phlorotannin-rich fractions displayed a decrease in a maximum number of writhing at the dosage of 100 mg/kg. In the hot plate test, phlorotannin-rich fractions of *Cladostephus spongeosis* and *Cystoseira sedoides* displayed maximum activity (Abdelhamid et al. 2018). Bhatia et al. (2019) investigated the various extracts (aqueous, acetone, ethanol and chloroform) of *Porphyra* *vietnamensis* against the anti-nociceptive effect. Acetone extract of *Porphyra vietnamensis* exhibited higher antinociceptive activity and also displayed positive results in formalin and hot plate test.

13.10 Hepatoprotective Effect

Manoharan et al. (2008) investigated the *Gracilaria corticata* for their hepatoprotective effect in mice administrated with aflatoxin B1. The aqueous extract of *Gracilaria corticata* normalized the hepatic parameters stimulated by the aflatoxin B1. The aqueous extract at a dosage of 250 mg/kg was potentially in normalizing the lactate dehydrogenase, aspartate aminotransferase, total bilirubin and alanine aminotransferase levels elevated by aflatoxin B1. Kim et al. (2011) investigated ethanol extract and serial solvent fractions (aqueous, ethyl acetate, methylene chloride, n-butanol and n-hexane) were isolated from *Eisenia bicyclis* to examine their hepatoprotective effect in HepG2 cells induced with tert-butyl hyperoxide. The n-butanol and ethyl acetate fractions displayed a protective effect in HepG2 cells. Five phlorotannin compounds derived from ethyl acetate fraction were further tested for hepatoprotective effect. Among them, phlorofucofuroeckol A compound displayed greater protective activity in HepG2 cells.

Makhmoor et al. (2013) studied aurone derivatives and cinnamic acid esters from *Spatoglossum variabile* to evaluate their hepatoprotective effect on Wistar rats injected with carbon tetrachloride. The findings indicated that the compounds displayed a hepatoprotective effect by reducing the hepatic biomarker levels in serum. Meenakshi et al. (2014) studied fucoidan extracted from *Turbinaria decurrens* to investigate their hepatoprotective effect in rats intoxicated with ethanol. Fucoidan reduced the levels of the hepatic markers and also increased the body weight and protein level in serum.

Gao et al. (2018) studied the polyguluronate sulphate for their hepatoprotective effect in HepG2 cells. Polyguluronate sulphate displayed an increase in cell viability and also reduced the IL-6, TNF-α, lactate dehydrogenase, malondialdehyde and oxidative stress induced by H_2O_2. In vivo studies showed that polyguluronate sulphate was significant in suppressing the elevation of alanine aminotransferase, total bilirubin and aspartate aminotransferase in mice with liver injury induced through Concanavalin A. Liu et al. (2018) studied the four fucoidans (KF1-KF4) extracted from *Kjellmaniella crassifolia* in mice with liver damage induced through carbon tetrachloride for their hepatoprotective activity. The KF4 fraction was found to be strong in reducing the alanine aminotransferase level. All fractions at concentrations of 500 and 300 mg/kg significantly inhibited the aspartate aminotransferase levels. KF2 and KF4 fractions were potential in decreasing the lactate dehydrogenase level. Quintal-Novelo et al. (2018) examined the ethanolic extract *of Sargassum fuitans* for their hepatoprotective effect in Wistar rats injected with carbon tetrachloride. The results indicated that ethanolic extract significantly normalized the serum enzyme marker elevated by carbon tetrachloride.

Hira et al. (2021) investigated the hepatoprotective effect of various extracts (methanol, n-hexane and chloroform) of *Sargassum ilicifolium* in Wistar rats administrated with acetaminophen to induce liver dysfunction. The methanolic and n-hexane fractions decreased the liver marker enzymes and creatinine, triglycerides, bilirubin, glucose and urea levels which are elevated by liver damage induced through acetaminophen administration.

13.11 Hypolipidaemic Effect

Huang et al. (2010) evaluated hypolipidaemic effect of fucoidan derived from *Laminaria japonica* in rats induced with high-fat emulsion diet. Fucoidan decreased the low-density lipoprotein, total cholesterol and triglyceride and elevated the high-density lipoprotein concentration. Cuong et al. (2015) investigated the hypolipidaemic effect of fucoidan isolated from *Sargassum henslowianum* in mice fed with high-fat diet. The results revealed that fucoidan aided the reduction in low-density lipoprotein, total cholesterol and triglyceride.

Fucoidan isolated from brown seaweeds was tested for their protective effect against dyslipidaemia. Fucoidan decreased the total cholesterol, non-high-density lipoprotein cholesterol, lipid levels in the blood and elevated the high-density lipoprotein and lipoprotein lipase in plasma (Yokota et al. 2016). Ren et al. (2019) tested the Fucoidan fractions obtained from *Saccharina sculpera* for their hypolipidaemic effect. The findings displayed that fucoidan fractions had significantly increased the high-density lipoprotein and reduced the low-density lipoprotein, total cholesterol and triglycerides. In addition, they inhibited the accumulation of lipid in the liver. The findings also revealed that fucoidan down-regulated the SREBP-1c and HMG-CoA-R to prevent the synthesis of fatty acids and cholesterol synthesis, respectively. Yim et al. (2019) examined the alginate isolated from *Saccharina japonica* for their hyperlipidaemic activity in rats. The results displayed that alginate-free residue has the ability to decrease low-density lipoprotein, total cholesterol and triglyceride. Li et al. (2020) investigated the polysaccharide of low molecular weight obtained from *Laminaria japonica* in mice. The findings demonstrated that *Laminaria japonica* had reduced the accumulation of lipid in liver and white adipose tissues.

13.12 Wound Healing Properties

Sellimi et al. (2018) evaluated the efficiency of laminaran derived from *Cystoseira barbata* in mice induced with the full-thickness wound. The findings suggested that *Cystoseira barbata* laminaran is a bioactive compound in the cream which enhanced the wound healing capacity. After treatment for a period of 13 days, 98% of wound contraction was achieved. The results also suggested that treated mice displayed an increase in vascular densities and fibroblast and improvement in the deposition of collagen. Kavitha et al. (2019) tested the wound healing property of collagen along with the sodium alginate extracted from *Turbinaria conoides*. The results demonstrated that the microfilm had a significant swelling index which is a key factor for wound dressing agent and it also displayed anti-inflammatory activity. 1*Halomonas venusta,* a melanin-producing bacteria, was extracted from *Callyspongia* sp. (Poulose et al. 2020). Along with *Gelidium spinosum* was tested wound healing property through scratch assay. The findings suggested cream formulated with melanin and *Gelidium spinosum* was found to be effective in wound healing in mouse fibroblast cell.

Wound healing properties of aqueous extract of *Ulva lactuca* and *Sargassum ilicifolium* seaweeds were tested in vivo (mice with excision wound) and in vitro (L929 cell line). The results demonstrated that *Sargassum ilicifolium* displayed a maximum wound healing effect than *Ulva lactuca*. In vitro studies revealed that *Sargassum ilicifolium* has maximum cell proliferation. In vivo condition, *Sargassum ilicifolium* displayed a remarkable wound healing effect in 3 days (Premarathna et al. 2021).

13.13 Cardioprotective Effect

Matanjun et al. (2010) studied the cardioprotective effect of *Sargassum polycystum*, *Kappaphycus alvarezii* and *Caulerpa lentillifera* in rats fed with a high-fat diet. The results suggested that *Caulerpa lentillifera*, *Sargassum polycystum* and *Kappaphycus alvarezii* display cardioprotective through lowering the total cholesterol level in plasma and atherogenic index (ratio of low-density lipoprotein/high-density lipoprotein) found to be decreased. *Sargassum polycystum* was significant in anti-obesity activity and *Kappaphycus alvarezii* displayed maximum antioxidant activity. Kammoun et al. (2018) evaluated the cardioprotective activity of ethanolic extract of *Ulva lactuca* in mice with a hypercholesterolaemic regime. The findings demonstrated improved atherogenic index, biochemical parameters and heart oxidative stress (superoxide dismutase, monoaldehydes, glutathione and reduced glutathione levels). They also decreased the gene expression of interleukin-1b (IL-1b), TNF- α and IL-6 in the heart. Prabha et al. (2019) studied the *Gracilaria corticata* for their cardioprotective activity in mice administrated with isoproterenol. The findings demonstrated that *Gracilaria corticata* normalized the biochemical (high-density lipoprotein, low-density lipoprotein, total cholesterol and triglyceride) parameters. They also normalized the cardiac marker enzymes like roponin I and troponin T.

13.14 Anticoagulant Activity

Sulphated polysaccharide derived from 15 from tropical seaweeds was evaluated for anticoagulant activity. The results displayed that *Udotea flabellum* had significant activity. F-I and F-II (sulphated homogalactans) had anticoagulant activity in the presences of HCII, F- I had higher activity than F-II (Mendes Marques et al. 2019). Okamoto et al. (2019) extracted Rhamnan sulphate from *Monostroma nitidum* to study their anticoagulant activity in human endothelial cells. The results displayed that Rhamnan sulphate potentially exhibited enhanced thrombin inhibition.

13.15 Antidepressant Effect

Abreu et al. (2018) studied lectin from *Solieria filiformis* to evaluate their antidepressant effect in mice. The results exhibited lectin had antidepressant activity without inducing anxiolytic-like effect and psychostimulant. Siddiqui et al. (2017) screened methanolic extracts *Nizamuddinia zanardinii*, *Sargassum swartzii* and *Stoechospermum marginatum* for their antidepressant activity in rats. The results displayed that all methanolic extract exhibited antidepressant activity by reducing the immobility time in the forced swim test. Violle et al. (2018) studied hydrophilic extract of *Ulva* sp. to test their antidepressant activity in Wistar rats. The hydrophilic extract significantly reduced the immobility behaviour in the forced swim test in comparison with imipramine (reference drug). Li et al. (2020a, b) tested the fucoidan derived from brown seaweed for their antidepressant activity against chronic restraint stress induced in mice. The findings suggested that fucoidan displayed a decrease in depressive-like behaviour in the forced swim test and tail suspension test. Mesripour et al. (2019) investigated methanolic and hexane extracts of *Sargassum plagyophylum* for their antidepressant effect in mice. Methanol partition elevated antidepressant effect by decreasing the immobility after 7 days of treatment. The hexane partition also exhibited antidepressant behaviour in the forced swim test.

13.16 Summary

The marine algae are naturally available resource but some of them are cultivated because of the enormous bioactive compounds like fuciodan, carrageenan, rhamnan sulphate, lectins, polyguluronate sulphate, ulvan, alginate, phlorotannin, fucoxanthin, fucosterol, laminarin, 24(S)-Saringosterol, porphyran, bisindole, sulphated polygalactans, dieckol and meroterpenoids present in seaweeds. Seaweeds are versatile products widely used in pharmaceutical products, functional foods, fertilizers, cosmetics industries (skin and hair care products, sunscreen cream and antiageing cream) and as nutraceuticals.

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Chapter 14
Probiotics and Its Application in Aquaculture

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Abstract During the preceding two decades, there has been a tremendous rise in both finfish and shellfish aquaculture. Even while there have been positive impacts, some of these improvements have also led to environmental deterioration, the spread of illness, and a decreased rate of productivity. Research and probiotics have both been stimulated by the demand for improved growth performance, enhance immune system, feed efficiency, and safety in the aquatic environment. Antibiotics are very effective at treating ailments, but they are also utilized to fuel the expansion of antibiotic-resistant bacteria and resistance genes and accumulate antimicrobial residues in fish tissues. The effect could be detrimental to human health, the fish population, and the environment. A newly used approach to combat bacterial illnesses in fish involves using vaccinations. Probiotics, due to increased demand, help further push the need for ecologically friendly aquaculture. Nonetheless, we need to work on our comprehension of gut microbiology, produce adequate probiotic amounts, and ensure the safety of these supplements.

14.1 Introduction

Aquaculture is vital for providing an adequate supply of nutritious food for a growing population. Occasional invasions of opportunistic pathogenic microorganisms lead to substantial disease losses and environmental degradation in intensive aquaculture operations. Crowding, contaminated water, pesticides, and nutrition shortages all increase the risk of microbial infections in animals who are allowed to live in large groups (Banerjee and Ray 2017). Until preventive and therapeutic

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methods address all of these concerns, the aquaculture sector will not grow. The use of medications and chemical additives is becoming essential in modern aquaculture. Cheap chemotherapeutic medications are detrimental to the ecosystem because they are frequently used. Because antibiotics can affect the gut microbiome, they are a point of discussion for preventing illness. Because antibiotics, which have long been used in aquaculture, have helped create antibiotic resistance, their usage has further worsened the problem. Due to the overuse of antibiotics in aquaculture, an increasingly large spectrum of antibiotic-resistant human illnesses, including *Vibrio cholerae, Escherichia tarda, E. coli, Aeromonas sp., Vibrio vulnificus,* and others, have been found (Allameh et al. 2016; Brogden et al. 2014).

For farmers, the commercial aquaculture system uses a feed containing a balanced combination of probiotics to help improve better growth, flesh quality, productivity, immune system, protein quantity, carcass quality, and intestinal health. In turn, consumers see benefits because of better growth, flesh quality, productivity, and protein quantity. This, however, is true for farmers in low-income and impoverished countries, many of whom continue to rely on naturally derived feeds to decrease production costs (typically phytoplankton and zooplankton). The approach, however, decreases growth, and mortality, all of which have a negative impact on profitability (Ige 2013). Due to the deleterious consequences on fish health, disease outbreak management is no longer recommended. A detailed chapter on probiotics discusses their use in aquaculture, application areas, numerous methods of administration, and the underlying mechanisms of action.

14.2 Alternatives for Medicines and Antibiotics

Bacteria that aid the human gastrointestinal tract have been recommended as environmentally safe alternatives to antibiotics. In recent years, environmental-friendly feed additives, such as microbial supplements, have increased popularity (Jahangiri and Esteban 2018). Aquatic environments harbour various microorganisms, including the ability to recycle nutrients, process organic garbage, and keep fish healthy. Probiotics are compounds that are beneficial to aquaculture. They are used to help avoid infections and, at the same time, aid animals in gaining weight, staying alive, and growing.

Additionally, there are no ill effects on the animals receiving the treatment. Increasing concern about discovering safe, non-antibiotic, and environmentally acceptable options for treating infections is another cause of the rise in infections. To combat the widespread issues with antibiotics and broad-spectrum insecticides in aquatic animals, the rapid development of probiotics in aquaculture has occurred (Lakshmi et al. 2013). There are alternatives to antibiotics, such as probiotics, that can help with infectious agent management and disease treatment. While this is certainly the case, proper usage of probiotics in aquaculture is critical for their effectiveness. At present, everyone agrees that the correct type of bacteria in the gut is crucial for maintaining good health (Nayak 2010). Microorganisms proliferate in reaction to disease, regardless of the host species.

14.3 Definition

Probiotics are defined as “live bacteria that impart health benefits to the host when provided in sufficient doses”. Probiotics are microbiological origin factors that boost the growth of other organisms. Probiotic organisms can be alive or dead, or they can be part of the microbial cell composition that benefits the host by helping to boost the individual’s disease resistance, overall health, growth performance, feed usage, stress response, or overall vitality, all in part by helping to maintain the microbial balance of the host or the overall microbial balance of the surrounding environment (Merrifield et al. 2010). These non-pathogenic bacteria reside in the gastrointestinal tract, and they may contribute to health and well-being by helping maintain gut health. Since this microflora is needed to avoid sickness, it is essential to save this microflora to protect against illness, particularly GI tract infections. Probiotics are a healthy microbiome, which take time to develop and provide health benefits to the host. A healthy population of intestines in the stomach expands with the addition of probiotic strains.

14.4 History of Probiotics

Lilly and Stillwell coined the word “probiotics” in 1965. Roy Fuller proposed in 1989 that probiotics have a favourable effect on the host. He described probiotics as live bacteria that promote the host’s health by improving the gut’s balance in the intestine when given in sufficient quantities. According to FAO and the WHO, probiotics are live bacteria that are ingested orally and have been shown to have substantial health benefits (Hotel and Cordoba 2001). The term “probiotics” derives from the Greek terms “pro” and “bios”, which mean “for life”, and was coined in 1953 by German scientist Werner Kollath to describe “active substances that are required for the healthy development of life”. Unlike antibiotics, he advocated the term “all organic and inorganic food complexes” to elevate these food complexes to the level of supplements. According to him, probiotics are an “active substance necessary for the healthy growth of life”. In a different context, Lilly and Stillwell used this word in 1965 to refer to “substances released by one organism that stimulate the growth of another”. Parker (1974) defined probiotics in the context of animal feeds a few years later as “Organisms and chemicals that have a favourable influence on the host by contributing to its intestinal microbial balance”. Fuller, in 1992, defined probiotics as “a live microbial feed supplement that benefits the host animal by enhancing its gut microbial balance” (McFarland 2015). Due to their unusual functional capability, it is hypothesized that probiotics may influence the gut microbiota of any aquatic species. Probiotics have already been revealed to conduct a different function in the host body by researchers (such as reducing disease and stress, improving immunity, modulating microbiota in the gut, assisting in nutrition, improving water quality). Furthermore, probiotics' favourable effects giving to higher feed value and animal growth and higher spawning and hatching rates in aquaculture systems. In this section, we discussed each function of probiotics and attempted to compare them with prior knowledge.

14.5 Salient Features of Probiotics

Probiotics with specified properties are commonly accepted as being necessary for the appropriate development of successful medications (Priyodip et al. 2017; Thakur et al. 2016). A nutritious diet with enough probiotic intake is essential for maintaining optimal gastrointestinal functioning. To be sure, non-invasive and non-carcinogenic and effective in a targeted manner must be provided; plasmid-free antibiotic and virulence resistance genes should be avoided, and it must be proven to successfully colonize and multiply within the host for an extended period (Hasan and Banerjee 2020).

14.5.1 The Essential Properties of a Probiotic Encompass

1. It should be capable of dealing with the acidic and bile salt conditions that exist in the stomach and small intestine (Pereira et al. 2003). According to WHO and FAO recommendations, probiotic organisms that are used in food must be able to survive the travel through the digestive system, including exposure to gastric acids and bile (Senok et al. 2005). They must also be able to colonize and increase in the digestive tract and maintain their safety, effectiveness, and strength throughout the product's life cycle.
2. Probiotics must have a high level of adhesion to epithelial cells (Kravtsov et al. 2008). Because adhesion is necessary for colonization, it is a critical selection factor for probiotic bacteria (Beachey 1981). The main protection mechanisms against pathogenic strains owing to competition on site of connection and food are the adhesion and colonization on mucosal surfaces (Westerdahl et al. 1991). Mucus binding proteins are found in most probiotic bacteria, which aid in the binding process.
3. It should prevent pathogen adhesion by specific rivalry (Reid et al. 2006).
4. Competition Exclusion: G. F. Gause, a Russian ecologist, established the competitive exclusion principle. This idea claims that species cannot coexist if they exploit the environment in the same way. In order to protect health, specific strains of bacteria are introduced into fish cultures to enhance the expression of various immune components and reduce pathogen burden on the intestinal mucus

layer (Banerjee and Ray 2017). Lactobacilli were found to compete with and diminish the attachment of *Yersinia ruckeri*, *Aeromonas salmonicida*, and *Carnobacterium piscicola* to rainbow trout intestinal mucus (Balcázar et al. 2006b).

5. It is feasible to stop pathogen colonization by stimulating decolonization of pathogenic microorganisms using one of several techniques. Bacteria must be able to cling to mucus and wall surfaces to establish themselves in the intestines of fish (Roeselers et al. 2011). Thus, competition for adhesion receptors with pathogens may be the first probiotic action because bacterial attachment to the tissue surface is crucial in the early stages of pathogenic infection (Chabrillón et al. 2006).

14.5.2 Characteristic Feature

1. Probiotics assist fish to grow, develop, and resist bacterial infection.
2. The probiotic microorganisms should have no adverse effects on the host.
3. Probiotics should not develop drug resistance; instead, they should maintain inherited features.
4. Probiotics should have the following characteristics in order to be used as an efficient feed:
    - Tolerance to acid and bile.
    - Gastric juice resistance.
    - Adherence to the surface of the digestive system.
    - Antagonism to pathogens.
    - Immune stimulation.
    - Increased intestinal motility.
    - Mucous survival.
    - Enzyme and vitamin production.
5. They should have good sensory qualities, fermentative action, freeze-drying tolerance, and viability in feed during the packaging and storage procedure (Chauhan and Singh 2018).

14.6 Selection Criteria

Probiotics are used to develop a friendly contact between the beneficial microbiome and pathogenic strains, to preserve the mucus constituents of freshwater fish intestines or skin. In order to prevent undesired effects in fish administration, probiotics must be non-pathogenic and non-toxic. Antagonism, adhesion, and challenge, for instance, must be investigated in vitro using probiotics. Some crucial and unique characteristics contribute to the advantage of the host. In the fish farming industry, probiotics are most often found in the fish gut and are produced from this source. Streptococcus is among the most common bacteria found in the lab (Giri et al. 2013). Although probiotics are a relatively recent method, they have acquired popularity due to their capability to moderate various physiological activities in aquatic species. After that, a variety of probiotics, such as *Aeromonas media*, *Enterococcus faecium, Bacillus subtilis, Carnobacterium inhibens, Lactobacillus helveticus,* and others, are regarded to be particularly beneficial at the time. Another microbe that was found in the GIT of fish and shellfish probiotic candidates is Gram-negative facultative symbiotic anaerobes such as Pseudomonas, Vibrio, and Aeromonas (Lakshmi et al. 2013; Verschuere et al. 2000). In addition to bacteria, bacteriophages, microalgae, and yeast, all forms of probiotics are forms of bacteria (Llewellyn et al. 2014). Many mono- and multi-strain probiotics are already available on the market (Van Doan et al. 2017).

14.7 Types of Probiotics

Aquaculture industry used two types:

1. Gut probiotics are mixed with food and orally administered to maintain and improve the beneficial microbiome of the gut. Gut probiotics are generally used in finfish culture.
2. Water probiotics live in water and consume all nutrients available, except for dangerous bacteria. This can remove harmful strains via starving (Nageswara and Babu 2006; Sahu et al. 2008). Currently, shrimp farming uses water-based probiotics. Probiotics that can be utilized for feed or as supplements for water are considered to function in fish as biological controls.

14.8 Forms of Probiotics

Two kinds of aquatic probiotics are available:

1. Dry forms: Dry probiotics are available in bags. They can be fed food or mixed with water and have several advantages, including safety, ease of usage, and a longer lifespan (Decamp and Moriarty 2007).
2. Liquid forms: The hatcheries mainly use live and ready-to-act liquid forms. These fluid forms are either added directly to hatchery tanks or mixed with farm feed. Liquid forms show short-lived positive results instead of dry and spore bacteria, although lower in density (Nageswara and Babu 2006). There are no reports that probiotics have adverse effects. However, it may temporarily increase biological oxygen for probiotics. Therefore, a minimum dissolved level of oxygen of 3% during probiotic administration is desirable.

14.9 Benefits of Probiotics

Probiotics are non-toxic to the host animal and help the host animal resist pathogenic bacteria either directly or indirectly. Due to a lot of work that has been done on the topic of probiotics, many of the theoretical advantages of their use have yet to be proved. It is critical to identify which probiotic strains are being utilized, as each strain offers specific health benefits (Senok et al. 2005).

- Despite well-established effects of probiotics, new research on the benefits of probiotics is always being conducted, and there are numerous open questions about the accuracy of some of the claims. While different probiotic strains offer distinct health advantages, it is essential to keep this in mind when purchasing supplements. By supporting and enhancing the immune system of cultured organisms, this treatment helps organisms better resist disease and stimulate the production of chemicals that hinder harmful organisms from causing disease in the host (Verschuere et al. 2000).
- It is proposed that the probiotic bacteria's activities have a direct growth-stimulating effect on fish. This may be due to either directly feeding the fish nutrients or supplementing minerals and vitamins. On the other hand, probiotics have been demonstrated in experiments to increase the ability of fish organisms to overcome host ailments or stimulate growth without harming the host.
- According to Venkateswara (2007), probiotic supplements have been shown to regulate microflora, control pathogenic ones, promote the breakdown of undesired organic substances, and improve environmental performance by reducing hazardous gases such as H_2O_2, NH_3, N_2O, and methane, among other things. Following probiotics, water quality improved in several trials, most notably with *Bacillus* sp. (Verschuere et al. 2000). *Bacillus* species are better at converting Gram-positive carbon to biomass or slime when compared to their Gram-negative counterparts.
- Probiotics and their products have various health benefits for both aquatic animals and terrestrial animals, but all of these benefits are provided by aquaculture, terrestrial animals, and human disease control. We also use microbial additives that help prevent intestinal infections, skin infections, and the proliferation of microbes in their environment.

14.10 Modes of Action of Probiotics

Probiotics can be administered in various ways, including (1) Incorporate to the pellet feed and culture water (2) enriching via live feed. Additionally, a thorough understanding of the mechanism of action and appropriate application procedures may be necessary for successful probiotic use in aquaculture. While the actual mechanism of action is unknown, it frequently exhibits host and strain-specific changes in its actions. On the other hand, probiotics generate great scientific and economic interest on a global scale in aquaculture (Banerjee and Ray 2017). Many criteria are used to select suitable probiotic strains, including production of extracellular enzymes, survival in gastric juice, mucus growth, acid and bile tolerance, production of antimicrobial compounds that suppress pathogen growth in vitro, and bio-safety (haemolytic activity and antibiotics susceptibility).

1. Probiotics may influence the host's immune system, especially the innate and acquired immune systems. The ability of this mechanism to accomplish this is essential not only for the inhibition and treatment of infectious illness but also for resolving intestinal inflammation.
2. Additionally, probiotics can influence commensals and potentially harmful organisms. This is an idea of high importance in the field of infection prevention and treatment and restoring microbial homeostasis in animals' digestive tracts.
3. Toxin inactivation and detoxification of host and food components may occur in the gut due to the action of probiotics on microbial products, host products, and dietary factors.
4. Probiotic substances function as an antagonist to the quorum-sensing system.
5. The role of enzymes in digesting.
6. Iron competition: For harmful bacteria to survive in the host, iron acquisition is crucial. Because of this, a large number of genes that play a role in iron uptake are associated with pathogenicity. Furthermore, because siderophores have a great affinity for ferric ions, they inhibit the growth of dangerous bacteria by limiting iron availability.
7. Some recent studies have indicated that probiotics can significantly impact various key physiological systems in aquatic animals. Some studies have found that it helps raise the insulin-like growth factor (IGF-I) levels in European seabass (Carnevali et al. 2014).
8. As furthermore, research shows that probiotics lower cortisol levels and support antioxidative enzymes to help the body better cope with stress (Zolotukhin et al. 2018).
9. While the use of probiotics to enhance disease resistance is widely documented, research on the effects and mechanism of probiotics on aquatic animal reproductive function is sparse (Abasali and Mohamad 2011). Using diverse strains such as *Lactobacillus subtilis, L. acidophilus, L. casei* and *B. subtilis*, very few studies have attempted to demonstrate the effect of probiotic supplementation on reproductive performance in aquaculture. It is well established that probiotics affect reproduction in various ways, including fertilization, gonadosomatic index, fecundity, and fry production by females (Ghosh et al. 2007).
10. While probiotic bacteria studies reveal that many bacterial strains have antiviral properties in aquaculture, the exact mechanism by which they inhibit viruses has not yet been determined. In the scientific community, the effectiveness of probiotic agents such as *Pseudomonas* sp. and *Vibrios* sp. against the infectious haematopoietic necrosis virus is generally acknowledged (Sahu et al. 2008).
11. Probiotic bacteria play an essential function in limiting algal development, notably red tide plankton (Qi et al. 2009). While unicellular algae are being

developed in the culture pond, bacteria antagonistic to algae will be undesirable in green water larval-raising techniques in hatcheries, where bacterial antagonists to algae will be useful.

12. Probiotic bacterial populations can produce numerous chemical compounds bactericidal or bacteriostatic to both Gram-negative and Gram-positive bacteria. These inhibitory molecules are of various origins, including proteinaceous substances (lysozyme and other types of proteases), chemicals (hydrogen peroxides), and iron-chelating compounds such as siderophores (Giri et al. 2013). These inhibitory chemicals are vital in pathogen suppression and growth, reducing pathogen load.
13. Physical competition for attachment sites on the host's gut mucosal layer is one putative strategy for inhibiting pathogen colonization. It is well understood that the capacity to cling to mucus and wall surfaces is required for bacteria to establish themselves in fish intestines (Roeselers et al. 2011).
14. Probiotics have a favourable role as immunostimulants in protecting cultured aquatic species by lowering the impact of illnesses and pathogen entry. As a result, its usage as an immunostimulant is an efficient method to improving aquaculture success. Many authors have validated the use of probiotics in carp species to improve immune response, disease resistance and minimize abnormalities (Chi et al. 2014; Dawood and Koshio 2016; Wu et al. 2015). Unfortunately, the precise impact of probiotic supplementation in the expression of immunological parameters is still unknown.

14.10.1 Trials of Probiotics in Fish Culture

Probiotics in aquaculture may act similarly to those found in terrestrial animals. The interaction between aquatic animals and the culture situation is significantly more complicated than that of terrestrial animals. Probiotics are microbial supplements containing living microorganisms that benefit the host by altering the host's or its culture environment. The microorganisms found in water bodies are harmful bacteria that are opportunists, which means they reap the benefits of an animal's stress situation, which causes a decline in growth and survival (Verschuere et al. 2000). Consequently, probiotics in aquaculture aims not only at animal's significant benefit but also at the effect on the farming environment.

14.10.2 Role in Immune System

Probiotic bacteria are beneficial bacteria, which can prevent and regulate the host immune system as well as infections (Yousefi et al. 2018). Furthermore, a study on the GALT (gut-associated lymphoid tissue) of seabream found that oral administration of a probiotic combo (*Lactobacillus plantarum* and *Lactobacillus fructivorans*) increased the production of granulocytes and antibody (Picchietti et al. 2007). Fish fed with *Flavobacterium sasangense*, *Aeromonas veronii*, and *Vibrio lentus* reported the rises in the expression of TNF, lysozyme-C, and IL-1b (Dawood et al. 2016). Probiotics administration increased leucocyte numbers (Korkea-Aho et al. 2012), lymphocytes (Brunt et al. 2007), monocytes, and erythrocytes (Aly et al. 2008; Sun et al. 2010), and serum bacterial agglutination titres, albumin levels (Abbass et al. 2010), and serum peroxidase (Heo et al. 2013) have also been shown to increase. Immunological parameters such as tumour necrosis factor, serum lysozyme activity, and expression of the cytokine genes (interleukin-1, heat shock protein 70) and respiratory bursts were considerably improved in fish fed with *Rummeliibacillus stabekisii* (Tan et al. 2019). Innate immune responses are the initial line of defence against infections, and they involve many cells and mechanisms that secure the host organism from infectious diseases. *Clostridium butyricum* treatment improved the phagocytic activity of rainbow trout leucocytes (Sakai et al. 1995).

Probiotics have been reported to affect non-specific immune system factors such as neutrophils, macrophages, monocytes, and natural cell killers (Hoseinifar et al. 2020). Improved fish immunity is associated with a rise in granulocytes and lymphocytes, which is linked to cell-mediated mucosal defence system (Lazado and Caipang 2014). After the administration of probiotics such as, *Lactobacillus belbrüeckii, Bacillus subtilis*, and *Debaryomyces hansenii,* with *Sparus aurata* has been shown to increase innate immune responses (Salinas et al. 2005). When treated with the probiotic strain *Bacillus subtilis* and *Pseudomonas, Oreochromis niloticus* demonstrated notable improvements in growth performance and immunological response (Wang et al. 2010). Gatesoupe (1994) discovered that turbot larvae fed with rotifera supplemented with lactacid bacteria developed resistance to *Vibrio* spp. infection. The feeding trial of *Lactobacilllus fructivorans* and *L. plantarum* with sea bream larvae (*Sparus aurata*) showed increased in colonization of the intestine as well as growth and survival (Carnevali et al. 2014). Probiotic *Lactobacillus rhamnosus* was found to accelerate the respiratory burst in rainbow trout (Nikoskelainen et al. 2003).

14.10.3 Immunostimulants

Probiotics play a vital role as immunostimulants in the protection of aquatic animals by reducing disease and pathogen entry. As a result, its usage as an immunostimulant is a very practical method to improving aquaculture success. Numerous studies have validated the usage of probiotics in carp species to increase disease resistance, immune response and reduce abnormalities. The studies showed on probiotics indicated favourable effects on host gut defences, which is critical in disease prevention and treatment of digestive tract inflammation and producing inhibitory substances (Akhter et al. 2015; Azimirad et al. 2016; Dawood and Koshio 2016). Probiotics or their derivatives for host health benefits have been demonstrated to be effective in aquaculture disease management. These comprise microbial adjuncts that inhibit harmful bacteria from multiplying in the intestine, on the surface of the skin, as well as within the culture atmosphere of the species (Verschuere et al. 2000). Probiotics have an effect on culture organisms by improving their immune systems, increasing their susceptibility to pathogens, or generating inhibitory substances that can stop harmful organisms from infecting the host.

14.10.4 Colonization Capacity

Competitive exclusion and immunomodulation are the two basic modes of action in aquatic species. Probiotics have the potential to be an effective substitute to the preventive usage of antibiotics and additives. Oxygen, chemicals, and nutrition can be competitive with or even better than water and feed utilities in encouraging the health and growth of livestock. The intestinal tract's mucosal epithelium is inhabited by probiotics (Korkea-Aho et al. 2012), and exhibit adherence (Mahdhi et al. 2012) and proliferation in intestinal mucus (Sorroza et al. 2012). Probiotics such as *Bifidobacterium infantis*, *Bacillus coagulans*, and *Bacillus mesentericus* effectively colonized in the gut of freshwater ornamental fish *Puntius conchonius* (Divya et al. 2012). While Pseudoalteromonas RA7–14, Ruegeria RA4–1, Microbacterium ID3–10, and Vibrio RD5–30 formed in the intestines of Atlantic cod larvae, their colonization was just a temporary occurrence (Skjermo et al. 2015). Probiotics (*Bifidobacterium infantis, Lactobacillus rhamnosus, Bifidobacterium longum, Bacillus coagulans, and Bacillus mesentericus*) effectively colonized in the gut of fish, *Brachydanio rerio* larvae (Akbar et al. 2014b).

14.10.5 Antagonistic Activity

Few bacteria yield a variety of antibiotic and antagonistic components that can be used as probiotics. Probiotics are utilized as a substitute to antibiotics and pesticides for prophylaxis (Decamp et al. 2008). To compete for nutrients and habitats, they developed anti-bacterial compounds (Moriarty 1998). Probiotics produced enough organic acid and a decrease in pH to inhibit many harmful bacteria. *Bacillus licheniformis* and *Bacillus pumilus* both have antibiotic activity and could survive low pH and high bile levels (Ramesh et al. 2015). Bacteriocidal proteins, organic acids, diacetyl, and hydro peroxide were all generated by *Lactobacillus* spp. (Farzanfar 2006; Verschuere et al. 2000). These compounds activated animal immune systems, making them more resistant to pathogens or suppressed bacterial pathogens in aquaculture systems. In white leg prawns, *Bacillus licheniformis* displayed in vitro inhibitory properties contrary to *Vibrio alginolyticus* (Ferreira et al. 2015).

14.10.6 To Improve Disease Resistance

Probiotics have been demonstrated to be disease-resistant and are good pathogen-prevention measures. Probiotics are vital in the development of anti-bacterial compounds that prevent pathogenic bacteria from entering organisms and develop resistance to infectious diseases. Probiotics improved the gut flora's ability to fight infections. Because probiotics are a beneficial contributor to disease management policies in aquaculture (Balcázar et al. 2006a). Probiotics administration has been shown to increase disease resistance in fish by increasing both innate and adaptive immune responses. Several studies have shown that probiotics can protect aquatic animals from pathogenic infection. The probiotic *Pedioccus acidilactici* fed to *Litopenaeus stylirostris* demonstrated vibriosis resistance (Castex et al. 2008). Similar studies have been reported by *Vibrio* spp. vs. *Brevibacillus brevis* (Mahdhi et al. 2012), *Flavobacterium psychrophilum* vs. Pseudomonas M174 and M162 (Ambas et al. 2013), *Bacillus* spp. vs. *Strep. iniae* (Cha et al. 2013). *Pseudomonas aeruginosa* supplementation increased innate immunity and illness resistance in rats (Giri et al. 2012). *Streptococcus phocae* is a fish disease; however it increased the growth of *Penaeus monodon* post larvae and protection against *Vibryo harveyi* (Austin and Austin 2012). *Aeromonas hydrophila* and *A. sobria* have been shown to be fish pathogens (Austin and Austin 2012), and they have been shown to diminish infections of *Aeromonas salmonicida* (Irianto and Austin 2002). Studies have reported in *Bacillus pumilus* in *Nile* tilapia (Aly et al. 2008), *Oncorhynchus mykiss* (Brunt et al. 2007) and *Bacillus* S11 in white scampi (Rengpipat et al. 2000). Rohu was protected against *A. hydrophila* infection by *Bacillus licheniformis* and *Bacillus pumilus* (Ramesh et al. 2015). Dietary probiotic *Lactococcus lactis* on Japanese flounder (Heo et al. 2013), *B. pumilus* improved Nile tilapia health and disease resistance (Aly et al. 2008). The occurrence of white spot syndrome virus was reduced in white leg prawns fed a mixture of *Staphylococcus hemolyticus* and *Pediococcus pentosaceus* (Leyva-Madrigal et al. 2011).

14.10.7 Improve Water Quality

Probiotics have been proved to help enhance water quality in different ways. Probiotics have enhanced water quality by reducing pathogenic water bacteria (Dalmin et al. 2001), enhanced organic matter decomposition, reduced ammonia concentration, hydrogen sulphide, nitrogen and phosphorus, nitrite (Boyd and Massaut 1999; Cha et al. 2013). Probiotics reduced phosphate pollution in the sediments (Wang and He 2009), organic matter accumulation, mitigated nitrogen (Verschuere et al. 2000; Wang et al. 2005), probiotics reduced metabolic wastes in cardinal tetras during transportation (*Paracheirodon aexlrodi*) (Gomes et al. 2009).

14.10.8 Improvement in Nutrient Utilization and Digestion

Studies have demonstrated that administering probiotics to a diet will benefit the health and disease resistance of fish. Recent research has also shown that dietary probiotics can improve nutritional digestibility in aquatic animals by boosting the activity of digestive enzymes. *Bacillus* species have been widely used in aquaculture due to their ability to sporulate and beneficial impacts on feed utilization, immunological response, and digestive enzyme activity. Furthermore, some *Bacillus* species supplementation via water bath or live feed has been found to modify the gut microbial composition in fish. However, Lactic acid bacteria are the most often utilized probiotic formulations for this purpose. It enhanced nutrient digestibility and enhanced the production of digestive enzymes in the host (Ghosh et al. 2017). Few bacteria, for example, *Rhodobacter sphaeroides* and *Bacillus* sp., participate successfully in improving digestive enzymes and cellulase enzymes considerably in white shrimp (*Litopenaeus vannamei*) and bivalves (Sahu et al. 2008; Wang et al. 2008). Bacterial species *Staphylococcus* sp., *Brevibacterium* sp., *Pseudomonas* sp., *Microbacterium* sp., and *Agrobacterium* sp. have all been identified as essential to a charr's metabolic and nutritional well-being in the Arctic (*Salvelinus alpines*) (Ringø et al. 2014).

The probiotics like *Lactobacillus*, *Brevibacillus brevis*, *Vibrio harveyi*, and *Vagococcus fluvialis* are all known to help aid digestion and gastrointestinal health (Lazado et al. 2011; Mahdhi et al. 2012), adhere to the mucus, and also improve feed digestibility by increasing the activity of digestive enzymes (Zokaeifar et al. 2012). They also create body components, such as fatty acids, vitamins, antibiotics, bacteriocins, siderophores, and lysozyme, which are beneficial to host health (Vine et al. 2006). Probiotic supplemented food, including *Streptomyces* sp. and *Bacillus subtilis,* increased growth and survival rates in *Xiphophorus maculates*, *Poecilia reticulate*, and *Xiphophorus helleri* (Dharmaraj and Dhevendaran 2010). Khattab et al. (2005) reported Micrococcus luteus as probiotics in Tilapia, which exhibited improved feed conversion ratio and growth performance. Nile tilapia fed with *Rummeliibacillus stabekisii* for eight weeks resulted in greater digestive enzyme secretion, faster growth, and more significant weight gain (Tan et al. 2019). Feeding a probiotic strain of *Lactobacillus* sp. to Nile tilapia (*Oreochromis niloticus*) improved crude lipid, total protein, and body weight (Hamdan et al. 2016). It is generally accepted that probiotics help animals grow and develop strong immune systems and better digestion by increasing the number of beneficial microorganisms in the intestinal tract, resulting in improved feed quality, growth-promoting substances, and digestive enzymes. Probiotics alter the population of microbes in the gastrointestinal tract in several ways, one of which is to generate various types of fatty acids, minerals, vitamins, and important amino acids (Newaj-Fyzul et al. 2014). *L. vannamei* growth and feed efficiency are increased by probiotic *B. subtilis* E20. Bacillus contains probiotic strains that release extracellular enzymes that break down numerous nutrients into smaller molecules such as proteins, lipids, and carbohydrates (Ochoa-Solano and Olmos-Soto 2006). Van Hai and Fotedar (2009), for example, demonstrated that dietary probiotics (*Pseudomonas Synxantha* and *P. aeruginosa*) increased the growth performance of western king prawns. Rohyati (2015) recently discovered that feeding probiotics (Vibrio Alg3.1RfR-Abn1.2RfR-enriched protein) increased the growth rate of *Haliotis asinine*; probiotics may be a supplemental food source due to their availability of essential nutrients such as biotin, fatty acids, and vitamins (Verschuere et al. 2000).

14.10.9 Growth and Survival

Probiotics are live microbial cells that, when given in appropriate quantities, helps boost the growth and health of fish (Ringø and Gatesoupe 1998). Another important characteristic of probiotics is that it takes less time for gut loading of live feeds such as Artemia (Akbar et al. 2014a). Several research investigations reveal that probiotic supplementation has been shown to increase the rate of development, the strength of the immune system, and the resistance to sickness in fish. Kumar et al. (2006) discovered that *Bacillus subtilis* fed *Labeo rohita* had a greater survival rate when subjected to an intraperitoneal injection of *Aeromonas hydrophila*. Growth stimulation *Bacillus* spp. has been previously reported in *Paralichthys olivaceus* (Gatesoupe et al. 1989), Tilapia, *Scophthalmus maximus* (Gatesoupe 1991), and Nile tilapia (Aly et al. 2008). Probiotics have increased the growth rates of aquatic animals, the feed utility, and survival rates by modulating digestive enzyme activities (Ringø et al. 2010). Bergh et al. (1992) discovered that when the Atlantic halibut (*Hippoglossus hippoglossus*) started feeding for the first time, the intestinal flora changed from a predominance of *Flavobacterium* spp. to *Aeromonas* spp. demonstrating the impact of the external environment and food on the microbial community of this fish. Probiotics enhanced the growth of *Penaeus monodon* (Maeda and Liao 1992) and *Macrobrachium rosenbergii*, a giant freshwater prawn (Mujeeb Rahiman et al. 2010). The growth of western king prawns was boosted by *Pseudomonas aeruginosa* and *Pseudomonas synxantha* (Van Hai et al. 2010). Probiotic *Lactobacillus plantarum* helps the fish gut by creating antibiotic compounds that prevent dangerous intestinal bacteria from multiplying and suppressing the growth of competing bacteria (Kesarcodi-Watson et al. 2008). As a result, supplementation with *L. plantarum* resulted in immunological modulation, enhanced growth parameters, and elevated disease resistance in a wide range of fish species (Dawood et al. 2015; Son et al. 2009). Probiotics help in digestion by enhancing the production of extracellular enzymes like proteases, carbohydrases, and lipases, as well as providing growth factors. Flocculated algae (flocculated with probiotics *Bacillus subtilis* (MTCC 441) and *Lactobacillus acidophilus* (10,307 MTCC)) enriched Artemia has a remarkable effect on digestive enzyme production, growth, and gut microflora of freshwater fish *Catla catla* (Kandathil Radhakrishnan et al. 2020). Vibrio midae SY9 improved the digestive enzyme activity (protease), digestion and absorption of proteins, and growth performance of *Haliotis midae* (Huddy and Coyne 2015). With an increase in digestive enzymes, photosynthetic bacteria and *Bacillus* spp.

boosted the growth of white leg prawns (Wang 2007). Specific digestive enzyme (lipase, protease, and amylase) activities were elevated in probiotic-fed *Fenneropenaeus indicus* (Ziaei-Nejad et al. 2006). Furthermore, probiotics resulted in the production of vital nutrients such as fatty acids, biotin, and vitamin B12 (Vine et al. 2006). Bacteria are a key food component in natural environments by holothurian deposit-feeding, and probiotics can act as a supplementary food or meal digestion aid (Verschuere et al. 2000). Yassir et al. (2002) attempted to use probiotic bacteria as a growth promoter on *Oreochromis niloticus* and discovered that *Micrococcus luteus* resulted in the best growth parameters and the feed conversion ratio on Tilapia. As a result, probiotics can be used as growth promoters in aquaculture while also providing other profits. The growth and survival of *Haliotis rufescens* (Silva-Aciares et al. 2011) and *H. midae* was improved by Vibrio C21-UMA and *Vibrio midae*, respectively (Macey and Coyne 2006). *B. subtilis* or *Lactobacillus acidophilus* increased the survival rate of Nile tilapia (Aly et al. 2008), and *L. acidophilus* improved the survival rate of Nile tilapia (Villamil et al. 2014). *Pseudomonas aeruginosa* YC58 increased the survival of pearl oyster juveniles (Aguilar-Macías et al. 2010). Sahandi et al. (2012) found that direct inoculation of Bacillus probiotic into fish culture tanks has good impacts on the growth of *Oreochromis mossambicus, Ctenopharyngodon idella,* and *Cyprinus carpio*, and Tilapia had considerably increased growth performance when the commercial probiotic BZT® BIOAQUA was introduced to tanks, and probiotic treated fish inclined to perform well survival in all treatments (Mohamed et al. 2013). Devaraja et al. (2013) demonstrated that *Bacillus* sp. increased the growth of *Penaeus monodon* in a culture system with no water exchange. Bacillus is a frequent probiotic used in aquaculture. Due to the fact that *Bacillus* spp. are non-toxic and good to shrimp, this microbe is a popular one in aquaculture. For example, the introduction of *Bacillus* to the meal helped the tiger shrimp's survival, growth, and immunity (Rengpipat et al. 2000). Some beneficial bacterial strains (Micrococcus, Bacillus, Lactobacillus, Saccharomyces, Debaryomyces, Lactococcus, Enterococcus, and Photorhodobacterium) have been added shrimp diets or water to enhance their growth (Tseng et al. 2009; Wang et al. 2019). *Bacillus subtilis* E20 was used as a probiotic for *L. vannamei,* and the larval development was improved by either orally administering it to water or by adding it to water (Liu et al. 2010). Numerous studies have demonstrated that specific probiotics administration may be a substitute for preserving aquatic life. Farmers, however, cannot predict when sickness will strike to offer probiotic feed ahead of infection. Consider that in aquaculture, good in vitro results don't always translate to good in vivo effects. Also, the lifespan of probiotic benefits is unknown. Nobody knows what happens to living probiotics in aquatic environments. It is suspected that short-term cyclic probiotic feeding may avoid stimulating the immune system while sustaining some level of protection or immunostimulant. As a result, more research is required to explore this application technique. However, more research is needed to find out how often, in what manner, for how long, and with what kinds of symbiotic to be used. Probiotic bacteria can improve feed efficiency by producing digestive enzymes, which is especially useful in aquaculture because there is a shortage of fish meal. Probiotics are critical when animal protein alternatives such as plant protein sources are used as a supplement. It is critical to explore the metabolic abilities of probiotics when trying to deconstruct antinutrients, particularly in plant-based proteins. Dosage-dependent research is scarce and inconsistent. Additional investigation is also required before providing any level of certainty. As more in-depth research is done on every area of probiotics, more fruitful results are anticipated about the application of probiotics in aquaculture. In order to ensure the integrity of probiotics, they will have to be grown and developed in industry settings that follow strict quality control requirements. This would enable the worldwide distribution of organic aquaculture products, which are essential for human nutrition and wellness. A harvest that uses natural and sustainable methods can help to keep food fresh and safe for humans.

14.11 Conclusion

More recently, probiotics have enhanced fish health, water quality, disease prevention, and immune response support. Probiotics could be used in aquaculture, but there is much work ahead of us. Developing screening criteria for potential probiotics are critical, as understanding the mechanisms of action is essential to discovering new probiotics. To obtain a complete understanding of the microbiome and probiotics, further in-depth research on host-microbe interactions in vivo and the development of new tools for microbial surveillance is required. The effectiveness of probiotics is dependent on knowledge of species and strain competition.

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Chapter 15
Glimpse of Feed and Feed Additive Necessity and Mycotoxin Challenges in Aquaculture

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Abstract Aquaculture is one of the fastest-growing industries in the world, and it primarily helps to boost global production. The possibility of cultured fish demonstrating their genetic capacity for growth and reproduction major crucial factor determined by nutrition. Because of the limitation on the use of various antibiotics, as well as their cost-effectiveness, natural feed additives are an external source of aquaculture output. The enhancement in cost scarcity of supplies of fish feed has necessitated more study into replacements. Many researchers have recently demonstrated the benefits of medicinal herbs as feed additives. Even consumers are concerned about using antibiotics instead of natural feed additives to increase aquaculture quality. The determination of feed additives is to suppress pathogenic bacteria, improve growth, stimulate the immune system, and assure water purity. Essential oils, essential fatty acids, probiotics and prebiotics, synbiotics, and exogenous enzymes were all used in several types of feed additives. These additives were

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praised for their unique medical capabilities as well as their eco-friendly digestive system metabolisms. However, utilizing crops in feeds increases the risk of fungus and mycotoxins contamination, as well as the incidence of mycotoxicosis in fish. Because mycotoxicosis causes decreased bodyweight, growth impairment, and greater rates of disease and mortality in fish, this could reduce aquaculture productivity. This chapter highlights the cumulative research findings on various feed additives, and aquaculture production quality and mycotoxins impair the animal immune systems.

15.1 Introduction

The annual growth rate of the aquaculture industry has experienced a faster expansion during the last decades compared with the other animal protein food-producing industries such as poultry, pork, and beef (Balamuralikrishnan et al. 2017; Choi et al. 2020). However, how seriously sustainable development standards considered will have a significant impact on the aquaculture industry's future growth. Hishamunda et al. (2009) demonstrated that the feed is a critical component for the aquaculture industry's long-term viability, as it has a substantial impact on production costs (50–70%) and trophic transfer efficiency. Fish meal is an excellent source of essential amino acids with high palatability and digestibility among the several protein sources utilized in the fish diet. In 2005, Zhou et al. stated that fish meal is an ideal protein source in aquafeeds because of its reasonably balanced nutrient diet and low levels of anti-nutritional agent. Other nutrients found in fish meal include important fatty acids, nucleotides, vitamins, minerals, and phospholipids (Tacon and Metian 2015). However, due to overexploitation of fishery resources, high demand and insufficient supply of fish meal have resulted in higher fish meal prices (Li et al. 2021). As a result, many scientists have been searching for alternate protein sources to reduce fish feed costs and improve long-term aquaculture development.

Several animal and plant protein sources have been investigated for entire or partial substitution of fish meal in aquafeed (Govindharajan et al. 2020a, b; Wen-Chao et al. 2020). Gatlin et al. (2007) stated that soybean meal is one of the most extensively used plant proteins in fish feed and livestock animals because of its global availability, low price, strong nutritional content, and generally balanced amino acid outline (Balamuralikrishnan et al. 2021). Due to their high digestibility, nutritional content, and low cost, poultry by-product meal, animal generated by-products such as meat and bone meal, and tankage meal have also been used as fish meal substitutes (Galkanda-Arachchige et al. 2019; Govindharajan and Vairakannu 2022). As a result, in order to reduce costs and boost profitability in an aquaculture business, the introduction of plant-based protein that can compete well with conventional protein sources in terms of nutritional quality is unavoidable (Francis et al. 2012). Although, there are certain restrictions to using plant sources in fish nutrition due to specific issues such as poor protein content and the presence of anti-nutrients, which will undoubtedly impair the palatability and digestion of feed for fish (Ali et al. 2003; Dorothy et al. 2018a, b). Previously, Encarnação (2016) reported that the wide range of feed extracts are used to target the health and growth of aquaculture species, as well as feed utilization and stress resistance. Antibiotics and synthetic minerals based on chemicals can have serious adverse effects and degrade water quality (Hoseinifar et al. 2019). Moreover, we focus on the use of natural feed additives in the diet of aqua culture in terms of their antioxidant role, antimicrobial action, beneficial impact on growth efficacy, and their effect on enhancing the digestibility.

15.2 Nutritional Factors for Fish Feeding

Fish feeding is done to meet nutritional and energy needs, and most aquatic species' feed intake is negatively associated with feed energy and protein levels. The fishing industry plays a critical role in the country's economic development, job creation, export revenues, and food and nutritional security (Ayoola 2010). Paul and Giri (2015) stated that after China, India's fisheries are the world's second-largest producer of fish. The first two quarters of 2015–16 had an increase in fish production, which was predicted to be 7.54 MT (DAHAD 2015–16). Due to the rapid expansion of the aquaculture business and the advancement of culture techniques, the need for fish feed has skyrocketed. The provision of low-cost, high-quality feeds is critical to the global aquaculture sector. As the worldwide fish farming industry grows, the demand for alternative, cost-effective, and high-quality protein sources grows as well (Fig. 15.1). Feed expenditures alone account for 60% of overall fish production costs (Paul and Giri 2015). Because of its balanced amino acid profiles, high protein content, and rich source of critical fatty acids, fish meal is a prominent

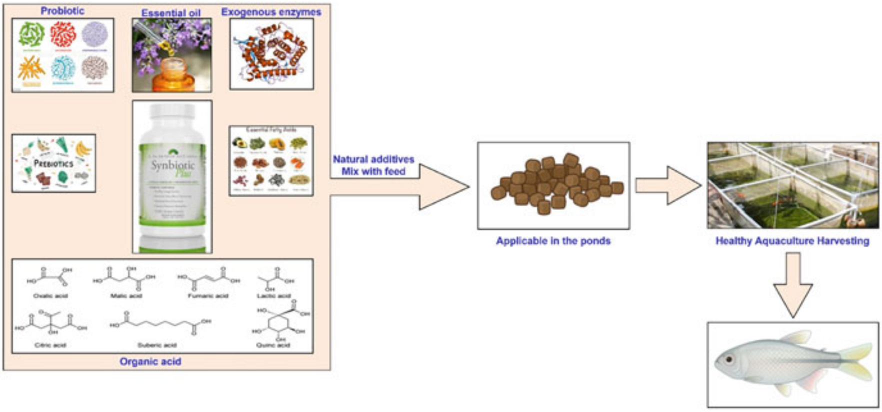

Fig. 15.1 Schematic diagram of natural feed additives

protein source in aquafeed businesses (Abdelghanty 2003). Proteins, lipids, vitamin E, carbs, cholesterol, nutritional attractants, and dietary preferences are among the nutritional elements identified in feed consumption. Cho and Kaushik (1990) discovered that raising the dietary protein-to-energy ratio reduces rainbow trout feed intake. The maximum amount of kingfish consumed depends on the protein-to-energy ratio, which fluctuates depending on body weight. The effects of dietary protein and energy levels on kingfish were investigated by Pirozzi et al. (2010). Their natural dietary patterns, which are classed as herbivorous, omnivorous, or carnivorous, account for this variance. As a result, there is a link between natural feeding patterns and protein requirements in the diet. Some carnivorous species require more protein in their diet than herbivorous and omnivorous species (NRC 1993). Carnivorous species are particularly efficient at converting food protein and lipid into energy, but not so much at converting carbohydrates into energy.

15.2.1 Proteins and Amino Acids

Proteins are made up of a variety of amino acids, the makeup of which determines the features of each protein. For numerous fish species, quantitative dietary requirements for the 10 essential amino acids have been determined (Wilson 1994). Although fish do not have a precise dietary requirement for carbs, providing these compounds in diets is a low-cost source of energy for them. Many carnivorous species use dietary carbohydrate for energy less effectively than herbivorous and omnivorous species (Wilson 1994). Some carbohydrate is stored as glycogen in tissues like the liver and muscle, where it provides an immediate source of energy. Some carbohydrate is stored as glycogen in tissues like the liver and muscle, where it provides an immediate source of energy. Small amounts of soluble carbohydrate in the form of glycogen, which is structurally identical to starch, are found in animal tissues such as liver and muscle.

15.2.2 Lipids

This nutrient category is made up of a variety of substances. For aquatic animals, neutral lipids (oils and fats) in the form of triglycerides provide a concentrated source of energy. Dietary lipids also supply essential fatty acids that the body cannot create on its own (Sargent et al. 1995). Fish require more of the linolenic acid (n-3) family of fatty acids than the linoleic acid (n-6) family. Dietary protein and dietary fat have the maximum weight increase rate in *S. meridionalis* Chen. The feed consumption of both catfish decreased as the dietary protein level increased. Feed consumption was lowered in both catfish species. It was inversely proportional to protein content, and the feed efficiency ratio was likewise lowered. Furthermore, the protein efficiency ratio improved. Dietary protein had a considerable impact on the protein efficiency ratio of both catfish. The effects of dietary lipid levels on the growth and feed consumption of juvenile all-female rainbow trout (*Oncorhynchus mykiss*) were studied by Eya et al. (2013). A meal containing 42% crude protein and a minimum of 20% dietary lipid promotes rapid growth and feed utilization in young all-female rainbow trout (*O. mykiss*).

15.2.3 Vitamins and Minerals

Inorganic elements that the body requires for a range of processes are included in this nutrition group. For tissue development, osmoregulation, and other metabolic activities, fish requires the same minerals as terrestrial animals (Lall 2002). Dissolved minerals in the water, on the other hand, may be able to meet some of the metabolic needs of fish. Huang and Huang (2003) conducted a feeding experiment on young hybrid tilapia fed oxidized oil meals to see how vitamin E levels affected growth, tissue lipid peroxidation, and liver glutathione levels. In addition, Ogwuegbu et al. (2022) reported that 15 vitamins are required for terrestrial animals and numerous fish species that have been studied so far. Ascorbate-induced lipid peroxidation was much lower in the muscle and liver of fish fed diets containing more than 80 IU vitamin E/kg than in those fed lower amounts of vitamin E (0 and 40 IU/kg). Vitamin E deficiency causes tissue vitamin E and liver glutathione levels to rise.

Vitamin premixes can now be added to prepared meals to ensure that fish get proper quantities of each vitamin regardless of dietary ingredient levels. This provides producers with a buffer against losses caused by processing and storage. Protective coatings and/or chemical modifications have enhanced vitamin stability during feed manufacturing and storage throughout the years. This is especially noticeable in the synthesis of several stabilized forms of ascorbic acid, which is extremely labile (Ogwuegbu et al. 2022). As a result, vitamin inadequacies in commercial manufacturing are uncommon.

15.3 Environmental Impact of Aquaculture Feed

Aquaculture is a rapidly expanding business that accounts for about half of all fish consumed by humans globally (Santos and Ramos 2018; Govindharajan et al. 2020b). Aquaculture activities contribute significantly to the rise of organic waste and hazardous compounds in the aquaculture industry (Cao et al. 2007a, b). Mycotoxin are abiotic hazards produced by certain fungi that can grow variety of animal feed. Period of unfavorable storage condition can led to changes in quality of fish. A relevant toxin of mycotoxin could be developed in the fish feed, here the mycotoxin of ochratoxin A (OTA) was found in samples after warm and humidity treatment (Pietsch et al. 2020). The presence of aflatoxin B1 (AFB1), OTA, and ZEN in fish feed, as well as the chemical composition of the diet, was investigated. The higher the risk of mycotoxins exposure and invitro digestion, the younger the fish. Bioaccessibility level of 85% to assess high levels of AFB1 (Nogueira et al. 2020; Bol et al. 2016). The small-scale fish farmers, cost of feed and its quality of nutritional value were concerned to produce own feed from agricultural by-product which has bed impact on the aquaculture production to reduce the growth, nutritional quality, and fish growth time.

15.4 Feed Additives Developments

Which function does the European Union Reference Laboratory (EURL) for feed additives play in the European Union's licensing system for feed additives, Regulation (EC) No.1831/2003 entirely altered the authorization method and established the EURL (von Holst et al. 2015). The study of photosynthetic study of *Isochrysis zhangiangenesis* of microalgae cultivation to be aquaculture applications, this low-cost microalga production technology, as well as superior microalgae production and aquaculture integration (Zhu et al. 2018, 2019). Zhu et al. (2018) demonstrated that used a low-cost floating photobioreactor (PBR) with no aeration or agitation mechanism to scale up spirulina platensis culture is a common difficulty. The use of additives in the fish feed industry has been focused on synergetic energy with more than one molecule of taurine (500 ppm) and silymarin (100 ppm) in the salmon farming industry with cell line CHSE-214 on that data which indicate that the preparation of diets carried out synergy between antioxidant additives and the reduction of additive antagonistic effects (Olivares et al. 2020). Because farmed animals lack access to carotenoid sources, the red and pink ketocarotenoids, canthaxanthin and astaxanthin are employed as feed additives in the poultry and aquaculture sectors as sources of egg yolk and flesh coloration (Pierce et al. 2015). The alternative drug of emamectin benzoate (EMA) as well as food safety concerns about the Nile tilapia's potential in freshwater fish (*Oreochromis niloticus*) residue limit 100 μg/g for fish muscle, its answer to the well-known desire for new alternative veterinary medications for aquaculture is well known. With dietary addition of hydrated sodium aluminum silicates nanoparticles or nano copper, pathological lesions caused by OTA-contaminated feed on growth would be reduced (Fadl et al. 2020). The effects of phytogenic feed additives (PFA) on the livestock industry are inconsistent, posing a risk.

15.5 The Role of Natural Feed Additive

Natural food can make a significant contribution to the nutrition of particular life stages of fish in certain cultural systems (e.g., ponds). Producers should encourage the growth of natural foods whenever possible, supplementing with prepared feeds. Fish will require more nutrition as they get older than their environment can provide, especially in high production conditions, and should be fed nutritionally complete prepared meals. The use of nutritionally adequate prepared feeds is crucial in culture systems such as raceways, cages/net pens, and recirculating systems, where natural food is limited.

15.5.1 Compounds That Are Phytogenic or Phytobiotic

Phytobiotic or phytogenic compounds are plant derivatives that are added to fish feed to improve their growth and health. Antioxidant, analgesic, antibacterial, anticarcinogenic, antiparasitic, insecticidal, appetite enhancer, anticoccidial, stimulator of bile production, and digestive enzyme activity are some of the qualities of these plant compounds (Asimi and Sahu 2013). These phytobiotic components are a diverse group of feed additives derived from many parts of plants, including leaves, tubers, roots, spices, and fruits. Because of its economic and important uses, researchers have recently focused on *Moringa oleifera,* also known as the drumstick tree. It is a fast-growing *Moringaceae* family member that can be found throughout the tropics and subtropics.

Saponins and phenols (anti-nutrients) in *M. oleifera* leaf (Egwui et al. 2013) can be removed using a variety of procedures, including soaking in water, air drying at room temperature, or grinding (Lochmann et al. 2011). These nutrients boost fish health, increase growth, and so increase aquaculture productivity (Dorothy et al. 2018a, b; Nsofor et al. 2012). Other fish species, such as Nile tilapia and common carps, have had their diets supplemented with Moringa leaf meals up to 30% of the time without seeing any negative effects on growth (Afuang et al. 2003; Yuangsoi and Masumoto 2012).

15.5.2 Probiotics

Probiotics are live microbial feed supplements that boost fish growth by influencing the gut flora population (Balasubramanian et al. 2016a, 2017). A single type of microbe or a mixture of species can be used as a probiotic. The bacteria in the supplement colonize the intestines and prevent harmful microbes from flourishing. Allowing the fish to avoid wasting metabolic energy resisting the harmful organisms' effects. After pelleting, probiotics must be introduced to the diets (Kavita et al. 2015).

15.5.3 Fatty Acids

To enhance growth, 1% of highly unsaturated fatty acids (HUFA) such as docosahexaenoic acid (22:6 W3) and eicosatetraenoic acid (20:5 W3) can be added (Nasseri et al. 2011).

15.5.4 Prebiotics

Prebiotics are non-digestible feed elements that have a positive effect on the host by encouraging the growth or activity of one or a small number of bacterial species that are already present in the gut, thereby attempting to improve host health (Gibson and Roberfroid 1995). Xylooligosaccharide, inulin, fructooligosaccharide, oligofructose, mannan oligosaccharide, b-glucan, and galactooligosaccharide are some of the most commonly utilized prebiotics in aquaculture. Prebiotics are largely fibers that are non-digestible food elements that benefit the host's health by encouraging the growth and activity of certain microorganisms in the colon, primarily lactobacilli and bacteria (Kavita et al. 2015). For prebiotics used in feed, the following characteristics should be met: (i) Resistance to the fishes' upper gut tract. (ii) It should be easily fermentable by the microorganisms in the intestine. (iii) It should be good for the host's health. (iv) Probiotics should be stimulated selectively. The following criteria should be a must for prebiotics, which is used in feed: (1) Resistance to the upper gut tract of the fishes. (2) It should be easily fermentable by intestinal microbiota. (3) It should be beneficial to host health. (4) It should selectively stimulate the probiotics (Crittenden and Playne 2009).

15.5.5 Organic Acids

Organic acids are carboxylic short-chain fatty acids with a low molecular weight because they breakdown into a hydrogen ion and a carboxylate ion in water (Balasubramanian et al. 2016b; Lim et al. 2015). Citric acid, formic acid, benzoic acid, acetic acids, lactic acid, malic, propionic, and sorbic acids, as well as their salts, are examples of organic acids (Ng et al. 2009). Previously, Nates (2016) sated that fish growth, nutritional use, and disease resistance were all improved by organic acids. It lowers the pH in the stomach and intestine while enhancing the activity of digesting enzymes. Organic acids permeate bacteria cell walls, disrupting their normal function and inhibiting their growth (Nates 2016). Its activities are influenced by a variety of parameters including fish species, size, age, organic acid kinds and levels, feed management, and water quality (Fefana 2014).

15.5.6 Enzymes

Anti-nutritional factors have a negative impact on the growth performance and digestion of dietary components of fish and livestock animals (Balamuralikrishnan et al. 2018; Kwang Yong et al. 2018). Lin et al. (2007) and Ebru et al. (2016) demonstrated exogenous enzymes which can help with these issues. Phytase, protease, carbohydrase, alpha-amylase, lipase, pepsin, and papain are some of the most commonly utilized enzymes in aquaculture feeds. Phosphorus is found in plant seeds in the form of phytate, which accounts for 80% of the phosphorus. The digestion and bioavailability of phytate phosphorus in fish are quite low. As a result, phytase in fish feed improves phytate phosphorus digestion, lowers phosphorus excretion and phosphorus utilization, and improves protein (Cao et al. 2007a, b). The addition of non-starch polysaccharides degrading enzymes to feeds improves the digestibility of non-starch polysaccharides (Sinha et al. 2011).

15.5.6.1 Aqua Feed Additives and Replacement of Fish Meal

Poultry by product meal (PPM) is an animal-based protein source that can be used to replace fish meal (FM) because of its high protein content and low cost, furthermore, seafood wastage was used for the animal and aquaculture feed for avoiding the fish wastages which cause environmental pollution (Galkanda-Arachchige et al. 2020; Afreen and Ucak 2020). Soybean meal and pea protein concentrate were shown to be the optimum quality for replacing commercial fish meal and narrow-leafed lupin in salmon from the Atlantic (47 g) study (Carter and Hauler 2000). Purple phototropic bacteria (PPB) produced a high protein proportion of biomass from wastewater, with potential added value as ingredients for replacing fish meal in Asian sea bass diets with PPB microbial biomass (Lates calcarifer), a hugely valuable carnivorous fish. The study discovered that replacing fish meal in bulk with PPB is possible and commercially viable at 33% and 6%, respectively (Delamare-Deboutteville et al. 2019). In this study was revealed that brewers by-products arise as alternative potential ingredients to fish meal in aqua feed due to their availability of higher nutrient content, enzymatically processed brewery (LIFE16ENV/ES/000160) to improve digestibility obtained the results that inclusion of brewers spent yeast and good protein digestibility could be successful replacement of fish meal in aqua diet. Sesbania leaf meal was fermented to form fermented Sesbania meal (FSLM) to replace of de-oiled rice bran (DORB). While the therapy was ongoing, a 30% FLSM inclusion level had a negative impact on growth and metabolic response at the cellular and molecular level, even if FLSM utilizing *B. subtilis* could be replaced 50% in the diet (Anand et al. 2020). In Atlantic salmon, rainbow trout, and white leg shrimp, single cell protein (SCP) products and protein meals based on microbial algae and biomass were determined to have the greatest potential for rapid growth (Jones et al. 2020). This study was revealed that marine microalga of *Nannochloropsis oceanica* NIOF15/001 were isolated for dual application as biodiesel and aquaculture feed, even (Ashour et al. 2019). The hydrophobic fraction from housefly (HF) (mucus domestica) larvae was regarded to be a crucial mechanism for sustained aquaculture (Hashizume et al. 2019). In a new open pond, the microalga *Nannochloropsis oceanica* SCS-1981 was investigated, and different biochemical compositions were found to be as important as they dictated the species' applicability and profitability, as well as the great promote the low-cost cultivation of microalgae for aquaculture feed purposes (Li et al. 2020). A lipid free biomass of *Nannochloropsis oceanica* with 50% of soybean mixing with gave to the Nile tilapia fish got better results than control lipid rich feed, accordingly which gives cost effectives promotes form the source of bio diesel content (Abugrara et al. 2019). *Nannochloropsis oceanica* was optimized with Walne's medium to increase the carbohydrate, nitrate (160 mg/L), biomass (760 ± 0.01 mg/L) compared with non-optimized condition of biomass (580 ± 0.01 mg/L) (Kumaran et al. 2021). Here, the *N. oceanica* was biochemical component ratio (lipid, carbohydrate, and protein) which has been improved via the recent articles. Ashour and Kamel (2017) were revealed that different concentration of algal media from marketable agricultural fertilizer (CAGF) compared to the F/2 Guillard's normal medium as control that have demonstrated as carbohydrate, protein with especially lipids (18.40–46.12%) were depending on nutritional limitation than compared from f/2 lipid percentage (30.70%). Pre-pupa meal (HM) is used in diets for European seabass Dicentrarchus labrax juveniles, whereas fish meal is controlled. While calculated the apparent digestibility coefficient (ADC) of protein, valine, histidine as well as amylase and protease activity were not lower in HM 6.5 diet than control (Magalhães et al. 2017). *Oncorhynchus mykiss* rainbow trout was provided with a 28% defatted insect as black solider fly, Hermetia illucens (HIM) and compared to a control (certified organic and fish meal), in this study was initially fish weight 66.5 ± 2.3 g was grown to 125 ± 4.5 g, which HIM meal helps in improved utilization of protein and lipid (Stadtlander et al. 2017). Moreover, *Oncorhynchus mykiss* fed one of three isonitrogenous and isocaloric diets (46% protein, 16% lipid) of bioprocessed soybean meal served as a trail control diet for the reference diet, in which 80% of fish meal was replaced with bioprocessed soy protein (60% or 80%) according to percent gain, feed conversion (Voorhees et al. 2019). Soybean meal and cotton seed (Jiang et al. 2018) were used to replace fish meal in a juvenile diet containing Macrobrachium nipponense for 8 weeks. The amino acid makeup of the meals and prawn muscle were compared, with soybean meal having the superior in vitro digestibility (Huang et al. 2018). The dietary supplement of algae, on the growth performance, body consumption, and feed utilization of Nile tilapia, Gracilaria arcuata were found to have a 20% effect (G20) exhibited significantly higher growth performance than other 40% (G40) and 60% (G60) of algae concentration (Younis et al. 2018). Even though, lupin seed meal (*Lupinus angustifolius*) 100 g kg^{-1} were tested with white leg shrimp (*Litopenaeus vannamei*) as control local protein supplement the use of fish meal, which results obtained that 100 g 100 g kg^{-1} was not affected haemolymph protein content, and glucose and acyl glyceride concentration in significantly higher than 200 and 300 g kg^{-1} among the different concentration, which were described that low inclusion of luppin mela don't induce negative consequences in juvenile *L. vannamei* and appear to boost the immune system (Weiss et al. 2020). Soybean meal, cotton seed, and rapeseed meal on the growth, feed utilization, and body composition of juvenile Acipenser baerii × Acipenser schrenckii with an initial weight of 8.630.24 g were used to substitute fish meal, and one of the concentrations of 300 g kg^{-1} could be substituted. (Jiang et al. 2018).

15.6 Antibiotics Risk in Aquaculture

Antibiotics are widely used in aquaculture, and intensive farming encourages the use of antibiotics indiscriminately in farmed aquatic products, leading to bacterial resistance (Chen et al. 2020). Antibiotic resistance threats were found in both mariculture and natural water using trimethoprim and enrofloxacin, according to the study's findings and exerted selection pressure on the bacterial community in the environment (Han et al. 2021). Distribution and combined pollution of risk assessment of antibiotics of sulfadiazine, sulfamethazine, and enrofloxacin as well as high Mn concentrations were combined contamination to aquaculture environment (Han et al. 2020). To found to be bioaccumulation of trimethoprim into Lutjanus russellii, a juvenile species having a bioaccumulation factor of 6488 L/kg affects the human dietary exposure (Chen et al. 2015). Antibiotic residues added just 2–35% to the hazard, despite the fact that high levels of other pharmacologic residues of caffeine were greater than medium ecological concerns (Zhou et al. 2021). Antibiotics' use, environmental fate, and ecological dangers in a tilapia cage, the antibiotics oxytetracycline (OTC) and enrofloxacin (ENR) were tested utilizing river water and sediment samples. Sediment concentrations up to 6908 g/kg d.w. for OTC and 2339 g/kg d.w. for ENR were found insignificant short-term risks for primary producers and invertebrates (Rico et al. 2014). Furthermore, the ecological risk of antibiotics ENR and ciprofloxacin (CIP) in a pangasius catfish in a farm in Vietnam, where the Chlorella sp. had EC50 values of 111,000 and 23,000 g L (-1) for ENR and CIP, respectively, and 69,000 and 71,000 g L (-1) for M. After treatment, macrocopa was unlikely to have substantial harmful impacts on exposed aquatic ecosystems (Andrieu et al. 2015). The contamination of antibiotic confrontation genes (ARGs) in aquaculture environments had increased, and the farm in south China was explored, with the results indicating much greater ARG abundances are higher in ARG samples than in water samples from shrimp (Wang et al. 2019; Su et al. 2017, 2020). However, more research is needed to refine the water treatment procedure so that ARGs from aquaculture can be reduced.

15.7 Mycotoxins Impair Animals' Immune System

The risk of animals being exposed to mycotoxins increases when more plant-derived components are utilized in commercial fish formulations. While the biological effects of mycotoxins in aquatic species vary depending on their quantity in the feed, age, and species, they generally include decreased performance, worsening pond conditions, and increased disease susceptibility (Pandey et al. 2015). In order to tackle these harmful fungal toxins, mycotoxin risk management is essential.

Several scientific research show that eating mycotoxin-contaminated foods inhibits the immune system and lowers disease resistance. This can happen even at low concentrations due to the acute toxicity of mycotoxins. White blood cells

Table 15.1 US FDA Guidelines for acceptable levels of total aflatoxins in food and animal feeds

Action level (ppb)	Commodity	Target species	comments
0.5 (AFM[a])	Milk from daily cows fed aflatoxin feeds	Human	
20	Any food except milk	Human	
20	Animal feeds	All species	
Exceptions to above guidelines			
300	Cottonseed meal used in mixed feeds	All species	
300	Corn	Finishing beef cattle	Must be fed to designated animal species
200	Corn	Finishing swine	Same as above
100	Corn	Breeding cattle, swine, or mature poultry	Same as above

Source: US FDA Guidelines
[a]Metabolite of AFB

(erythrocytes and leukocytes) were found in significantly larger concentrations in the blood of animals fed Mycofix® Secure. Hepatopancreatic lesions were discovered, with the severity of the lesions increasing as the concentration of AFB_1. We were able to establish from the challenge test that pangasius fed AFB_1 contaminated diets had decreased survival rates. The use of Mycofix® Secure can help to prevent AFB_1's harmful effects.

Mycotoxins are obviously a source of concern for farmers who raise cereal grains and oil seed crops. Producers that feed these plant-based feeds to animals reared for human consumption should also be worried. Grain elevators, storage facilities, and feed mills should all have a mycotoxin testing program in place, especially if the growth circumstances are prone to cause mold impairment. At this point, the only mycotoxin regulated by the federal government is aflatoxin. A 20-ppb upper limit (action level) for aflatoxin in foods and most animal feeds and feed additives has been set by the US Food and Drug Administration (FDA). Cottonseed meal and corn meal which contain greater amounts of total aflatoxins have been exempted, as shown in Table 15.1. Feed containing corn carrying greater levels of total aflatoxins (100 to 300 ppb) must be fed to a specific animal species (Fig. 15.2).

Furthermore, the FDA has published fumonisin guideline and DON recommendation on the allowable quantities of these mycotoxins in feeds and feed additives. Total fumonisin concentrations in maize (containing no more than 50% of the completed feed) utilized in the manufacture of catfish feeds should not exceed 20 ppm, according to the recommendation, and concentrations in completed catfish feeds should not exceed 10 ppm. Wheat, wheat by-products, and other small grains used in fish diets are subject to a 5-ppm limit, according to the DON, as well as a 2-ppm upper limit in finished fish feeds. Following these limitations should prevent difficulties in warmwater fish or rainbow trout, according to research done with these two mycotoxins. Moldy feeds and feed ingredients with a known mycotoxin may also contain undiscovered chemical compounds produced by mold organisms. These

Fig. 15.2 Source: Expected productivity of aquaculture in worldwide 2018–2025 from allied market research of fish forming market

chemical compounds may harm target animals or exacerbate the toxicity of a known mycotoxin. Fusaric acid, for example, is a toxin generated by numerous fungi in the Fusarium genus. Fusaric acid is considered a phytotoxin with modest toxicity to farm animals. Despite the fact that fusaric acid's toxicity has yet to be determined in fish, there is reason to be concerned about the combined effects of fusaric acid and fumonisin or DON, with which it may come into contact (Fig. 15.3).

15.8 Feed Additives Reduce Chronic Heavy Metal Toxicity

As an aquaculture field that is growing faster than other food production sectors, worldwide aquaculture manufacture, including aquatic plants, is 110.2 million tons, with a USD 243.5 billion sale value. Today's increase in aquaculture growth has been primarily attributed to the development of good aqua feeds, which are derived from proper and essential feed additives (Bharathi et al. 2019). Although, Wang et al. (2019), the potential benthic transfer of titanium oxide nanoparticles from calm

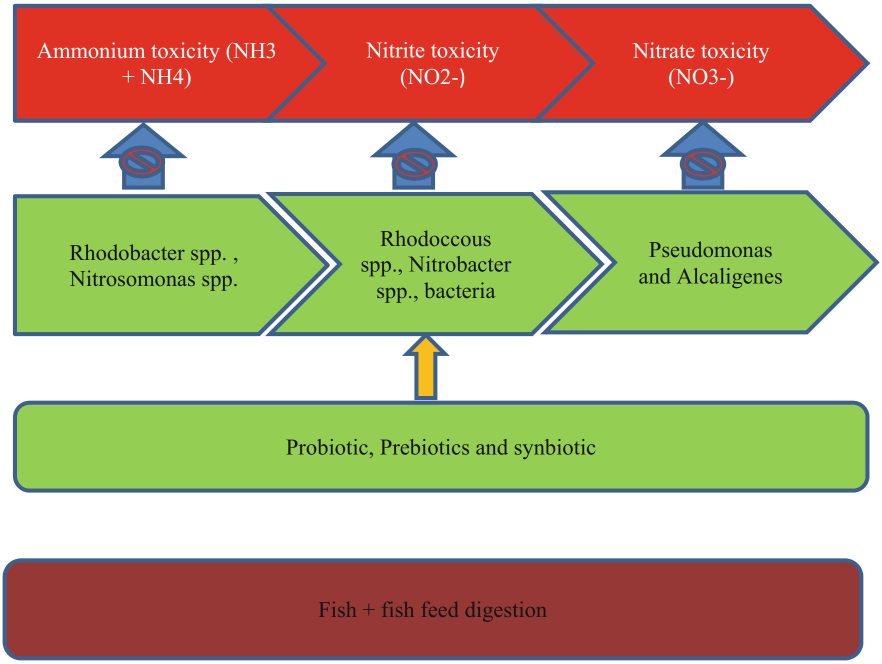

Fig. 15.3 Schematic diagram of aquaculture feed additive control heavy metal toxicity

worms (Perinereis aibuhitensis) to juvenile turbots exposed to much reduced protein and greater fat contents, in comparison to the nutrition quality, reduction of turbots was investigated (Wang et al. 2019). Heavy metals contaminate in an aquatic environments via various sources of industrial waste and e-waste and metal based industrious wastewater, mixing to river water and groundwater, where metal toxicity is responsible for the degradation of the population of aquaculture polluting the aquatic environment. As fishes are part of human consumption, it is indirectly affecting human also (Sonone et al. 2020). Probiotic (bactocell$^{®}$), vitamin E, and vitamin C supplement would be helpful for reducing the cadmium pollution (Munksgaard et al. 2017) and increasing the body weight (Ayyat et al. 2020). Lactobacillus plantarum CCFM8610 had been reduced the abundance of flavobacterium and pseudomonas, which was significantly reduced cd-exposed level and improved in the gut microbial diversity in Nile tilapia (Zhai et al. 2017a, b). The study was used probiotic microorganism of Pediococcus pentosaceu had been control the cadmium and lead concentration as a bioremediation, which was obtained that reduce the cadmium level along with MIC test had been showed 25–50 ppm in the concentration of 52.17–11.25% and same concentration of Lead had 62.10–68.39%, finally the isolated microorganism was used as an aquaculture probiotic considered as fishpond study area (Jaafar 2020). The probiotic Akkermansia muciniphila was found to minimize cd exposure, while the presence of several cd inhibitory bacteria was found to rise after cd exposure and induce the community diversity of their gut microbiota in common crap (Cyprinus carpio; Chang et al. 2019). The water pollution created by heavy metal pollutions of cadmium and used to adopted to the tilapia (oreochromis niloticus) which is received the probiotic (lactic acid bacteria (LAB)), Vitamin C, had been demonstrated that significantly control the cd- exposed and toxic environment and sperm quality (sperm motility) with total intestinal bacteria of fish exposed by cadmium were obtained. (Hayati 2020). The genus od bacillus cereus dominated probiotics would be obtained with Carassius auratus gibelio *(*C. gibelio*)* for exposure of cadmium, here which genes had been controlled exposed cadmium by the antioxidant enzyme including super dioxide dismutase (SOD), total antioxidant (T-AOC), catalase (CAT), and glutathione-S-transferase (GST) (Wang et al. 2019). Fenugreek (*Trigonella foenum-graecum*) seed was used on Nile tilapia for cd-exposed with control of the oxidative stress, here the results showed that 1% of fenugreek concentration on Nile tilapia to alleviate some toxic effects of cd and fish tissue reduces the fish cd accumulation (Abbas et al. 2019). Mercury (Hg) is poisoning in human, and fish represents unique global problem, whereas hg is the most dangerous to the ecosystem due to its high toxicity. The rapid expansion of industrial society has resulted in a great quantity of energy and resource use, which has resulted in some mercury contamination (Hg) (Jadán-Piedra et al. 2017). Pollution problems present in an aquaculture field for purpose to control such study of selenium and mercury combined and among with bacillus subtilis probiotics demonstrated to common carp to obtained the selenium (se) rich bacillus subtilis could be reduced the mercury buildup in organs after hg exposure, despite the fact that selenium-rich *B. subtilis* outperformed the selenium-only group in terms of lowering Hg accumulation (Shang et al. 2021). Market samples of carp and trout taken in 2019 were evaluated for mercury Hg accumulation levels in maritime wild fish, they found that undetectable concentration of total Hg in muscle tissue than 2006–2008 at collected samples, that results concluded environmental pollution; food webs and biological factors were main causes of Hg accumulations in fish and its impact on human health value indicate of ecosystem. Trash of fed harvested fish black seabream (acanthopagrus schlegelii) obtained total mercury and methyl mercury bioaccumulation higher the muscle than commercial feed pelleted used perch (*Perca fluviatilis*) and red snapper (*Lutjanus campechanus*); which results obtained mercury contamination and bioaccumulation were associated due trash fed and heavy metal associated level different location in the muscle (Gao et al. 2017). Furthermore, Allium sativum and lactic acid bacteria (LAB) were evolved with Nile tilapia (*Oreochromis niloticus*) to reduce the gonadal somatic index (GSI) and gonadal size could be reduced as well as increase the duration of mortality and viability of fish sperm exposed to Hg (Hayati et al. 2020). A El-Matary et al. (2021) showed that use of plant waste of Moringa Oleifera, Opuntia ficus-indica, *and* Telfairia occidentalis supplementary diet improves the hematobiochemical blood and increases the ability of resist effects of mercury toxin level in Oreochromis niloticus. A toxic effect of a variety of Lead (Pb) from soil and the atmosphere contaminates water bodies and has an impact on aquatic animals. Therapeutic effect of dietic feed additives *Lactobacillus reuteri p16* (10^8 CFU g^{-1}) against the *Cyprinus carpio*, buildup of Pb tissues reduced motility and increased growth performance (Giri et al. 2019). The Bacillus subtilis (109 cfu/g) administration could be provided to combat dietary lead toxicity in Carassius auratus gibelio (*C. gibelio*) among with significantly reduce the lead bioaccumulation and increase the antioxidant enzymes activity. In this study, selected probiotic of lactobacillus plantarum CCFM8661 against the waterborne Pb exposure was evaluated in Nile tilapia (*Oreochromis niloticus*) which ameliorated the growth performance and prevented the death (Zhai et al. 2017a, b). *L. reuteri P16* was isolated from *Cyprinus carpio* and studied in vitro to see if it may boost antioxidant capacity and high-level survival after 4 h of exposure to both low and high pH (2.5–3.5) (Giri et al. 2019). Ld- heavy metal may affect the many physiological process and including serum index and the immune response. Here, Carassius auratus gibelio (*C. gibelio*) from the explore the toxin effects of Pb was identified via used bacillus subtilis (10^9 cfu/g) among the analysis had been verified sIgA activity increases, expression of immune repones genes, including HSP70, IgM, HSP90, IL-6, TNF-α as well as pb- exposed significantly affects in the dietary fish (Cai et al. 2019). Bentonite with mannan oligosaccharide (MOS), organic selenium (OS), or vitamin C applied to that pb contaminated Nile tilapia diet confirmed that significantly reduce the Pb residual in the fish body (Ayyat et al. 2020). The effect of probiotic *Lactobacillus acidophilus* (5 × 107 CFU/g) on the lead contaminated to the rainbow trout fish (O*ncorhynchus mykiss*), the results were concluded a positive impact of improved growth performance and significant expression of digestive enzymes (lipase, trypsin, and alpha amylase), in addition that reduce the lead toxicity (Mohammadian et al. 2018). Antioxidative properties of dietary supplement garlic, propolis, and wakame had been significantly increased recuperation of cd-exposed and tissue functions in Japanese medaka (Ujeh and Kurasaki 2020).

15.9 Feed Additives and Reduce the Pesticide Toxicity

Pesticide pollutions have common health hazards over the last several decades because of the widespread in the world (Yuan et al. 2019). Most countries have banned organophosphate and organochlorine pesticides due to their high toxicity and bioaccumulation in non-target animals. Pyrethroid insecticides have been used in agriculture and aquaculture to substitute traditional insecticides although pyrethroids are 1000 times more hazardous to fish than animals and birds (Yang et al. 2020). The pesticides to act in the fish to affect neurological, reproductive, and growth factor. Biocides are chemical compounds that can destroy the bacteria that cause biofouling, but as the industry increases, so does the need for them, more medications, disinfectants, and antifouling biocide chemicals are needed to eradicate germs in aquaculture facilities (Guardiola et al. 2012). Because sediment-dwelling invertebrates are at risk of pyrethroid exposure in urban streams, the presence of pyrethroids is critical to the ecology (Hartz et al. 2019; Deanovic et al. 2018).

Formaldehyde is used in freshwater aquaculture facilities to avoid external parasites, and it is discharged into the receiving environment without treatment, However, values of 1.61 mg/L are harmful to aquatic life, and these findings suggest that formaldehyde released from land-based facilities may have long-term consequences (Lalonde et al. 2015). Trichlorfon and fenitrothion pesticide contamination in feed had no influence on health or bioaccumulation in grass crops, but they were related with poor physiological responses and may enhance susceptibility to illnesses (Pucher et al. 2014).

15.10 Feed Additives and Reduce the Nitrogenous Toxicity

One of the economic activities in the aquaculture area faces the inescapable problem of water quality degradation, which is mostly caused by inefficient production pond management. Even, an accumulation of undigested feed residues, metabolic by-products excreted by organism cultivation, and others (Robles-Porchas et al. 2020). The most cost-effective contemporary aquaculture area necessitates high-density cultivation. Aquaculture with high density will be exposed to higher nitrogenous waste concentrations (Tomasso 1994). Ammonia, nitrite, and nitrate are among the nitrogenous wastes that are becoming a global issue in aquatic ecosystems as a result of increased anthropogenic activity, and they are a common concern in aquaculture. (Romano and Zeng 2013). This pollution is irrelevant through the nitrogen cycle with direct metabolism of ammonia being a most toxic with high species specificity. Adversely, the mechanism of nitrite toxicity had large parallel development of an aquatic organism (Tomasso 2012). Nitrate, a by-product of the nitrification cycle, causes nitrate concentrations to rise during the culture period, potentially reducing fish growth and mortality (Furtado et al. 2015; Yang et al. 2019). The feed additives are suitable for clear on greenhouse gas and are important to ensure sustainable aquaculture protein production (Adegbeye et al. 2019).

15.11 Feed Additives and Reduce the Ammonia Toxicity

Ammonia waste discharged into the production pond environment by aquatic animals; this ammonia is excreted directly from the fish gill into the water. The toxicity of ammonia is affected by the pH of the pond, unionized ammonia (NH_3) and ionized ammonia (NH4+) are less harmful at low pH, but ammonia-nitrogen (NH_3-N) is more poisonous at high pH (Wurts 2003). The presence of high levels of ammonia poses a significant issue for the aquaculture business in terms of maintaining the fish population. Scophthalmus maximus was treated to continual ammonium chloride solutions for 4–6 weeks in three batches of turbot to study the long-term effects of ammonia, the results showed that chronic lethal and TAN (total ammonia nitrogen) levels in chronic adaptation were around 20 mg/L plasma and

13–15 mg/L urine, respectively, and that only the highest ammonia concentrations examined had significantly increased urea-N excretion daily rates (Person-Le Ruyet et al. 1997). In a tank of Oreochromis mossambicus fingerlings, a consortium of three cultures identified as Bacillus cereus, Bacillus amyloliquefaciens, and Pseudomonas stutzeri, respectively, converted ammonia to nitrite and then to the deadlier nitrite (John et al. 2020). Herbal menthol essential oil was indicated to improve Nile tilapia growth performance; the final growth rate, weight gain, and feed conversion ratio (FCR) were all significantly improved; and MNT inflammatory genes (IL-8, TNF-, and IL-1) were reduced, whereas ammonia stimulated HSP70 expression (heat shock protein 70) is reduced (Magouz et al. 2021). The potential effects of olive oil leaf (olea europea L.) extract (0, 1, 5 and 10 g/kg) supplementation on common carp health for 60 days significantly increased blood white and red blood cells in the 5–10 g OLE/kg supplemented treatment, but there was no significant difference between the treatments after ammonia exposure, The results of that study revealed that a diet of 1 gram of OLE per kilogram of fish could improve fish health and prevent ammonia toxicity in common carp (Rajabiesterabadi et al. 2020). An inclusion of yucca powder on common crop as tested dose as control, 075 mg/L with yucca extract, and 10 mg/L without ammonia treated yucca powder and fourth group ammonia treated with yucca powder, after 30 days while treatment was confirmed according to their parameter the yucca extract was recommended to protect common carp from the toxicity of water borne ammonia (Dawood et al. 2021). The antioxidant capacity of artemisia (artemisia annua) leaf extract (AE) was tested in common carp during ammonia exposure, and AE significantly reduced/inhibited ammonia-induced increase in hepatic antioxidant enzyme activities and gene expression, implying that AE could be used as a feed additive to prevent hepatotoxicity in common carp during ammonia exposure (Mirghaed et al. 2020). Although a dietary anthocyanin on the Nile tilapia parameter was analyzed that significantly increased the respiratory burst activity, phagocytic activity, phagocytic index, lysozyme activity, 80 mg/kg group of anthocyanin induces the ammonia stress in fish (Nile tilapia) (Yilmaz et al. 2019). On rainbow trout, *Oncorhynchus mykiss*, a dose of 0.5–1% dietary Roselle, Hibiscus sabdariffa, was recommended for trout feed formulation, which lowered ammonia exposure toxicity and attenuated alanine aminotransferase (ALT) and aspartate levels in the blood. Tumor necrosis factor alpha (TNFA), interleukin-1 beta (il1b), interleukin-8 (il8), and heat shock protein 70 (hsp70) genes are all expressed in the liver, as well as aminotransferase (AST) activities (Yousefi et al. 2021). This study looked into the effects of dietary arginine supplementation on common carp that had been exposed to ambient ammonia and had much lower plasma ammonia and urea levels, as well as immunological and antioxidant gene expression (Yousefi et al. 2021).

Yucca schidigera is capable of reducing the ammonia gas and because it could be available or grown in the desert area, which makes potential to reduce the odor from manure (Adegbeye et al. 2019). Furthermore, Yucca schidigera (YE) combined with saccharomyces cerevisiae (1 g/L) as feed additives on Nile tilapia could be increased the growth and significantly improved the water quality by reducing pH and ammonia level (Abdel-Tawwab et al. 2021). The preventive effects of dietary garlic on common carp (Cyprinus carpio) were good effects to improve plasma antioxidant and immunological parameters, especially when administered at 1–1.5% (Yousefi et al. 2020). Microporous of aluminum silicates as zeolites was used in aquaculture industry to improvement of water quality at fish farm and fish transportation tanks by particulate ammonia emission and toxic complexity to reduce, respectively (Ghasemi et al. 2018). The dietary effects of 1,8-cineole were investigated on common carp (*Cyprinus carpio*) to suppress stress response, augment thyroid hormone levels, and mitigate ammonia toxicity level (Mirghaed et al. 2019).

15.12 Feed Additives and Reduce the Nitrite Toxicity

In stringent and recirculatory aquaculture systems, high nitrite levels are common, and significant progress on nitrite and its implications in the aquaculture business has been accomplished over the previous few decades (Ciji and Akhtar 2020). In the past four decades, researchers have gained environmentally aquaculture nitrite management and how to control the nitrite toxicity had been understating the mechanism of nitrite toxicity largely parallel to the development (Tomasso 2012). An intensive recirculating aquaculture system (RAS) is needed for high level of water recirculating treatment to maintain good water quality and remove the ammonia, nitrite, and yellow substances and total bacterial biomass in seawater (Schroeder et al. 2011). Furthermore, an *Aliivibrio fischeri* sustainability of RAS would require parameters of temperature variation effects which were also considered to reduce the nitrite level (da Silva et al. 2018). In light of biosecurity concerns, the only approach to ameliorate these nitrogenous wastes without water exchange is to establish in situ nitrification or denitrification via biofilters or bioreactors triggered with bioaugmentors (Preena et al. 2021). Despite the fact that the false clownfish, Amphiprion ocellaris, is one of the world's most common fish species, excessive stocking densities used to optimize fish production can result in hazardous amounts of ammonia and nitrite. The increase in ammonia or nitrite exposed to 0.57 mg/L NH_3-N or 100 mg/L NO_2-N is based on that demonstration (Medeiros et al. 2016). According to Yun et al. (2019), the bacterial strain LPN080, which was isolated and evaluated from a shrimp *Litopenaeus vannamei* culture pond, was able to efficiently remove both ammonia-nitrogen and nitrite and has potential economic utility in aquaculture and even wastewater treatment. An autotrophic bio floc technology (ABFT) system involving simultaneous microalgae co-culturing with juvenile-farming-stage fish and shrimp in aquaculture and microalgae-based water treatment was recently developed, and it could be integrated with existing industrial systems to achieve sustainable aquaculture (Kim et al. 2019). The intermediate nitrification of nitrite production was found to be controlled by two nitrite-oxidizing bacterial consortia, collected from marine and brackish water, with the more diverse flora and higher potential to be used as start-up cultures for activating nitrifying bioreactors following salinity acclimation (Preena et al. 2018). Neissi (2020) revealed the Cold resistant hetrotropic ammonium and nitrite bacteria Dyadobacter sp. (no. 68) and Janthinobacterium sp. (no.100) were identified, removing bacteria that were chosen for their adaptation to culture at ^{150}C and drastically lowering ammonia-nitrogen and nitrite levels (Fig. 15.4).

Nitrate (NO_3^-), a potentially harmful substance for aquatic organisms, is found in almost every aquatic habitat on the planet. (Yu et al. 2021). Nitrate is the end product of the biological nitrification process, and it can induce aquatic toxicity in fish in a variety of ways depending on how long they've been exposed to it. Chronic nitrate exposure harms the health of juvenile turbot (Scophthalmus maximus) through altering their intestinal shape, immunological state, barrier function, and microbiota (Yu et al. 2021). In juvenile turbot culture, nitrate exposure causes growth retardation and poor health, and endocrine abnormalities or dysfunction may blame for the growth inhibition. Furthermore, in light-limited settings, chemoautotrophic bacterial nitrification (CBN) and heterotrophic bacterial assimilation are the two main ammonium pathways in aquaculture water (HBA) (Luo et al. 2020). Mirissa was used to measure potassium nitrate exposure concentrations, and it was discovered that freshly hatched fish in the external gill stage was more sensitive to nitrate contamination than fish in the internal gill stage (Balangoda et al. 2018). RAS assessed the acute and chronic toxicity of nitrate–N to spotted knifejaw, Oplegnathus punctatus, subjected to 165 mg/L nitrate –N for 96 h and 28 days, and advised more research. Nitrate poisoning is prevented by lowering the toxicity level (Yang et al. 2019). In contrast, RAS fish growing the possible impacts of excessive nitrate accumulation on Nile tilapia development and health status at suggested not surpassing concentrations of 500 mg/L of NO_3-N to ensure Nile tilapia health and growth (Monsees et al. 2017). Isolated microorganism of *Bacillus sp*-TO3, TO-10, TO-12 from West coast of Korea were controlled or eliminated as hydrogen sulfide, ammonia- nitrogen and nitrite- nitrogen to suggested that as probiotics used for water quality improvement reduces above-mentioned toxicity (Hong-Kook et al. 2020).

15.13 The Prospective of Feed Additives Agents

Nanoparticle and microparticles are promising carrier systems for oral delivering of drugs or vaccines. Nanoparticles of trace minerals in aquaculture use nanotechnology for a developing technology with enormous promise and a wide range of applications in human and animal nutrition and agriculture. The living systems and nanoscale components such as proteins, DNA, and enzymes, nanotechnology has a wide range of biological applications (Hassan et al. 2020). Marine-derived chemicals are linked to improved immunity, intestinal health, and antioxidative levels in aquatic animals, and are known to have a significant impact on their performance. Here, the role of chitosan marine-derived nano particles on the gray mullet (Liza ramada) improved the health and immune response and enhanced intestinal histo-morphometry and intestinal health (Dawood et al. 2020a, b). The effects of selenium nanoparticles and vitamin E on Nile tilapia development, blood health, intestinal histology, oxidative state, and immune-related gene expressions

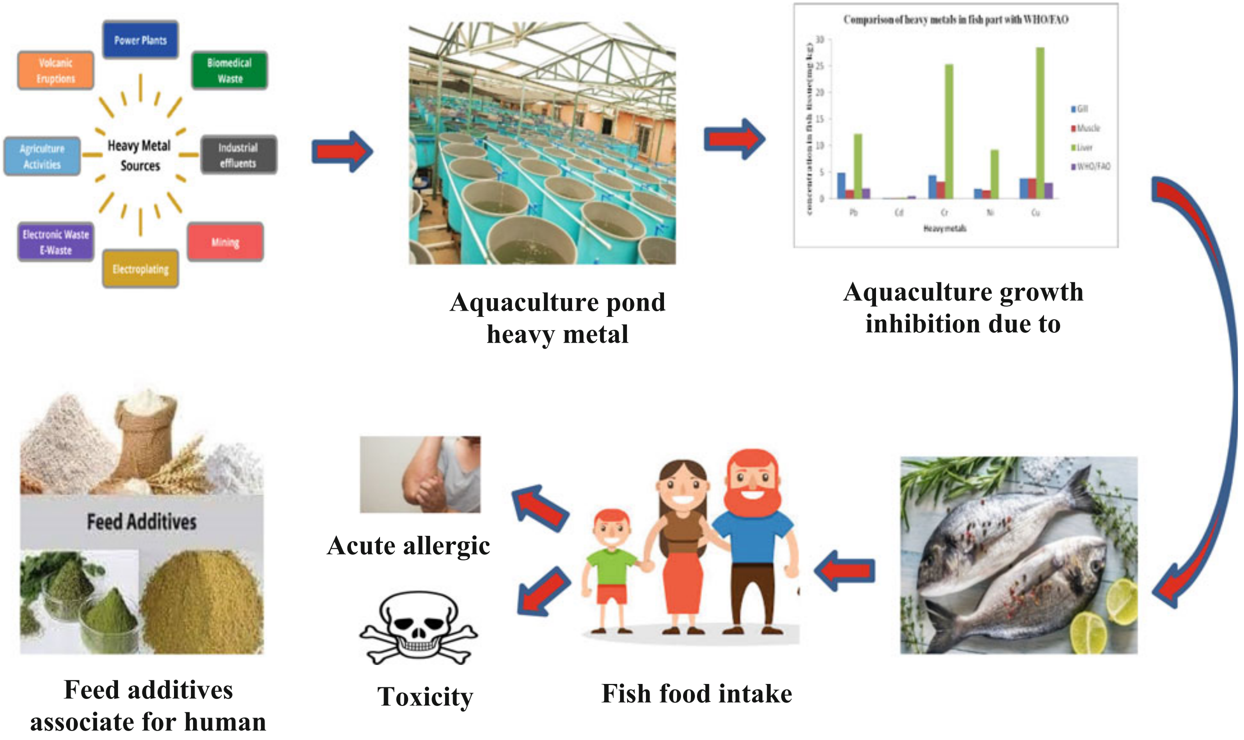

Fig. 15.4 Schematic diagram of contamination by mycotoxins can cause the nutritional content of the materials and/or aquafeed generated to deteriorate, as well as pose a significant health risk to both fish and humans

were investigated (Dawood et al. 2019). Even, nano-selenium and vitamin C were employed to induce immune and antioxidative responses in Nile tilapia aquatic animals without posing any environmental risks. (Dawood et al. 2020a, b). Furthermore, nano-selenium (nano-Se) were studied on the Nile tilapia to be significantly increased the transcriptomic profile and antioxidant, stimulate the growth and against streptococcus iniae infection (Neamat-Allah et al. 2019). This study investigated sodium butyrate (SB) at 1–2 g/kg diet on the Nile tilapia, and results obtained significantly improved growth performance, feed efficiency, and immune response as well as tolerance to heat stress (Dawood et al. 2020a, b).

15.14 Summary and Perspectives

For human consumption, fish is valuable and economical sources of omega fatty acids and a variety of other essential elements. The need to expand aquaculture production and management is always present. Investigated eco-friendly feed additives have numerous beneficial effects on the aquaculture. The increased risk of mycotoxin contamination caused by the use of plant-based components in aquafeed could represent a severe threat to aquaculture productivity. Mycotoxins increase disease and mortality rates in fish, as well as the incidence of reproductive difficulties and reduce weight gain, resulting in significant economic losses. As a result, the presence of mycotoxins in aquafeed has serious economic and public health consequences. According to the previous literature, eco-friendly feed additive has positive effects in improving the performance of aquaculture production. Some researchers have recommended that the improving effects of dietary inclusion of eco-friendly feed additives are partially associated with enhanced feed consumption probably due to improved palatability of the diet.

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Chapter 16

Potential Role of Dietary Minerals in Fish and Crustaceans

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Abstract Minerals are essential nutrients that play a key role in all living organisms. Minerals act as a catalyst for many biological functions of organisms including skeletal formation, maintenance of colloidal systems, acid-base equilibrium, and other biologically important compounds like enzymes, hormones, vitamins, etc. Aquatic animals can obtain essential minerals from diet and water. However, the quantitative requirement of each dietary mineral is species dependent. The quantitative requirement of dietary minerals has attained significant improvements in the aquaculture industry, particularly in fish and crustaceans. The adequate level of essential dietary minerals like calcium, potassium, sodium, iron, zinc, copper, selenium, etc., can promote survival, growth, proximate composition, nonspecific immunity, and disease resistance against the pathogen in fish and crustaceans. Regarding this, the optimum dietary requirement of minerals in fish and crustaceans has been studied and reported by earlier researchers. This chapter deals on the role of 11 dietary minerals such as calcium, magnesium, potassium, phosphorus, sodium, copper, chromium, fluorine, iron, selenium, and zinc) on survival, growth, feed index, digestive enzymes, proximate composition, immune response, antioxidants and disease resistance of fish and crustaceans.

Keywords Minerals · Survival · Growth · Fish · Crustaceans · Immunity · Disease resistance

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16.1 Introduction

Aquaculture is one of the largest food production sectors in the world next to agriculture which provides food security and blue economy of the nations. Fish and shellfish are the most valuable edible species which supply nearly 50% of total animal protein (FAO 2020). Globally, 82,095 thousand tonnes of fish and shellfish species were produced during 2018 which includes 54,279, 9387, 17,511, and 919 thousand tonnes of fish, crustaceans, mollusks, and other species, respectively (FAO 2020). Inland culture fisheries like fish and crustaceans are one of the major components in the world fish trade. Feed is one of the major components in the production of fish and crustaceans which accounts for nearly 50% of total fish production cost (Adikari et al. 2017). The feed which has an adequate level of nutrients like protein, lipid, essential amino acids, fatty acids, pigments, vitamins, and minerals is considered as the quality diet for aquaculture production. Minerals are a crucial and minimum requirement to secure the optimal health and growth of all living organisms. Aquatic animals including fish, crustaceans, and mollusks can acquire minerals from the diet and water currents. Absorption and functional forms of the minerals are maintained constantly for normal cellular metabolic functions. This is facilitated by the homeostatic mechanisms operating in the animal and creating to the fluctuations in nutritional intake. The dietary source of 20 minerals has been demonstrated as essential for animals including fishes and crustaceans. Among these, calcium, potassium, sodium, magnesium, chlorine, phosphate, and sulfur are macro minerals and zinc, copper, iron, selenium, chromium, cobalt, fluorine, iodine, manganese, molybdenum, nickel, silicon, tin and vanadium are trace minerals (Underwood 1963; Davis and Gatlin 1996; Hasan 2001; Goopla 2006; NRC 2011; Antony Jesu Prabhu et al. 2016).

Minerals are responsible for the skeletal formation, balancing of osmotic pressure, maintenance of colloidal systems, nerve impulse, muscle impulse, regulation of acid-base equilibrium and also serving as an essential component for vitamins, pigments, enzymes, hormones, production of blood cells, and antioxidants, etc. in aquatic animals (FAO 1987; Watanabe et al. 1997; Lall 2002). The biological role of minerals in fish, crustaceans, and other animals is given in Table 16.1. Earlier research findings reported that the minerals such as calcium, sodium, potassium, phosphorus, magnesium, zinc, iron, copper, and selenium have been identified and recommended as an essential dietary component for growth, normal physiological process, immune system, tolerance to stress, and disease resistance against pathogens in fish (Davis and Gatlin 1996; Keshavanath et al. 2003; Sudhakar et al. 2009; Yu et al. 2013; Liang et al. 2018; Neamat-Allah et al. 2019; Mondal et al. 2020; Musharraf and Khan 2020; Siqwepu et al. 2020; Afshari et al. 2021; Zhang et al. 2021) and crustaceans (Davis et al. 1993a, b; Lee and Shiau 2002; Cheng et al. 2005a; Ambasankar et al. 2006; Roy et al. 2007a; Nugroho and Fotedar 2013; Muralisankar et al. 2015; Srinivasan et al. 2016). Figure 16.1 depicts the role of dietary minerals on fish and crustaceans. Dietary mineral requirement studies normally engage the experiments where animal responses or performance

Table 16.1 Biological roles of minerals in fish and crustaceans

Mineral	Biological role	References
Calcium (Ca)	Calcium is a vital component for bone, crucial for normal blood clotting, formation of crustacean exoskeleton, and activator of many important enzymes (ATPases, pancreatic lipase, cholinesterase, succinic dehydrogenase, and acid phosphatase). Ca promotes cobalamin absorption in the gastrointestinal tract and regulates normal heartbeat. Ca is crucial for ionic regulation of fish as it influences the biological membranes permeability by preventing diffusive efflux and high ionic loss to water	FAO (1987), Wood and McDonald (1988), Lall (2002)
Phosphorus (P)	Plays a central role in energy and cell metabolism, it is an essential bone component, gristle, and exoskeleton of crustaceans. P is a component of phosphoproteins, nucleic acids, phospholipids, creatine phosphate, higher energy phosphate esters hexose phosphates, and key enzymes. P regulates the acid-base balance of fluids	FAO (1987), Kreisberg (1977), Håglin (2001), Lall (2002)
Potassium (K)	Potassium is a major cation of intracellular fluid which regulates normal intracellular acid-base balance and osmotic pressure. K is essential for breakdown of glucose in metabolism, protein and glycogen synthesis	FAO (1987), Marshall and Bryson (1998), Shiau and Lu (2004), Evans et al. (2005)
Sodium (Na)	Like potassium, sodium is also a main monovalent ion of extracellular fluids and plasma. Although the principal role of Na in animals is maintenance of acid-base balance, and the regulation of osmotic pressure. Na also essential for carbohydrate absorption	FAO (1987), Shiau and Lu (2004)
Chlorine (Cl)	Chlorine is important for the regulation of acid-base balance and osmotic pressure. Cl plays a unique role in oxygen and carbon dioxide transport in the erythrocytes and the maintenance of pH in digestive juice	FAO (1987), Costa et al. (2012)
Magnesium (Mg)	Magnesium is a necessary component of bone and cartilage of fish, and the exoskeleton of crustaceans. Mg is an activator of several key enzyme including mutases, kinases, muscle ATPases, alkaline phosphatase, cholinesterase,, enolase, isocitric	FAO (1987), Lall (1989), Nielsen (1990a), Davis and Gatlin (1996), Shivakumar and Kumar (1997), Lall (2002), Tam et al. (2003), Vormann (2003)

(continued)

Table 16.1 (continued)

Mineral	Biological role	References
	dehydrogenase, arginase, glutaminase, and deoxyribonuclease. Mg involved in intracellular acid-base balance regulation, stimulates muscle and nerve irritability. It also plays an essential role in metabolism of proteins, lipids, and carbohydrates. Mg also plays a key role in antioxidation and immunity mechanisms	
Sulfur (S)	Sulfur is an important element of several key amino acids (cystine and methionine), vitamins (biotin and thiamine), insulin, and the exoskeleton of crustaceans. Enzymes like glutathione and coenzyme A activities are depending on free sulfhydryl groups. S involved in the detoxification of aromatic compounds in animals	FAO (1987), Murthy and Gatlin (2006)
Iron (Fe)	Iron is one of the crucial elements of the respiratory pigments hemoglobin and myoglobin. Fe is an essential component of various enzyme systems including the catalases, cytochromes, peroxidases, xanthine, succinic dehydrogenase, and aldehyde oxidase. Fe is vital for transporting of oxygen and electrons	Robbins and Pederson (1970), FAO (1987), Watanabe et al. (1997), Lall (2002)
Zinc (Zn)	Zinc is an essential element of more than 80 metalloenzymes, including glutamic dehydrogenase, carbonic anhydrase, alcohol dehydrogenase, superoxide dismutase, alkaline phosphatase, pyridine nucleotide dehydrogenase, pancreatic carboxypeptidase, tryptophan desmolase, etc. Zn also serves as a cofactor in many enzyme systems like enolase, arginase, oxalacetic decarboxylase, and peptidases. Zn plays a vital role in metabolism of protein, lipid, and carbohydrate. It is being particularly active in the synthesis and metabolism of nucleic acids (RNA) and proteins. Zn has wound healing properties and associated with prostaglandin metabolism and structural role in nucleoproteins	FAO (1987), Watanabe et al. (1997), Lall (2002)

(continued)

Table 16.1 (continued)

Mineral	Biological role	References
Copper (Cu)	Copper is main component of numerous oxidation reduction enzymes, such as cytochrome c oxidase, superoxide dismutase, uricase, tyrosinase, caeruloplasmin, amine oxidase, and lysyl oxidase. It is intimately involved in iron metabolism, hemoglobin synthesis, and erythrocytes production. It is believed that Cu is necessary for the formation of the pigment melanin, formation of bone and connective tissue, and maintaining the integrity of the myelin sheath of nerve fibers. Cu is also involved in metabolism of normal connective tissue	O'Dell (1976), Lall (1977), FAO (1987), Davis (1987), Turnlund (1994), Watanabe et al. (1997), Lall (2002)
Manganese (Mn)	Manganese is essential element for activating the enzymes such as phosphate dehydrogenases and phosphate transferases. Mainly the enzymes concerned with the citric acid cycle including alkaline phosphatase, arginase, and hexokinase. Mn is an essential component of the enzyme pyruvate carboxylase. It also essential for regeneration of erythrocytes, bone formation, carbohydrate metabolism, etc.	FAO (1987), Watanabe et al. (1997), Lall (2002)
Nickel (Ni)	Nickel is essential for normal biological functions and plays a key role in the processes related to the vitamin B_{12}-dependent pathway in methionine metabolism	Uthus and Poellot (1996), Barceloux and Barceloux (1999), Phipps et al. (2002)
Cobalt (Co)	Cobalt is a vital component of vitamin B_{12}, and as such is essential for formation of erythrocytes and nerve tissues maintenance	Sherrell and Percival (1984), FAO (1987)
Selenium (Se)	Selenium is an essential component for the growth and maintenance of homeostatic functions and the enzyme glutathione peroxidase. Se with the vitamin E defenses the cellular tissues and membranes against reactive oxygen species (oxidative damage). Se participates in the biosynthesis of ubiquinone (coenzyme Q) which involves in cellular electron transport. Se also has influence on vitamin E absorption and retention. Se plays an important role in the normal functioning of the immune system, cellular immune response, and helping the body to resist viral infection	FAO (1987), NRC (2011), Köhrle et al. (2000), Rayman (2000), Lall (2002), Lin and Shiau (2005)

(continued)

Table 16.1 (continued)

Mineral	Biological role	References
Chromium (Cr)	Chromium has a crucial role in metabolism of carbohydrate (glycogen synthesis) and trivalent Cr (Cr^{3+}) is believed to play a significant role in metabolism of amino acids and cholesterol. Cr is an integral factor of the glucose tolerance and acts as a cofactor for the hormone insulin. Cr plays a crucial role in the nutritional and physiological responses of fishes	FAO (1987), NRC (2011), Küçükbay et al. (2006), Liu et al. (2010b)
Iodine (I)	Iodine is an essential component for biosynthesis of thyroid hormones like thyroxine, and tri-iodothyronine. It is crucial for regulating all the metabolic events	FAO (1987), Sutija and Joss (2006)
Fluorine (F)	Fluorine has a crucial role in the defense mechanism against fluoride intoxication because of the removal of fluoride from body circulation. In addition, fluoride accumulation can play an important role in the hardening of hard tissues mainly the exoskeleton of marine crustaceans due to the combination of fluoride with calcium and phosphorous which forms fluorapatite	Sigler and Neuhold (1972), Kessabi et al. (1984), Sands et al. (1998)
Tin (Sn)	These trace elements are essential for the normal growth, development, and biology of organisms. As they may have a physiological role that influences methionine/methyl metabolism in animals, however, there are no specific reports available in fish and crustaceans	FAO (1987), Yokoi et al. (1990), Nielsen (1990b), Nielsen (1996), Lall (2002), Jugdaohsingh (2007)
Silicon (Si)		
Vanadium (Va)		
Arsenic (As)		
Molybdenum (Mo)		

characteristics have been studied relative to the feeding at graded levels. These levels of essential minerals are over a wide range, from zero to levels far beyond optimal. This is because the requirement of each mineral is based on the type of mineral and selected species. In this line, many studies have reported the optimum dietary requirement of minerals including trace elements in fish (Table 16.2) and crustaceans (Table 16.3). In the present chapter, the role of dietary macro minerals and trace elements on survival, food index, growth, digestive enzymes activity, proximate composition, nonspecific immune response including antioxidants, and disease resistance against pathogens in fish and crustaceans is summarized and discussed.

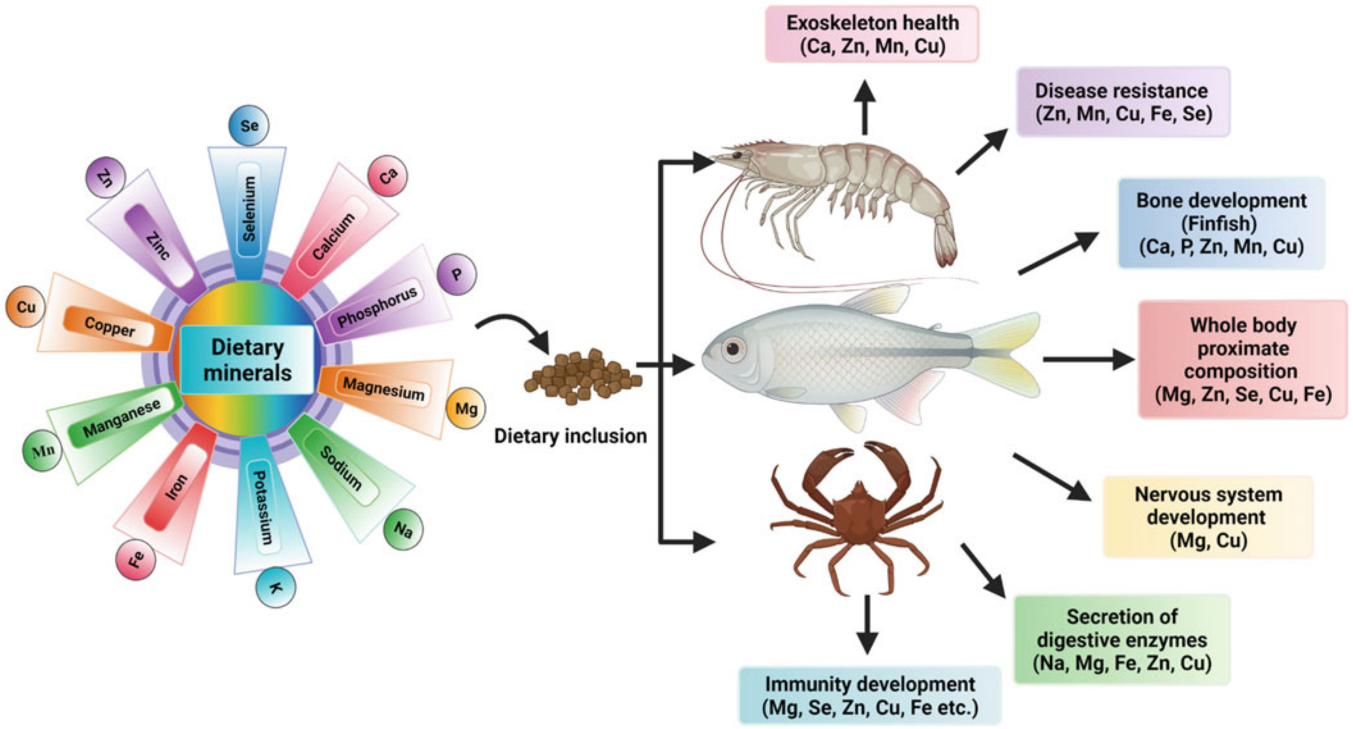

Fig. 16.1 Role of dietary minerals in fish and crustaceans

Table 16.2 Role of dietary minerals in fishes

Mineral	Species	Optimized dose	Exp. duration	Initial size of the organism	Survival, growth, proximate composition and Digestive enzymes	Immune response	References
Ca	*S. marmoratus*	2 g kg^{-1}	84 days	0.82 g	WG, FE (↑), SR (↔)	–	Hossain and Furuichi (2000)
	C. idella	16 g kg^{-1}	56 days	3.96 g	WG, FI, FE, PER (↑)	–	Liang et al. (2012)
	E. coioides	6.0 g kg^{-1}	70 days	29.8 g	WG, FI, FE, SR (↑) Protein, lipid (↔)	–	Ye et al. (2006)
	A. nobilis	12.6 g kg	56 days	105.52 g	WG, SGR, SR (↑) FCR (↓) Protein, lipid, ash (↔)	RBC, HCT (↔)	Liang et al. (2018)
	O. niloticus × *O. aureus*	10 g kg^{-1}	56 days	0.52 g	WG, SGR, FE, SR (↑)	–	Shiau and Tseng (2007)
	L. rohita	5.11 g kg^{-1}	56 days	1.94 g	WG, FI, PER (↑) FCR (↓) Protein, lipid (↑)	–	Musharraf and Khan (2020)
	I. punctatus	15 g kg^{-1}	150 days	6 g	WG (↑)	–	Andrews et al. (1973)
K	*C. idella*	9.87 g kg^{-1}	56 days	3.96 g	WG, FE, SGR, FI (↑)	–	Liang et al. (2014)
	O. niloticus × *O. aureus*	3 g kg^{-1}	56 days	0.77 g	WG, FE, PER, SR (↑)	$Na^{+} - K^{+}$ ATPase (↔)	Shiau and Hsieh (2001a)
Na	*L. rohita*	10 g kg^{-1}	120 days	1.6 g	WG, PER (↑) Protein (↔), lipid (↑) Protease (↑) Amylase (↑) Lipase (↑)	–	Keshavanath et al. (2003)
	C. mrigala	10 g kg^{-1}	120 days	1.2 g			
	C. carpio	10 g kg^{-1}	120 days	0.5 g			
	O. niloticus × *O. aureus*	0.2 g kg^{-1}	56 days	0.69 g	WG, FE, SR (↑)	–	

							Shiau and Lu (2004)
	P. fulvidraco	0.5 and 1 g kg^{-1}	56 days	1.26 g	SGR, hepatic trypsin, stomachic lipase, intestinal lipase, crude protein, ash (↑) FCR (↓)	ALP (↑)	Zhao et al. (2021)
P	*H. bidorsalis*	0.7 g kg^{-1}	56 days	3.33 g	WG, SGR, PER (↑) FCR (↓) Protein, lipid (↑)	–	Adekunle (2012)
	O. niloticus	0.75 g kg^{-1}	56 days	25.02 g	WE, SR (↑), FCR (↓) Protein (↔), lipid (↓)	ALP (↑)	Phromkunthong and Udom (2008)
	C. leather	7.1 g kg^{-1}	70 days	7.94 g	WG, SGR, PER, SR (↑) FCR (↓)	–	Yu et al. (2013)
	I. punctatus	5.2 g kg^{-1}	77 days	145–155 g	WG, FE, SR (↑)	–	Wilson et al. (1982)
	C. chanos	0.88 g kg^{-1}	112 days	2.5 g	WG, SGR, FE, SR (↑)	–	Borlongan and Satoh (2001)
	S. salar	17 g kg^{-1}	63 days	9.4 g	WG, FE (↑)	–	Åsgård and Shearer (1997)
	C. gariepinus	8 g kg^{-1}	84 days	10.2 g	WG, SGR (↑), FCR (↓) Protein (↑), ash (↔)	–	Nwanna et al. (2009)
	A. baerii	6 g kg^{-1}	56 days	14.50 g	WG, SGR (↑), FCR (↓) Protein, lipid (↔)	–	Xu et al. (2011)
	O. mykiss	10.5 g kg^{-1}	56 days	120 g	WG, FI (↑)	–	Morales et al. (2018)
	S. macrocephalus	8.9 g kg-1	56 days	11. 44 g	WG, FE, PER, SR (↑) Lipid, ash (↔)	LYZ (↑)	Shao et al. (2008)

(continued)

Table 16.2 (continued)

Mineral	Species	Optimized dose	Exp. duration	Initial size of the organism	Survival, growth, proximate composition and Digestive enzymes	Immune response	References
	C. idella	7.10 g kg^{-1}	60 days	256.22 g	WG, SGR, FE, FI (↑)	–	Chen et al. (2017)
	S. ocellatus	11.6 g kg^{-1}	77 days	1.3 g	WG, SR (↑)	–	Davis and Robinson (1987)
Mg	*I. punctatus*	0.57 mg kg^{-1}	70 days	107 g	WG, FE, SR (↑)	RBC, HCT, Hb (↑)	Gatlin et al. (1982)
	C. idella	5.92 g kg^{-1}	56 days	5.56 g	WG, FE, FI, SR (↑) Lipid (↑)	ALP (↑)	Wang et al. (2011)
	A. schrenckii♀ × *A. baerii*♂	0.5 g kg^{-1}	56 days	7.63 g	WG (↑)	SOD, CAT, GPx (↑) MDA (↓)	Zhang et al. (2021)
	S. gairdneri	0.54 mg kg^{-1}	140 days	1.5 g	WG, SR (↑)	–	Knox et al. (1981)
Fe	*I. punctatus*	30 mg kg^{-1}	70 days	7.1 g	WG, FE, SR (↑)	Hb, HCT (↑) RBC, MCV (↑)	Gatlin and Wilson (1986a)
		20 mg kg^{-1}	91 days	4.7 g	WG, FE, SR (↑)	TBC, HCT (↑)	Lim and Klesius (1997)
		0.3 g kg^{-1}	98 days	10.6 g	WG, SR (↑)	TBC, HCT, Hb (↑)	Lim et al. (2000)
		60 mg kg^{-1}	56 days	8.5 g	–	SR against (↑) *E. ictaluri*	Sealey et al. (1997)
	O. mykiss	25 mg kg^{-1}	140 days	1.5 g	WG, SR (↑)	Hb, HCT (↑) Plasma protein (↑)	Desjardins et al. (1987)
	O. niloticus × *O. aureus*	1.5 g kg^{-1}	56 days	0.63 g	WG, FE, SR (↑)	RBC, Hb (↑) MCV, MCH, HCT (↑)	Shiau and Su (2003)
	O. niloticus	14. 58 g kg^{-1}	84 days	3.75 g	WG, SGR, PER (↑) FCR (↓)	RBC, Hb, HCT (↑)	El-Saidy and Gaber (2004)
	C. carpio	147.4 mg kg^{-1}	60 days	11.4 g		ALP (↑)	

					WG, FE (↑) Protein (↑) Lipid, ash (↔) Trypsin, lipase, α-amylase (↑)		Ling et al. (2010)
	C. gariepinus	30 g kg^{-1}	96 days	112 g	WG, SGR, SR (↑) FCR (↓) Protein, lipid, ash (↔)	HCT, Hb, RBC, WBC, MCH, MCV (↔)	Siqwepu et al. (2020)
Zn	*C. auratus*	60 mg kg-^{1}	63 days	0.51 g	WG, FCE, SR (↑)	SOD, ALP (↑)	Hasnat et al. (2012)
	I. punctatus	60 mg kg^{-1}	56 days	78 g	WG, FE, SR (↑)	–	Gatlin III and Wilson (1983)
	L. rohita	20 mg kg^{-1}	45 days	5.06 g	WG, SGR (↑), Protease, lipase, amylase (↑)	SGOT, SGPT, PO, PHA, RB, SOD (↑)	Mondal et al. (2020)
		42 mg kg^{-1}	90 days	3.15 g	WG, FI, SGR, SR (↑) Protein, lipid (↔)	–	Akram et al. (2019)
		10 mg kg^{-1}	120 days	368 g	WG, (↑), FCR (↔)	RB, SOD, LYZ, SR (↑) against *A. hydrophila*	Swain et al. (2019)
	S. rivulatus	30 mg kg^{-1}	56 days	0.20 g	WG, PER, SR (↑),FCR (↓)	SOD, CAT, GPx, LYZ, PO (↑) MDA (↓)	Sallam et al. (2020)
	H. huso	15, 30, and 60 mg kg^{-1}	84 days	8.4 g	FW, WG, SGR (↑)	GST (↑) SGOT, SGPT (↓)	Mohseni et al. (2021)
Cu	*E. malabaricus*	6.56 mg kg^{-1}	56 days	13.35 g	WG, FE, SR (↑)	Cu-Zn SOD (↑) MDA (↓)	Lin et al. (2008)
	Salmo salar	5 mg kg^{-1}	84 days	7.5 g	WG (↑)	–	

(continued)

Table 16.2 (continued)

Mineral	Species	Optimized dose	Exp. duration	Initial size of the organism	Survival, growth, proximate composition and Digestive enzymes	Immune response	References
							Lorentzen et al. (1998)
	I. punctatus	8 mg kg^{-}	91 days	83 g	SR (↑)	SOD, cu-SOD, Mn-SOD (↔)	Gatlin and Wilson (1986b)
		80 mg kg^{-}	28 days	6.7 g	Growth (↔)	SR (↑) against *F. columnare*	Farmer et al. (2017)
	M. amblycephala	3 mg kg^{-1}	56 days	30.8 g	EG, SGR, SR (↑) FCR (↓)	Cu- Zn-SOD, CAT, GSH-Px (↔)	Shao et al. (2012)
	S. zarudnyi	3 mg kg^{-1}	84 days	23.97 g	SGR, SR (↑)	RBC, HCT, Hb, serum protein, SOD, CAT, GPx, LYZ (↑), MDA (↓)	Afshari et al. (2021)
Se	*M. salmoide*	0.6–1 mg kg^{-1}	56 days	4.95 g	WG, PER (↑), FCR (↓) Protein, lipid (↔)	GPx (↑), MDA (↔)	Zhu et al. (2012)
	S. gairdneri	41.1 mg kg^{-1}	140 days	4 g	WG, FI, SR (↑)	HCT, RBC, GPx (↑) against *E. ictaluri*	Hilton et al. (1980)
	A. regius	3.98 mg kg^{-1}	63 days	3.2 g	WG, SR (↑),FCR (↓)	SOD, CAT (↑) SGOT, SGPT (↑)	Khalil et al. (2019)
	E. malabaricus	0.5 mg kg^{-1}	56 days	12.20 g	WG, FE, SR (↑)	GPx, GSH (↑)	Lin and Shiau (2005)
	I. punctatus	1–5 mg kg^{-1}	98 days	70–72 g	WG, SR (↑) Protein, lipid, ash (↑)	Se-GSH, non se-GSH (↑)	Gatlin and Wilson (1984)
	S. aurata	0.2 mg kg^{-1}	636 days	6.2 g	WG, FI, SR (↑) Protein, lipid (↔)	–	Mechlaoui et al. (2019)
	R. canadum	1 mg kg^{-1}	70 days	6.27 g	SGR, PE, SR (↑)	Se- GPx (↑)	

							Liu et al. (2010a)
	C. carpio	1.2 mg kg^{-1}	56 days	125.26 g	WG (↑) FCR (↓)	Serum protein (↑) MDA (↓)	Luo et al. (2021)
	L. rohita	0.3 mg kg^{-1}	120 days			RB, SOD, LYZ, SR (↑) against *A. hydrophila*	Swain et al. (2019)
	O. niloticus	0.7 mg kg^{-1}	70 days	33 g	WG, SGR (↑)	RBC, Hb, HCT, LYZ (↑) against *S. iniae*	Neamat-Allah et al. (2019)
Cr	*C. carpio*	0.5 mg kg^{-1}	56 days	4.95 g	WG, PER, SGR (↑) FCR (↓) Protein, lipid (↑)	–	Ahmed et al. (2012a, b)
	O. mykiss	4 mg kg^{-1}	58 days	225 g	–	Blood glucose and fat	Küçükbay et al. (2006)
	C. idella	0.8 mg kg^{-1}	70 days	13.60 g	WG, FER, PER (↑) Protein, lipid ash (↔)	–	Liu et al. (2010b)
	L. rohita	0.3 mg kg^{-1}	90 days	1.81 g	WG, SGR (↑)	–	Asad et al. (2019)
	O. niloticus	0.4 mg kg^{-1}	58 days	225 g	–	Blood glucose and fat	Mehrim (2012)
F	*A. baerii*	75.2 mg kg^{-1}	84 days	11.99 g	WG, SGR (↑)	–	Shi et al. (2013)
	S. quinqueradiata	160 mg kg^{-1}	95 days	57.3 g	WG, SGR, FI, FE (↑) SR (↔) Protein, lipid (↔)	–	Yoshitomi and Nagano (2012)

ALP alkaline phosphatase, *CAT* catalase, *FCR* feed conversion ratio, *FE* feeding efficiency, *FI* feed intake, *GPx* glutathione peroxidase; *GSH* glutathione, *GSH-Px* plasma glutathione peroxidase, *GST* glutathione S-transferase, *Hb* hemoglobin, *HCT* hematocrit, *LYZ* lysozyme, *MCH* mean corpuscular hemoglobin, *MCV* mean corpuscular volume, *MDA* malondialdehyde, Na^+/K^+ATPase– Sodium–potassium adenosine triphosphatase, *PAH* phagocytic activity, *PER* protein efficiency ratio, *PO* phenoloxidase, *RB* respiratory burst, *RBC* red blood cells, *SGOT* serum glutamic oxaloacetic transaminase, *SGPT* serum glutamic pyruvic transaminase, *SOD* superoxide dismutase; *SR* survival, *TBC* total blood cells, *WBC* white blood cells, *WG* weight gain, *Ca* calcium, *K* potassium, *Na* sodium, *P* phosphorus, *Mg* magnesium, *Fe* iron, *Zn* zinc, *Cu* copper, *Se* selenium, *Cr* chromium, *Fe* fluoride, (↑) significant increase compared to control, (↓) significant decrease compared to control, (↔) insignificant alteration compared to control.

Table 16.3 Role of dietary minerals in crustaceans

Mineral	Organisms	Optimized dose	Exp. duration	Initial size of the organism	Survival, growth, proximate composition & digestive enzymes	Immune response	References
Ca	*P. indicus*	13 mg kg^{-1}	45 days	0.15 g	WG (↑), FCR (↓), SR (↔)	–	Ali (1999)
	P. vannamei	20 mg kg^{-1}	33 days	0.027 g	SR (↑)	–	Davis et al. (1993a, b)
K	*L. vannamei*	5–25 g kg^{-1}	46 days 56 days	0.5 g 0.8 g	WG, SGR, PER, SR (↑)	–	Zhu et al. (2006), Roy et al. (2007b)
	P. monodon	15 g kg^{-1}	56 days	0.75 g	WG, FE, PER, SR (↑)	–	Shiau and Hsieh (2001b)
Na	*M. rosenbergii*	20 g kg^{-1}	120 days	1.11 g	WG, PER, SR (↑), FCR (↓) Protein, lipid (↔) Protease, amylase, lipase (↑)	–	Keshavanath et al. (2003)
	L. vannamei	10 g kg^{-1}	49 days	0.5 g	WG, SR (↔)	–	Cheng et al. (2005b)
P	*P. monodon*	15 g kg^{-1}	45 days	2.54 g	WG, SR (↑), protein (↑)	–	Ambasankar et al. (2006)
	P. vannamei	20 mg kg^{-1}	33 days	0.027 g	SR (↑)	–	Davis et al. (1993a, b)
	E. sinensis	9.9 g kg^{-1}	56 days	0.38 g	WG, FI, SGR, SR (↑) Protein, lipid, ash (↔)	–	Lei et al. (2021)
Mg	*L. vannamei*	3.2 g kg^{-1}	49 days	0.5 g	WG (↑), SR (↓), protein (↑)	Na^+ / K^+ ATPase (↓) under salinity stress	Cheng et al. (2005a); Roy et al. 2007a, b)
	M. rosenbergii	0.5 g kg^{-1}	90 days	0.11 g	WG, SGR, SR (↑) Protein, lipid carbohydrate (↑) Protease, amylase, lipase (↑)	SOD, CAT, LPO, GOT, GPT (↔)	Srinivasan et al. (2017)
Fe	*P. vannamei*	20 mg kg^{-1}	28 days	26–32 mg	WG, SR (↑)	–	Davis et al. (1992)
	M. rosenbergii	10–20 mg kg^{-1}	90 days	0.11 g	WG, SR, FI, SGR (↑) Protein, lipid, carbohydrate, EAA, FA (↑) Protease, amylase, lipase (↑)	SOD, CAT, LPO, GOT, GPT (↔) THC, DHC (↑)	Srinivasan et al. (2016)
	E. sinensis	177 mg kg^{-1}	56 days	0.83 g			Song et al. (2021)

					WG, SGR, SR (↑) Protein, lipid (↔)	SOD, CAT, GSH (↑)	
Zn	*P. monodon*	40 mg kg^{-1}	56 days	0.44 g	WG, SR (↑)	SOD, CAT, LPO (↔) THC, DHC (↑)	Shiau and Jiang (2006)
	P. vannamei	15 mg kg^{-1}	33 days	0.058 g	WG, SR (↑)	–	Davis et al. (1993a)
	P. vannamei	65.5 mg kg^{-1}	56 days	1.34 g	WG, FI, SGR, SR (↑)	ProPO (↑)	Shi et al. (2021)
	M. rosenbergii	60 mg kg^{-1}	90 days	0.19 g	WG, SR, FI, SGR (↑) Protein, lipid, carbohydrate, EAA, FA(↑) Protease, amylase, lipase (↑)	SOD, CAT, LPO, GOT, GPT (↔) THC, DHC (↑)	Muralisankar et al. (2015), Thirunavukkarasu et al. (2019)
	E. sinensis	20 mg kg^{-1}	56 days	49.68 mg	WG, SR (↑)	–	Li et al. (2010)
Cu	*L. vannamei*	32 mg kg^{-1}	42 days	0.057 g	WG (↑)	–	Davis et al. (1993a)
	P. monodon	20 mg kg^{-1}	56 days	0.29 g	WG, FE, PER, SR (↑) MUFA, PUFA (↑)	–	Lee and Shiau (2002)
	L. vannamei	39.70 mg kg^{-1}	56 days	1.86 g	WG, FE, PER, SGR, SR (↑) Protein, lipid (↔)	–	Yuan et al. (2019)
	M. rosenbergii	40 mg kg^{-1}	90 days	0.18 g	WG, SR, FI, SGR (↑) Protein, lipid, carbohydrate (↑) Protease, amylase, lipase (↑)	SOD, CAT, LPO, GOT, GPT (↔)	Muralisankar et al. (2016)
	P. indicus	10 mg kg^{-1}	75 days	70 mg	WG, SR (↑)	–	Ali (2000)
	E. sinensis	40 mg kg^{-1}	56 days	0.45 g	WG (↑)	PO, THC (↑) SR (↑) against *A. hydrophila*	Sun et al. (2013)
Se	*M. rosenbergii*	0.5–1 mg kg^{-1}	75 days	NS	–	GPx, GST (↑) THC, PO, RB, PHA	Chiu et al. (2010)

(continued)

Table 16.3 (continued)

Mineral	Organisms	Optimized dose	Exp. duration	Initial size of the organism	Survival, growth, proximate composition & digestive enzymes	Immune response	References
						(↑) against *D. hansenii*	
	M. nipponense	0.47 mg kg^{-1}	56 days	0.133 g	WG, SR (↑)	SOD (↑)	Kong et al. (2017)
	P. chinensis	20 mg kg^{-1}	28 days	5–6.5 cm	WG, SR (↑)	–	Yuchuan and Fayi (1993)
	P. vannamei	0.3 mg kg^{-1}	35 days	1–2 g	WG (↑)	GH (↑) SR (↑) against TSV	Sritunyalucksana et al. (2011)
	C. cainii	0.2 g kg^{-1}	90 days	3.29 g	WG, SGR, SR (↑)	THC, DHC (↑) THC (↑) against *V. mimicus*	Nugroho and Fotedar (2013)

ALP alkaline phosphatase, *CAT* catalase, *DHC* differential hemocyte counts, *FCR* feed conversion ratio, *FE* feeding efficiency, *FI* feed intake, *GH* granular hemocytes, *GOT* glutamic oxaloacetic transaminase, *GPT* glutamic pyruvic transaminase, *GPx* glutathione peroxidase, *GSH* glutathione, *GST* glutathione S-transferase, *LPO* lipid peroxidation, *MDA* malondialdehyde, Na^+/K^+ATPase–sodium–potassium adenosine triphosphatase, *PER* protein efficiency ratio, *PO* phenoloxidase, *ProPO* prophenoloxidase, *RB* respiratory burst activity, *SOD* superoxide dismutase, *SR* survival, *THC* total hemocyte counts, *TSV* Taura syndrome virus, *WG* weight gain, *Ca* calcium, *K* potassium, *Na* sodium, *P* phosphorus, *Mg* magnesium, *Fe* iron, *Zn* zinc, *Cu* copper, *Se* selenium, *Cr* chromium, *Fe* fluoride, (↑) significant increase compared to control, (↓) significant decrease compared to control, (↔) insignificant alteration compared to control.

16.2 Effects of Dietary Minerals on Food Index, Survival, and Growth

Feed index, survival, and growth are major factors affecting the economy of the cultivable fish and crustaceans. In the aquaculture industry, feed is one of the primary factors which affect survival and growth. Minerals are part of an essential nutrient in aquafeeds. Minerals act as catalysts for many biological reactions within the body, including muscle response, hormones, digestion, transmitting senses through the nervous system, and utilization of nutrients from diets. The optimal level of each mineral is required for better feed intake and growth of aquatic animals. The optimal level of minerals are necessary for maintain normal physiological function of aquatic animals which leads to better growth and survival. The macromineral calcium (calcium lactate, calcium chloride, calcium carbonate, and calcium phosphate) incorporated feed fed fish, *Sebastiscus marmoratus, Ctenopharyngodon idella, Epinephelus coioides, Oreochromis niloticus* × *Oreochromis aureus, Labeo rohita, Ictalurus punctatus* and the shrimps, *Penaeus indicus*, and *Penaeus vannamei* had shown significant improvements in survival, weight gain, feed intake, feeding efficiency and protein efficiency ratio (Andrews et al. 1973; Davis et al. 1993b; Ali 1999; Hossain and Furuichi 2000; Ye et al. 2006; Shiau and Tseng 2007; Liang et al. 2012; Kalantarian et al. 2013; Musharraf and Khan 2020). Shiau and Hsieh (2001a, b), Zhu et al. (2006), Roy et al. (2007a, b), and Liang et al. (2014) reported that the dietary addition of potassium had produced better survival and significant improvements in weight gain, feeding efficiency, specific growth rate, and protein efficiency ratio in the white shrimp, *L. vannamei*, black tiger shrimp, *P. monodon,* and the fish grass carp, *C. idella*. Similarly, the fish *L. rohita*, *Cyprinus carpio, Cirrhinus mrigala,* and *O. aureus* × *O. niloticus* and the crustaceans *M. rosenbergii* and *L. vannamei* showed significant elevations in survival, growth (weight gain and specific growth rate), and feeding efficiency after fed to sodium incorporated diet (Keshavanath et al. 2003; Shiau and Lu 2004; Cheng et al. 2005a; Zhao et al. 2021). Further, dietary addition of phosphorus significantly promoted the survival, weight gain, specific growth rate, feed intake, and feeding efficiency in the fish *Heterobranchus bidorsalis*, *O. niloticus, Pelteobagrus fulvidraco, Clarias leather, I. punctatus, Chanos chanos, S. salar, Clarias gariepinus, Acipenser baerii, Sciaenops ocellatus, O. mykiss, C. idella, Sparus macrocephalus* and crustaceans *P. monodon, L. vannamei,* and *Eriocheir sinensis* (Davis and Robinson 1987; Davis et al. 1993b; Åsgård and Shearer 1997; Borlongan and Satoh 2001; Ambasankar et al. 2006; Phromkunthong and Udom 2008; Shao et al. 2008; Nwanna et al. 2009; Xu et al. 2011; Adekunle 2012; Yu et al. 2013; Chen et al. 2017; Morales et al. 2018; Lei et al. 2021). Moreover, it has also been observed earlier that the dietary inclusions of magnesium greatly promoted the survival of *O. mykiss*, *I. punctatus, C. idella, Salmo gairdneri, Acipenser schrenckii* × *A. baerii, M. rosenbergii*, and *L. vannamei* (Knox et al. 1981; Gatlin et al. 1982; Cheng et al. 2005a, b; Roy et al. 2007a, b; Wang et al. 2011; Srinivasan et al. 2017; Zhang et al.

2021). These reports indicate the influence of dietary macro minerals on the survival, feed intake, and growth of fish and crustaceans.

Trace elements also play a pivotal role in maintaining normal physiological functions which led to the survival of aquatic animals. The studies on various fish species, such as *I. punctatus*, *O. mykiss*, *O. niloticus*, *O. niloticus* × *O. aureus, C. gariepinus,* and *C. carpio*, and crustaceans, *P. vannamei* and *M. rosenbergii,* indicated significant improvements in survival, growth, and feeding efficiency after fed to dietary supplementation of iron (Fe_2O_3, $FeSO_4$, and $FeC_6H_6O_7$) (Gatlin and Wilson 1986a; Desjardins et al. 1987; Davis et al. 1992; Lim and Klesius 1997; Lim et al. 2000; Shiau and Su 2003; El-Saidy and Gaber 2004; Ling et al. 2010; Srinivasan et al. 2016; Siqwepu et al. 2020). The maximum level of feed intake, feeding efficiency, followed by increased survival, weight gain, and specific growth rate have been noticed in fishes like *O. niloticus*, *Carassius auratus*, *Siganus rivulatus, Huso huso*, *L. rohita,* and *I. punctatus* and crustaceans like *P. monodon, P. vannamei, M. rosenbergii,* and *E. sinensis* fed to graded level of zinc supplemented diets (Gatlin and Wilson 1986b; Shiau and Jiang 2006; Li et al. 2010; Hasnat et al. 2012; Muralisankar et al. 2015; Akram et al. 2019; Thirunavukkarasu et al. 2019; Mondal et al. 2020; Sallam et al. 2020; Mohseni et al. 2021; Shi et al. 2021). Also, the dietary copper ($CuSO_4$ and $CuCl_2$) supplemented feed fed fishes (*Epinephelus malabaricus*, *Schizothorax zarudnyi*, *Salmo salar*, *I. punctatus, Megalobrama amblycephala*) and crustaceans (*M. rosenbergii*, *P. indicus, L. vannamei,* and *P. monodon*) attained maximum feed intake, survival, and growth (final weight, weight gain, and specific growth rate) have been reported previously (Gatlin III and Wilson 1986; Lorentzen et al. 1998; Ali 2000; Lee and Shiau 2002; Lin et al. 2008; Shao et al. 2012; Muralisankar et al. 2016; Yuan et al. 2019; Afshari et al. 2021). Further, the dietary incorporation of selenium showed a higher survival rate, increased feed intake, weight gain, feeding efficiency, and specific growth rate in fishes such as *Micropterus salmoide*, *S. gairdneri*, *Argyrosomus regius, Sparus aurata, E. malabaricus*, *I. punctatus, C. carpio,* and *Rachycentron canadum* and crustaceans such as *P. vannamei, Macrobrachium nipponense,* and *Penaeus chinensis* (Hilton et al. 1980; Gatlin and Wilson 1984; Yuchuan and Fayi 1993; Lin and Shiau 2005; Liu et al. 2010a; Sritunyalucksana et al. 2011; Zhu et al. 2012; Chen et al. 2013; Kong et al. 2017; Khalil et al. 2019; Mechlaoui et al. 2019; Luo et al. 2021). Furthermore, the fishes like *Cyprinus carpio*, *O. mykiss*, *C. idella,* and *L. rohita* showed better survival, protein efficiency ratio, feeding efficiency, and growth (weight gain and specific growth rate) when fed on chromium supplemented diets (Küçükbay et al. 2006; Liu et al. 2010b; Ahmed et al. 2012a, b; Asad et al. 2019). It is reported that the manganese enriched *Artemia* diets gently promoted the survival of sea bream, *Pagrus major*. A significant increment in feed intake, feeding efficiency, total weight gain, and specific growth rate has been recorded in the fishes *A. baerii* and *Seriola quinqueradiata* fed to dietary fluoride (Yoshitomi and Nagano 2012; Shi et al. 2013). Therefore, above mentioned studies clearly indicate that minerals including trace elements have a significant role in the maintenance of physiological functions in fish and crustaceans, it leads to reduced stress and increased feed intake, growth, and survival.

16.3 Influence of Dietary Minerals on Digestive Enzymes

Activities of digestive enzymes in the fish and crustaceans play a central role in nutritional physiology and may directly or indirectly regulate survival and growth (Lovett and Felder 1990). The fish and crustaceans have digestive enzymes such as proteolytic enzymes (trypsin and carboxypeptidase), carbohydrate enzymes (maltase and amylase), and lipolytic enzymes (lipase) which are essential for the hydrolysis of proteins, carbohydrates, and lipids, respectively (Bone and Moore 2008). Animals rely on a functional digestive system to efficiently utilize the nutrients present in the food and the capability to hydrolyze, absorb, and assimilate the nutrients (Fernández-Reiriz et al. 2001). In another hand, the quality and nutritive value of formulated feeds depend on the digestibility of the individual components (D'Abramo and Sheen 1994; del Carmen González-Peña et al. 2002). Dietary supplementation of minerals can influences the digestive enzyme activities of fish and crustaceans. Dietary additions of sodium showed significant elevations in the intestinal and hepatopancreatic enzymes such as protease, amylase, and lipase in the fish (*L. rohita, C. mrigala, C. carpio,* and *P. fulvidraco*) and prawn (*M. rosenbergii*) (Keshavanath et al. 2003; Zhao et al. 2021). Srinivasan et al. (2017) recorded substantial elevations in the activity of digestive enzymes protease, lipase, and amylase of *M. rosenbergii* fed to dietary magnesium. Also, it has been noticed that the trace element iron included feed fed fish *C. carpio* and prawn *M. rosenbergii* had produced an elevated level of trypsin, lipase, and amylase (Ling et al. 2010; Srinivasan et al. 2016). Also, the fish *L. rohita* and the prawn *M. rosenbergii* had shown significant improvement in intestinal digestive enzymes activity (protease, lipase, and amylase) fed on dietary zinc (Muralisankar et al. 2015; Mondal et al. 2020) and copper (Muralisankar et al. 2016). The previous studies indicate the ability of different dietary minerals on the activity of the digestive enzymes. Nevertheless, the studies are scanty on the effects of minerals on the digestive enzymes of different species, hence, more studies are required to clarify the impact of minerals on different fish and crustaceans.

16.4 Effects of Dietary Minerals on Proximate Composition

The quality of the flesh is determined by analyzing the proximate composition (crude protein, lipid, nitrogen free extract, fiber, ash, and moisture) of the whole body and or muscle of any edible species. In aquatic edible species, the proximate composition is one of the most crucial factors to evaluate the nutrient quality of animals. Different levels of dietary minerals showed a correlation with the proximate composition of fish and crustaceans (Muralisankar et al. 2015, 2016; Musharraf and Khan 2020). The macromineral, calcium enriched feed fed edible fish *L. rohita* produced significant improvements in whole body crude protein and crude lipid (Musharraf and Khan 2020). In this context, the fishes such as *E. coioides* and *A. nobilis* fed to different levels of calcium enriched diets showed insignificant alterations in crude protein, lipid, and ash (Ye et al. 2006; Liang et al. 2018). Similarly, Keshavanath et al. (2003) reported from their findings, sodium enriched feed fed fish *L. rohita*, *C. carpio,* and *C. mrigala* showed significant elevation on muscle lipid content and an insignificant alteration in protein level. Also, the same study reported that there was no significant variation in the level of muscle protein and lipid in the freshwater prawn *M. rosenbergii*. Further, the *P. fulvidraco* fed to dietary sodium had produced significant elevations in muscle protein and ash contents (Zhao et al. 2021). The fishes like *H. bidorsalis* and *C. gariepinus* fed to the macromineral phosphorus incorporated feed have shown significant elevation in crude protein and lipid (Nwanna et al. 2009; Adekunle 2012). Whereas, Phromkunthong and Udom (2008), Shao et al. (2008), and Xu et al. (2011) reported insignificant alterations in protein, lipid, and ash content in the fish *O. niloticus, S. macrocephalus,* and *A. baerii* fed on dietary addition of phosphorus. In crustaceans, the shrimp *P. monodon* fed to dietary phosphorus gained maximum level of protein in the whole carcass contents (Ambasankar et al. 2006). While, insignificant alterations in protein, lipid, and ash have been noticed in the crab *E. sinensis* fed to dietary phosphorus (Lei et al. 2021). Further, Wang et al. (2011) noticed that dietary administration of magnesium had produced significant improvement in muscle lipid content of *C. idella* (Wang et al. 2011). While, the shrimp *L. vannamei* and the prawn *M. rosenbergii* fed to dietary magnesium showed significant elevations in muscle protein, amino acids, fatty acids, lipid, carbohydrate, and total ash (Cheng et al. 2005a; Roy et al. 2007a, b; Srinivasan et al. 2017).

The effect of trace elements on the proximate composition (protein, lipid, and ash) of fish and crustaceans has also been studied by various researchers. An increase in the crude protein in *C. carpio* fed with iron included diets has been reported by Ling et al. (2010), however, the same study indicated insignificant alterations in the crude lipid and ash contents. An insignificant alteration in muscle protein, lipid, and ash levels has been recorded in the fish *C. gariepinus* fed to different levels of iron (Siqwepu et al. 2020). Moreover, the freshwater prawn *M. rosenbergii* had gained better protein, lipid, carbohydrate, and ash content when fed to dietary iron (Srinivasan et al. 2016). In this context, insignificant elevations in whole body protein and lipid contents in *E. sinensis* fed to dietary iron have been noticed by Song et al. (2021). Similarly, the dietary administration of zinc did not produce significant alterations in the protein and lipid levels of *L. rohita* (Akram et al. 2019). While, different concentrations of dietary zinc and copper showed significant improvements in muscle protein, lipid, carbohydrate, essential amino acids, fatty acids, and ash contents in the prawn *M. rosenbergii* (Muralisankar et al. 2015, 2016; Muralisankar et al. 2019) and the shrimps *P. monodon* and *L. vannamei* (Lee and Shiau 2002; Yuan et al. 2019). In addition to this, 1:2 ratio of manganese and zinc complex supplemented enriched *Artemia* nauplii fed sea bream, *P. major* had significant elevation in carcass protein, lipid, fatty acid, and ash content (Nguyen et al. 2008). Dietary inclusion of selenium showed considerable improvements in proximate composition (whole body protein, lipid, and ash) of *I. punctatus* (Gatlin et al. 1982). Therefore, the above-cited studies showed that minerals can affect the proximate composition of fish and crustaceans. However, some studies reported reduction and insignificant alterations in proximate composition of aquatic animals, hence, more research has to be conducted to know the effect of each mineral on different stages of fish and crustaceans.

16.5 Role of Dietary Minerals on the Immune Response

The health status of an organism can be determined by immunological parameters like hematological measurements and antioxidants enzymes. In aquatic organisms, the immunity has been determined by total blood cells count (TBC), red blood cells count (RBC), phagocytosis activity (PHA), hemoglobin (Hb), hematocrit (HCT), etc., and antioxidant parameters such as superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LPO), glutamic oxaloacetic transaminase (GOT), glutamate pyruvate transaminase (GPT), glutathione peroxidase (GPx), etc. The effect of dietary minerals like potassium, sodium, magnesium, iron, zinc, selenium, copper, etc., on immune responses of fish and crustaceans has been proved by several researchers (Gatlin et al. 1982; Lim and Klesius 1997; Shiau and Hsieh 2001a; Cheng et al. 2005a; Muralisankar et al. 2015, 2016; Srinivasan et al. 2016; Khalil et al. 2019; Mondal et al. 2020; Afshari et al. 2021; Zhao et al. 2021). The increased alkaline phosphatase (ALP) and Lysozyme (LYZ) activities in the fish *P. fulvidraco, O. niloticus,* and *S. macrocephalus* fed to diets containing sodium and potassium have been observed earlier (Phromkunthong and Udom 2008; Shao et al. 2008; Zhao et al. 2021). Magnesium included diet fed fishes (*I. punctatus, C. idella,* and *A. schrenckii* × *A. baerii*) produced significant elevations in hematological parameters (RBC, HCT, Hb, and ALP) and antioxidant enzymes (SOD, CAT, and GPx); however, the hybrid fish *A. schrenckii* × *A. baerii* showed a significant decrease in the production of Malondialdehyde (MDA) (Gatlin et al. 1982; Wang et al. 2011; Zhang et al. 2021). In this context, some macro minerals like calcium and potassium did not produce any significant alterations in RBC, HCT, and Na^+ / K^+ ATPase levels in *A. nobilis* and *A. schrenckii* × *A. baerii* (Shiau and Hsieh 2001b; Liang et al. 2018). Moreover, Srinivasan et al. (2016) observed that the freshwater prawn *M. rosenbergii* showed insignificant alterations in the activity of antioxidant enzymes (SOD and CAT), LPO, and metabolic enzymes (GOT and GPT) fed to 0.5 g kg^{-1} of dietary iron, whereas prawn fed to beyond 0.5 g kg^{-1} of dietary iron showed significant alterations in these activities.

The dietary trace minerals are also playing a major role in the immune system of fish and crustaceans. The significant increments in TBC, RBC, HCT, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), ALP, and total plasma protein levels in the fishes such as *I. punctatus, O. mykiss, O. niloticus* × *O. aureus,* *O. niloticus, C. carpio*, and *C. gariepinus* fed on dietary iron have been reported in earlier studies (Gatlin and Wilson 1986a; Desjardins et al. 1987; Lim and Klesius 1997; Lim et al. 2000; Shiau and Su 2003; El-Saidy and Gaber 2004; Ling et al. 2010). Further, the dietary inclusion of iron improved the THC and DHC in the prawn *M. rosenbergii* (Srinivasan et al. 2016), and SOD, CAT, and glutathione (GSH) activities in the crab *E. sinensis* (Song et al. 2021). Administration of zinc in the diets of *C. auratus, L. rohita, S. rivulatus,* and *H. huso* attained significant improvement in phenoloxidase (PO), respiratory burst (RB), PHA, glutathione S-transferase, (GST), SOD, CAT, GPx, LYZ, ALP, serum GOT, and GPT activities (Hasnat et al. 2012; Mondal et al. 2020; Sallam et al. 2020; Mohseni et al. 2021). Moreover, the crustaceans (*P. monodon, P. vannamei*, and *M. rosenbergii*) had shown significant elevations in THC, DHC, and prophenoloxidase (ProPO) and an insignificant alteration in SOD, CAT, GOT, and GPT activities when fed after dietary zinc (Shiau and Jiang 2006; Muralisankar et al. 2015; Shi et al. 2021). The dietary inclusion of copper on the fishes (*E. malabaricus* and *S. zarudnyi*) showed significant improvements in RBC, HCT, Hb, Cu-Zn SOD, SOD, GPx, LYZ, and serum protein levels (Lin et al. 2008; Afshari et al. 2021). However, some fishes like *M. amblycephala* and *I. punctatus* and the prawn *M. rosenbergii* showed insignificant alterations in SOD, Cu-SOD, Mn- SOD, CAT GSH, and GPx activities fed after copper included diets has also been reported (Gatlin and Wilson 1986b; Shao et al. 2012; Muralisankar et al. 2016) which indicates the effects of dietary copper level on different species. Moreover, the significant elevations in antioxidants, such as SOD, CAT, GSH, Se-GSH, GPx, and serum protein levels were recorded in different fish species including *M. salmoide, A. regius, E. malabaricus, I. punctatus, R. canadum,* and *C. carpio* fed to dietary selenium (Gatlin and Wilson 1984; Lin and Shiau 2005; Liu et al. 2010a; Zhu et al. 2012; Khalil et al. 2019; Luo et al. 2021). In crustaceans, the freshwater prawn (*M. nipponense*) fed on dietary selenium showed significant improvement in SOD (Kong et al. 2017). Few studies have been reported that the dietary inclusion of chromium also improved the blood glucose and fat levels in the fish *O. mykiss* and *O. niloticus* (Küçükbay et al. 2006; Mehrim 2012). The above studies have indicated the effects of minerals on the immune system of fish and crustaceans. However, some studies reported that some minerals did not produce significant alteration in the immune parameters in some species of fish and crustaceans, hence, more studies are required to understand the immune stimulating mechanism of each mineral in fish and crustaceans at different life stages.

16.6 Influence of Dietary Minerals on Disease Resistance

The diseases caused by pathogens are considered as one of the major threats in aquaculture organisms. Aquatic animals including fish and crustaceans are mostly affected by pathogenic bacteria and viruses which leads to reduced immunity, followed by poor survival and growth. In culture systems, antibiotics are used in the diet of fish and crustaceans to mitigate the pathogen-mediated diseases.

However, the use of antibiotics may lead to the resistance of pathogens, suppressing the immune system of cultivable species, and also cause environmental pollution (Allameh et al. 2016). Hence, researchers are focusing to find the alternative for antibiotics to overcome the pathogen-related problems and enhance the immune system of aquatic animals. The optimal level of certain minerals, mainly the trace elements like zinc, copper, selenium, etc., can promote the survival, growth, immune system, and disease resistance against the various pathogen in fish and crustaceans (Hilton et al. 1980; Sun et al. 2013; Farmer et al. 2017; Swain et al. 2019). The fish *I. punctatus* fed to dietary iron (60 mg kg^{-1}) and copper (80 mg kg^{-1}) showed better survival against the pathogenic bacterium *Edwardsiella ictaluri* and *Flavobacterium columnare,* respectively (Sealey et al. 1997; Farmer et al. 2017). Dietary administration of zinc (10 mg kg^{-1}) produced significant elevations in RB, SOD, and LYZ activities and an increased survival rate against the pathogen *Aeromonas hydrophila* has been observed (Swain et al. 2019). Also, the fish *S. gairdneri* fed to dietary selenium (41.1 mg kg^{-1}), *L. rohita* (0.3 mg kg^{-1}), and *O. niloticus* (0.7 mg kg^{-1}) showed significant improvements in the hematological elements (RBC, HCT, and Hb), antioxidants (SOD and GPx), LYZ, and RB levels after challenged to *E. ictaluri* (Hilton et al. 1980), *A. hydrophila* (Swain et al. 2019), and *Streptococcus iniae* (Neamat-Allah et al. 2019). In crustaceans, the increased level of THC, PO, RB, PHA, and survival has been observed in the crab *E. sinensis* and prawn *M. rosenbergii* fed to dietary copper (40 mg kg^{-1}) and selenium (0.5–1 mg kg^{-1}) after challenged against the pathogens *A. hydrophila* and *Debaryomyces hansenii,* respectively (Chiu et al. 2010; Sun et al. 2013). Further, the dietary organic selenium fed smooth marron, *Cherax cainii* exhibited a significant increase in the production of THC challenged against *Vibrio mimicus* (Nugroho and Fotedar 2013). Moreover, the shrimp *P. vannamei* had shown improvements in granular hemocytes and survival against Taura syndrome virus after fed to 0.3 mg kg^{-1} of dietary selenium (Sritunyalucksana et al. 2011). The above studies showed the immune response of fish and crustaceans against pathogens.

16.7 Conclusion

The present chapter demonstrates that the optimum dietary supplementation of minerals can promote feed intake, feed efficiency, digestive enzymes secretion which leads to hydrolysis and utilization of nutrients from the diet by fish and crustaceans followed by better growth and muscle meat quality in terms of proximate composition. Also, minerals have potent to promote the nonspecific and specific immunity, antioxidants, and disease resistance against bacterial and viral pathogens, however, optimization of the dietary requirement of each macro and trace mineral needs to be studied for all edible cultivable aquatic species.

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Chapter 17
Future Therapeutic Approaches to Annihilate Bacterial Fish Diseases in Aquaculture

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Abstract The global emergence of bacterial fish diseases has been noticed due to the presence of infectious pathogens in the polluted environment. Bacterial fish diseases are reliable for serious economic losses in aquaculture and cause several clinical effects in human due to bacterial pathogens which are present in the aqua-environment worldwide. Hence, these contagious or zoonotic diseases and their outbreaks via aquatic animals have been treated by the development of advanced therapies like nano-biomaterials and bacteriophage-based approaches, which are rapidly demanded. Herein, we overwhelmingly emphasize on the bacterial fish disease causing pathogens such as *Aeromonas, Campylobacter, Edwardsiella, Erysipelothrix, Escherichia, Klebsiella, Mycobacterium, Salmonella,* and *Streptococcus* species and also highlight treatment including antibiotics, nanoparticles, antibacterial peptides, bacteriophage-based therapeutics and their scope in clinical

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and aqua-animal applications. Advanced therapeutic approaches can develop novel treatment patterns to overcome opportunistic or external or internal fish bacterial infections and are capable of reducing the emergence of multidrug-resistant (MDR) bacterial pathogens and inhibiting the major outbreaks of aquatic animals that could lead to unpredictable aqua-animals, humans, and economic losses.

Keywords Multidrug-resistant bacteria · Bacterial fish diseases · Antimicrobial phage components · Antibiotics · Antibacterial peptides

17.1 Introduction

Aquaculture or the fish farming sector is one of the major domains for the mass production of food, and granting maximum protein for human and animal consumption (Hoikkala et al. 2019). Various group of fishes such as finfishes (Koi, Salmon, Tilapia, and Trout), cartilaginous fishes (rays, sawfish, sharks, and skates), crustaceans (prawns, and shrimps) and molluskan shellfish (clam, mussel, oyster, and scallop) and Jawless fishes (hagfish, and lamprey) have been considered as the major global food from aquaculture (Maiti et al. 2020). Moreover, aquaculture raised shellfish and food fish production from nine million metric tons in 1990 to 60 million metric tons in 2010 due to escalating universal demand which is estimated at 119.4 billion US dollars worldwide. Therefore, it is very crucial to control or prevent emerging bacterial fish diseases.

The emergences of bacterial fish disease have been elevated in case of the presence of infectious bacterial pathogens. Bacteria are ubiquitous in water ecosystem, that means most of the fish pathogens can survive not only in fish but also outside the fish (de Oliveira and Margis 2015). In the presence of bacterial pathogens, the production of freshwater fishes, marine fishes, and other products has been facing a lot of challenges like reducing fish production by killing or infecting aquaculture organisms, leading to an economic overburden on the insufficient food products (Richards 2014). Stress can cause fish as a susceptible to bacterial infection that is raised by various parameters including irregular pH, temperature, salinity, poor water and oxygen quality. It is very important to detect, inhibit, and kill the emergence of bacterial fish pathogens with advanced and natural friendly approaches.

Vaccine is the suitable approach that can control or combat bacterial pathogens. Some of the limitedness have been identified due to diverse sorts of infectious diseases in different fish species (Arijo et al. 2005). On another note, chemical drug therapy commonly known as antibiotic therapy is a quick and efficient approach to kill or eradicate infectious pathogens. In case, it may enhance drug-resistant pathogen as a result of unrestricted use of antibiotics in each and every domain like aquaculture, agriculture, food safety, and medical sectors (Nakai and Park 2002). Antibiotic resistance associated bacterial diseases may be responsible for the death of around ten million people per year by 2050. This is why, eco-friendly approaches must be required to hold back fish disease in aquaculture. To address these issues, bacteriophages have been used in aquaculture that looks like a very gratifying and alternative approach than any other else (Shao 2001).

Bacteriophages or phages for short are bacterial viruses existing by nature that can infect specific bacterial pathogens and multidrug-resistant pathogens. Lytic and lysogenic types of phages have been spotted in phage life cycle. Phage attachment, genome insertion, amplification and congregation of phage components (nucleic acids, capsid proteins, and tail fiber proteins), and discharge young phages through host lysis are the unique process of phage lytic cycle. Single phage with novel characteristic features including narrow host specificity, rapid phage replication, and quick host eradication has been addressed as an ideal candidate for the development of phage therapy. On the other hand, lysogenic phages have similar replication pattern like the lytic phages. In addition, it has the potential to integrate their nucleic acid with the host's chromosomes. At certain time, those lysogenized cells were synthesizing and releasing new phage progenies. Among the type of phages, lysogenic type may not be involved in therapeutic approaches due to its ability to enhance bacterial virulence via the horizontal gene transfer from one bacterial cell to another (Richards 2014). Moreover, phage-encoded enzymes named endolysins are playing a vital role in case of lytic phages against Gram-negative and Gram-positive bacterial cells. Therefore, phage-encoded lytic proteins are considered as an efficient bactericidal agent specially against multidrug-resistant bacterial strains (Lin et al. 2017). Hence, phage-based approaches have been employed for agriculture, aquaculture, plants, food, human and veterinary medicine (Abdelrahman et al. 2021; Richards 2014).

This chapter predominantly focuses on various bacterial fish diseases in aquaculture, its global impact, conventional and advanced approaches to prevent or treat multidrug-resistant bacterial fish pathogens. In recent years, bacteriophage or phage component-based approaches have overcome the limitations of detection and eradication of fish pathogen. Herein, we significantly highlight the application of advanced phage and biotechnological approaches, specifically phage-encoded endolysin, and anti-phage defense system, for effective modalities to detect and fight against fish bacterial pathogens.

17.2 Fish-Borne Bacterial Diseases and Their Impact on Fish Health

The most common fish-borne bacterial diseases are like bacterial cold-water disease, bacterial gill disease, bacterial kidney disease, cotton mouth disease, dropsy, edwardsiellosis, fin rot disease, fish tuberculosis, furunculosis, piscine mycobacteriosis, piscine tuberculosis, Pseudomonasis, swim bladder disease, and vibriosis.

17.2.1 Bacterial Cold-Water Disease

Bacterial cold-water disease (BCWD) elicits considerable fish-borne diseases and is responsible for most of fish lethalness. *Flavobacterium psychrophilum* (*F. psychrophilum*) is a Gram-negative bacterium that causes an acute septicemic infection, bacterial cold-water disease (BCWD), and rainbow trout fry syndrome in *Salmonidae* family fishes such as chars, salmon, trout, freshwater whitefishes, and graylings. Common BCWD occurs in *Salmonidae* family fishes such as coho salmon (*Oncorhynchus kisutch*), lake trout (*Salmo namaycush*), rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), walleye (*Stizostedion vitreum*), and several species of whitefish, carp, dace, and suckers. The outbreaks of BCWD have been reported in the ranges between 4 and 10 °C and also proved that its severity has been reduced with increasing temperature (<15 °C) (Loch and Faisal 2015). As a consequence, some of the confines have been spotted in case of bacterial hosts and geographic area; nevertheless, no limitations in the sizes of fish, particularly fingerling-sized fish, are more susceptible to BCWD. BCWD can fetch a lot of clinical signs like external lesions on the fish dorsal region, erosion, and loss of the caudal fin and other fins. Most common BCWD has been characterized by blackened tails, erratic swimming behavior, and spinal deformities in fish. Rainbow trout fry syndrome (RTFS) is considered as another BCWD manifestation that arises few weeks after fish begin to feed, responsible for 50–60% mortality. Exophthalmia, loss of appetite, and dark skin pigmentation are signs of RTFS disease. An open lesion on the caudal peduncle has been considered as a significant clinical sign of diseased fishes. Lesions from the caudal region may spread into deep muscle tissues and also causes erosions in fins and deformed spinal regions including in the anterior, mid, and posterior regions. In addition, the septicemic infections have been stated by the spot of huge numbers of bacterial colonies in the heavily infected fish organs in particular air bladder, heart, intestine, liver, peritoneum, pancreas, and spleen, indicating of this disease.

17.2.2 Bacterial Gill Disease

Bacterial gill disease (BGD) has been considered as a common deadly disease that affects several fish species globally, particularly, it is responsible for serious problem in salmonid and fingerlings fishes. This disease can occur due to colonization of gill surfaces by *F. branchiophilum*, and has been shown to induce BGD under suitable conditions. Infected fish have a lot of clinical symptoms such as gasping at the surface of the water, low fright response, and no rivet in feed. Gills of fish exhibits excess mucus, detritus, and lamellar fusion as its clinical signs during microscopic examinations. Finally, dead fish have been spotted with flared opercula and lactic acid accumulation in the head muscles. More than 50% of mortality has been reached within 48 hours of infection.

17.2.3 Bacterial Kidney Disease

Bacterial kidney disease (BKD) is a chronic bacterial infection of salmonids fish that lives in freshwater and saltwater. *Renibacterium salmoninarum* (*R. salmoninarum*), Gram-positive and non-motile bacterium, causes inadequate growth rate and high mortality in chronically infected fish without age limit in fishes. This bacterium exists in most of the fish reproductive fluids and also intracellularly active within fish body cells. Other than that, this kind of bacterium has the potential like transfer between fishes and within the eggs. BKD was initially descripted in adult Atlantic salmon fish from Scottish rivers between 1930s and 1960s. Fish can transfer the bacterium responsible for BKD from fresh water to the seawater with latent infection; therefore this bacterium remains in both salt water and fresh water (Bruno 2004). Exophthalmia, fatigue, petechial hemorrhage, bloody ascites, and enlarged kidney and spleen have been recorded as clinical signs of BKD. Moreover, it shows lesions in the internal organ including kidney, and encapsulated membrane of heart, kidney, and spleen.

17.2.4 Columnaris Disease

Columnaris diseases (CD) have been caused by the most common, Gram-negative, rod-shaped *Flavobacterium columnare* (*F. columnare*). Other flavobacterium species such as *F. aquaticum, F. granuli, F. hercynium, and F. terrae* are responsible for most of the fish diseases (Verma and Rathore 2015). It arises erosion and necrotic lesions symptoms in catfish (*Clarias batrachus*), catla (*Catla catla*), carp (*Cyprinus carpio*), rohu (*Labeo rohita*), and other freshwater fishes. This disease frequently causes serious economic losses in the catfish industry globally (Declercq et al. 2013).

17.2.5 Dropsy

Pseudomonas punctata (*P. punctata*) is a Gram-negative bacterium that causes dropsy disease on the surface of fish scales. Infected fishes hold a lot of symptoms like difficulties in breathing and swimming due to swollen stomach. It can be simply identified in fishes those marked physical characteristics. Poor water quality, mass of water or body fluid in the body cavity can provoke dropsy symptoms in fish. Initially, massive mucus has been noticed by naked eye on the red-pale gills of Koi fish (*Cyprinus rubrofuscus*). With microscopic investigation, erosion and telangiectasia of primary and secondary lamella blood vessels, site for oxygen exchange, has been observed, respectively. Lamella damage may cause lack of oxygen in fish blood, a circulation problem, which leads to lethal effect on fish.

17.2.6 Edwardsiellosis

Edwardsiellosis, also known as popeye disease, is one of the significant bacterial diseases in tropical fish caused by *Edwardsiella* species. For instance, septicemic disease has been described with extensive lesions in the internal organs, and skins of commercially available fish verities including carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), chinook salmon (*Oncorhynchus tshawytscha*), eel (*Anguilliformes*), flounder (*Paralichthys dentatus*), mullet (*Mugilidae*), and tilapia (*Oreochromis niloticus*) (Park et al. 2012). Similarly, Edwardsiella disease-causing agents also entered the internal organ of fish through the gills, open wounds, mucous membranes, or skin. This kind of disease can be transferred from infected fishes generally by direct contact and also by waste matters like feces, mucus, reproductive fluids, and urine of diseased fish. Moreover, humans, animals, rodents, and birds can transfer fish diseases among different part of the locations by carrying diseased fish, contaminated fish feed, and contaminated water. Infected fish with *Edwardsiella* spp. exhibits abnormal swimming behavior like corkscrew swimming motion, spiral motion, and hang near the water surface.

17.2.7 Fin Rot Disease

Fin rot is one of the major common fish diseases in aquaculture and aquarium places. Winter flounder (*Pseudopleuronectes americanus*) fishes, also named as the black back, are more susceptible to fin rot disease. This kind of diseases are caused by various pathogenic bacteria such as Aeromonas, Pseudomonas, or Vibrio species. Specifically, *Pseudomonas fluorescens* (*P. fluorescens*) drives a ragged rotting of the fin. Fin tail rot disease occur by poor diet, poor water conditions, and stress. For instance, fishes like while flounder have been displaying fin rot and erosion symptoms when fishes are collected from the contaminated areas by industrial waste and chlorinated hydrocarbon residues, dichlorodiphenyltrichloroethane (DDT). It begins at the edge of the fins and continues to destroy its tissue (Ziskowski et al. 2008).

17.2.8 Fish Tuberculosis

Tropical fishes are growing in the consummate conditions like 25 – 35 °C which is more susceptible to fish tuberculosis diseases caused by mycobacterium species. Moreover, those mycobacteriosis may not present in cold zones fishes. Most of the mycobacterium species including *M. celatum*, *M. chelonae*, *M. diernhoferi*, *M. flavescens*, *M. fortuitum*, *M. gordonae*, *M. intracellulare*, *M. kansasii*, *M. marinum*, *M. terrae*, and *M. triviale* cause fish tuberculosis (Beran et al. 2006). Fish tuberculosis has not displayed any clinical signs for years even after post-infection similar like human tuberculosis. Unusual growth can be observed on the internal organs like spleen and liver in the early stage of disease and external signs such as lesions and rare fatigues are witnessed in the later stage of disease (Ramakrishnan and Falkow 1994). For instance, *M. marinum* holds liable for chronic diseases with the sign of granulomas and lesions in various internal organs in freshwater fish, specifically Zebra fish. The infected fishes may cause 44% and 83% mortality at low concentration (5 CFU) and high concentration (60 CFU) within 16 weeks. The declining fish were frequently marked with kidney failure and osmoregulatory stress (Swaim et al. 2006).

17.2.9 Furunculosis

Furunculosis causes acute or chronic hemorrhagic septicemic infection in salmonid fish by *Aeromonas salmonicida* (*A. salmonicida*), Gram-negative, rod-shaped bacterium and culpable for most of the economic losses in aquatic environment worldwide. These pathogens induce ulcerative lesions, hemorrhagic sepsis, bleeding disorder, boils, ulcers, liquefaction, and gastroenteritis that lead to death (Dallaire-Dufresne et al. 2014). Acute form of infection has been reported with a lot of clinical symptoms including melanosis (blackening of the fish), lethargy, and hemorrhages on the fins. Chronic form of infection has been noticed with lethargy, protrude eyes, and bloody discharge from the vents. Other *Aeromonas* species like *A. salmonicida* and *A. sobria* infections were observed in most of the infected salmonid fish (*Salmonidae*), goldfish (*Carassius auratus*), and koi fish (*Cyprinus rubrofuscus*) (Lim and Hong 2020; Richards 2014).

17.2.10 Hemorrhagic Septicemia

Aeromonas species is a Gram-negative, rod shape, motile pathogen that causes hemorrhagic septicemia, aeromonad septicemia, red spot disease, red sore disease, and tail and fin rot disease in fish. In aquaculture, the maximum outbreaks have raised by the various *Aeromonas* bacterium such as *Aeromonas hydrophila* (*A. hydrophila*), *A. caviae*, *A. sobria,* and *A. veronii* in case of septicemia disease. These diseases have comprised a lot of clinical symptoms such as erosion on the fins, lesions on surface, which leads to the loss of scales. Moreover, it is liable for abdominal expansion, ruin to internal organs, compilation of ascites fluid, and anemia. The virulence of bacteria was determined by virulence factors and the efficiency of biofilm formation (Das et al. 2020; Richards 2014).

17.2.11 Pseudomonas Infections

Pseudomonas is Gram-negative motile, aerobic, and non-spore-forming rod-shaped bacterium, classified among the most dangerous fish pathogens all over the world. For instance, *Pseudomonas plecoglossicida* and *Pseudomonas aeruginosa* have been considered as opportunistic pathogens, those causing serious illness including abdominal distension, friable liver, gill necrosis, hemorrhagic septicemia, and kidney congestion in various fishes such as rainbow trout (*Oncorhynchus mykiss*) (Sun et al. 2020), orange-spotted grouper (*Epinephelus coioides*) (Sun et al. 2018), Larimichthys crocea (*Pseudosciaena crocea*) (Huang et al. 2019), and ayu (*Plecoglossus altivelis*) (Nishimori et al. 2000). As a model, *P. plecoglossicida* may cause infection with abnormal tissue growth in the internal organs like liver, kidney, and spleen of diseased fish that leads to peak mortality, and severe economic losses particularly in China (Tao et al. 2016). Most of the bacterial infection has been recorded between 15 and 20 °C in the seawater. Other reports revealed that the conditions may prompt virulence, for example, the expression of a cold-shock protein in *P. plecoglossicida* has been significantly increased at 18 °C (Huang et al. 2018).

17.2.12 Vibriosis

Vibriosis is an emergent ubiquitous fish diseases caused by genus *Vibrio* in aquatic system. Vibrio group of bacteria are Gram-negative bacteria, pathogenic microbes to bivalves, crustaceans, and fishes (Pereira et al. 2011). In particular, this disease influences both warm and cold-water fish species, comprising carp, catfish, eel, flounder, salmon, and all other sea bass. Several *Vibrio* species such as *V. anguillarum, V. alginolyticus, V. carchariae, V. damsela, V. ordalii, V. parahaemolyticus, V. salmonicida,* and *V. vulnificus* have been engaged with most of the fish infections worldwide (Pereira et al. 2011).

17.2.13 Fish-Borne Zoonotic Diseases

Emerging zoonotic disease is a raising general health concern and requires close monitoring to conclude the way of disease diffusion between humans and animals. Fish-borne zoonotic diseases are happening due to first and foremost bacterial infections including *Aeromonas, Campylobacter, Edwardsiella, Erysipelothrix, Escherichia, Klebsiella, Mycobacterium, Salmonella, Streptococcus*, and *Vibrio* species. Fish-borne zoonotic diseases are described as diseases transferred from fishes to humans as a result of a contamination directly or indirectly by fish, fish tank water, or fish meat (Chlebicz and Śliżewska 2018). In some cases, these infections could not make any sick in fishes but may show a lot of serious sickness in humans. For instance, humans have been reported with crucial medical issues such as a chronic illness and immunodeficiency. In another way, pathogens can transfer one fish to another via aerosols, contaminated surfaces, fish feed, and the water (Richards 2014).

Piscine mycobacteriosis is a good example for zoonotic fish disease with deadly effect around the globe in both marine and freshwater fish. Over 20 different species of mycobacterium pathogens were identified that can persist so far in fish environment. Notably, *Mycobacterium marinum* (*M. marinum*) is a Gram-positive, non-motile, and slow-growing mycobacteria that elicits illness in fishes that had similar indication of tuberculosis. These most significant fish pathogens are associated with various signs such as abdominal bulge, loss of weight and color, exophthalmia, skin ulcer, scoliosis, and swim in disorganized way. Infected fishes were indicted with the conformation of granuloma in kidney, liver, and spleen (Hashish et al. 2018). This bacterium can cause infection in humans, when wounded skin of human may have a chance to expose with contaminated aqueous ecosystem (Parent et al. 1995).

Another important *Mycobacterium* species like *Mycobacterium ulcerans* (*M. ulcerans*) is a toxin-producing bacterium and causes necrosis and Buruli ulcers in human skin. More than that it is responsible for most of the infectious disease in major living organisms such as fish (Stragier et al. 2008), reptiles (Sakaguchi et al. 2011), and mammals (Fyfe et al. 2010) around the globe. Several factors like endurance of the bacteria, impractical screening, and asepsis techniques of pathogens in an aquatic environment may influence the clearance or eradication of fish pathogens. Experiments showed that the efficacy of antibiotics depends on the species of mycobacterium. For example, standard antibiotics such as erythromycin, rifampicin, and streptomycin could not eradicate mycobacterium completely in yellow tails (*Seriola quinqueradiata*) fishes (Keller et al. 2018).

Other freshwater and saltwater fish pathogens like Aeromonas and Vibrio may cause wound infections and gastroenteritis in human when human make contact with contaminated fish. Septicemia and some other life-threatening disease may also be possible in case of immunosuppressed human (Janda and Abbott 2010).

17.3 Antimicrobial Agents

Antibiotics, also noted as antimicrobial agents, are one of the best preventive and therapeutic approaches to combat the bacterial diseases in aquaculture farms. Over time, the efficiency of potent antibiotics may become limited due to antibiotic resistance. Nowadays the unsystematic use of antibiotics in aqua-farming sectors has led to the prevalence of antibiotic resistance in aquatic environment. Even though aquaculture is one of the major food-producing sectors, it frequently bears huge economic losses in case of multidrug-resistant bacterial infection in various fish species. Antibiotics have been eradicating the infectious pathogens in fish farm, aquaculture, etc. Therefore, several organizations have developed a lot of new antibiotics with overwhelmed efforts and invest a lot of funds for clinical trial of antibiotics to control the rapid emergence of antibiotic resistance.

Several organization like the Innovative Medicines Initiative (IMI), the Combating Antibiotic-Resistant Bacteria Biopharmaceutical Accelerator (CARB-X) and the Global Antibiotic Research and Development Partnership (GARDP) have been involved and supported by European Federation of Pharmaceutical Industries and Associations (EFPIA), the US Department of Health and Human Services (HHS) and Drugs for Neglected Diseases initiative (DNDi), respectively (Högberg et al. 2010; Tagliabue and Rappuoli 2018). In the past 20 years, only two antibiotics such as cyclic lipopeptides (CLPs) and oxazolidinone have been successfully produced as novel classes of antibiotics that focus on type IIA topoisomerases with a unique functional mechanism against methicillin-resistant *Staphylococcus aureus* (MRSA). Although, the development of new antibiotics with broad spectrum properties may be difficult due to the basic rationales like its risk and cost throughout the process (Coates et al. 2011). However, the effective treatment to control bacterial disease using various antibiotics in particular chloramphenicol, erythromycin, florfenicol, kanamycin, macrolides, nitrofurans, sulfonamides, quinolones, and fluoroquinolones has been turned into problematic due to its temporary suppression of bacterial growth. Most of the fish farms have been using chloramphenicol, oxytetracycline and erythromycin to eradicate the fish pathogenic bacteria.

17.3.1 Chloramphenicol

Since 1947, Chloramphenicol (2,2-dichlor-N- [(aR,bR)-b-hydroxy-a-hydroxymethyl- 4-nitrophenethyl] acetamide) has been employed in aquaculture as a disinfectant or prophylactic to block bacterial diseases with broad spectrum activity against both Gram-negative and Gram-positive bacterial infections in fish. While it used as chemotherapeutic agents, it could adversely penetrate fish body and affect the health of the fish. For example, chloramphenicol showed a considerable negative impact on the volume of erythrocyte, hematocrit and mean corpuscular volume (MCV) in the koi carp blood, when exposed with 10 mg L^{-1} for short time (10 days). This leads to immature erythrocytes, followed by anemic issues in fish. The functional group of chloramphenicol (CONH) has been assumed for suppress bone marrow during the process of blood cells and platelets production in humans and animals. Additionally, it causes severe toxic effects like hypoplastic anemia, aplastic anemia, granulocytopenia, and thrombocytopenia. Hence, these antibiotics are completely prohibited from the usage in food animals worldwide due to its life-threatening effects (Chanda et al. 2011).

17.3.2 Florfenicol

Florfenicol (FF) (2,2-dichloro-N-[1R,2S-fluoro-1-hydroxy-1-(4-methylsulfonylphenyl) propan-2-yl] acetamide) is an analog of thiamphenicol and chloramphenicol. According to previous report, the activity of FF had better host spectrum compared with chloramphenicol. For instance, FF had vigorous killing activity against *Escherichia coli*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*, those strains are resistant to chloramphenicol (Sin et al. 2015).

FF acts as antibacterial agent that inhibits peptidyl transferase, responsible for protein synthesis. With unique functional mechanism, FF is employed as therapeutic agents in aquaculture sectors to prevent bacterial infections and protect different types of fishes like cod, channel catfish, salmonid, tilapia, and trout (Sin et al. 2015). Hence, FF is one of the leading aquatic drugs mostly used in over 25 countries worldwide. For sample, Aquaflor is a commercially available feed premix for the treat of diverse pathogens in both marine and fresh water (Hayes 2013).

17.3.3 Nitrofurans

Nitrofurans (NFs) drugs are a class of antimicrobial/antibiotic drugs which have been used for many years administered in humans, especially in the treatment of urinary tract infection (UTI) (Huttner et al. 2015) and veterinary medicine, primarily for bacterial infections treatment. Nitrofurans, mainly furazolidone (FZD), furaltadone (FTD), nitrofurantoin (NFT), and nitrofurazone (NFZ), all contain a typical 5-nitrofuran ring (Chu et al. 2008). In aquaculture, these drugs were commonly administered as feed additives as prophylactic agents and for the promotion of growth in bacterial and protozoan infections treatment like gastrointestinal enteritis caused by *Escherichia coli* and *Salmonella* spp. (Barbosa et al. 2007).

Due to mutagenicity and carcinogenicity concerns, many countries have banned the use of NFs in food-producing animals. However, NFs continue to be used significantly for its prophylactic and growth regulating properties, as non-complaint residues are still present in animal food products. NF and its tissue metabolites were found in the muscle of channel catfish after oral administration (Chu et al. 2008). Similar studies have been reported for shrimp (Chu and Lopez 2005) and carp (Wang et al. 2020).

17.3.4 Kanamycin

Kanamycin is an antibiotic in the aminoglycosides that contains part of the molecule of an amino-modified glycoside. Kanamycin has shown to be an effective therapeutic agent in the control of bacterial disease caused by organisms such as *Aeromonas* *liquefaciens, Aeromonas hydrophila, Aeromonas punctata, Pseudomonas putida,* and *Pseudomonas fluorescens*, studied in freshwater fishes.

In aquaculture, kanamycin is thought to be effective against gastrointestinal bacterial infections when used as channel catfish feed. Kanamycin can interfere in the protein synthesis process by binding with the bacterial ribosomes (30S subunit) that leads to incorrect alignment with the mRNA, thus causing dislocate of amino acid (Martí et al. 2018). The antibiotic has shown good pathogenicity of these organisms studied in vivo and in vitro making kanamycin a good control and treatment of bacterial fish diseases such as dropsy, popeye, septicemia, and fin and tail rot.

17.3.5 Macrolides Antibiotics

Macrolides are the common ribosome-targeting antibiotics that are successfully applied to clinical purposes for the past 70 years. First- and second-generation macrolides such as erythromycin A, and clarithromycin, roxithromycin, and azithromycin have been used since the 1950s and 1980s, respectively. Functional mechanism of these antibiotics has been determined by a macrolactone ring with various ranges like 12–16 atoms for instance, erythromycin or clarithromycin (14-atoms) and azithromycin (15-atoms) (Vázquez-Laslop and Mankin 2018). For instance, erythromycin is an antibiotic used in the treatment of bacterial infections in human medicine, most effective against Gram-positive bacteria, such as *Streptococcus* (Liu et al. 2014). Erythromycin belongs to the macrolide family of antibiotics, which consist of a large macrocyclic lactone ring to which one or more deoxy sugars are bound. Erythromycin exhibits bacteriostatic activity, by means of inhibiting bacterial growth by binding to subunit 50s of the rRNA bacterial complex (Esposito et al. 2007).

In aquaculture, erythromycin has been used against streptococcal infection, which is the primary concern of Trout farming (*Oncorhynchus mykiss*) (Serdoz et al. 2011). Pharma-kinetics studies in rainbow trout show histological changes (Rodrigues et al. 2019) and depletion of erythromycin muscle plus skin in natural proportion after oral administration of the drug. Erythromycin is not very effective in a bath treatment, and it should only be administered by injection or in feed (Esposito et al. 2007). A new generation of macrolides is known as ketolides, for example, solithromycin, that significantly enhanced bactericidal activity against few resistant strains. Toxicity and secondary effect of macrolides have limited usage in the clinical sectors even though it has high antibacterial potency (Vázquez-Laslop and Mankin 2018).

17.3.6 Sulfonamides

Sulfonamides (sulfa drugs) are synthetic bacteriostatic drugs prepared by reaction of a sulfonyl chloride with ammonia or an amine in the presence of caustic alkali (Mujumdar and Poulsen 2015). The functional mechanism of the drugs showed killing bacteria by interference from cellular metabolism (Kadlec et al. 2011). A number of other sulfonamides are synthesized during the clinical assessment of sulfanilamide derivatives, including sulfadiazine, sulfamethizole, sulfamethoxazole, sulfasalazine, sulfisoxazole, and various other derivatives (Mujumdar and Poulsen 2015).

In aquatic environment, sulfonamides are widely used because of their cost-effectiveness, stability, bacteriostatic activity, and high mobility in the environment (Zou et al. 2011). Sulfonamides are highly effective against fish diseases caused by organisms like *Aeromonas hydrophila, Aeromonas sobria, Pseudomonas fluorescens,* and *Edwardsiella tarda*. The sulfonamides and the residual concentration of their derivatives were studied in different freshwater fishes such as shrimp, crab, bream, and perch, as well as other species, to confirm the presence or concentration of sulfonamides in the environment.

17.3.7 Quinolones and Fluoroquinolones

Quinolones including oxolinic acid, nalidixic acid, piromidic acid, enrofloxacin, and ciprofloxacin are bactericidal broad-spectra antibacterial agents that act especially against Gram-negative bacteria with a chemical nature of a bicyclic ring. Fluorine atom replacements at the C-6 position on the quinolone base structure produced various fluoroquinolones, namely norfloxacin, ciprofloxacin, levofloxacin, and moxifloxacin. Bacterial gyrase and topoisomerase IV enzymes are the target molecules for quinolones and fluoroquinolones. Fluoroquinolones are strong antimicrobial agents because of their wide spectrum and excellent tissue penetration (Kocsis et al. 2016).

Quinolones enter the bacterial cell by passive diffusion through porins located in the outer membrane and inhibit bacterial growth intracellularly by interfering with DNA gyrase (Wolfson and Hooper 1989). They are effective against all common bacterial infections like furunculosis (*Aeromonas salmonicida*), atypical furunculosis (*A. salmonicida subsp. salmonicida*), classical vibriosis (*Listonella (Vibrio) anguillarum*), cold-water vibriosis (*Vibrio salmonicida*), and yersiniosis (*Yersinia ruckeri*).

Pharma kinetics studies of freshwater species such as Channel catfish (*Ictalurus punctatus*), Eel (*Anguilla anguilla*), Rainbow trout (*Oncorhynchus mykiss*), and Brown trout (*Salmo trutta*) and sea water species such as Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*), Goldsinny wrasse (*Ctenolabrus rupestris*), and Turbot (*S. maximus*) have been extensively reviewed in Serdoz et al. (2011), showing the absorption and elimination of different quinolones and fluoroquinolones. Unfortunately, quinolone resistance has become a problem in areas in which use of the drugs in aquaculture is widespread.

17.3.8 Implications of Rising Antibiotic Resistance

In general, antibiotics have the potential to prevent the increment of pathogenic bacteria in fish, plants, animals, and humans. Naturally, antimicrobial agents have been snooped in the major molecular synthetic process of bacteria such as deoxyribonucleic acid (DNA) ribonucleic acid (RNA), cell-wall, and protein synthesis (Bengtsson-Palme et al. 2018). Most of the bacteria developed resistance in fast way due to its novel phenomenon comprising change bacterial cell membrane permeability, enhance mutation rate, and activate efflux pump system in the course of non-stop expose of antibiotics. In general, bacterium develops resistance under various molecular systems in particular by reshaping host target site and destruction of the antibiotics. Moreover, low antibiotic influx and high antibiotic efflux system of bacteria has been determined by the limited membrane permeability and the major role of efflux transporters against various antibiotics (Laws et al. 2019; Nikaido 2009). Even, antibiotics can make miserable effect while applied in low volume in the domain of clinical therapy of fishes, animals, and humans (Monserrat-Martinez et al. 2019). Even the escalation of fish production has been usually reached when treated with antibiotics; however, prolonged antibiotic approach has led to the formation and prevalence of antibiotic resistance among all the living organisms worldwide (Fig. 17.1). There are numerous pathogens; for example, *Aeromonas*, *Edwardsiella*, *Pseudomonas*, *Staphylococcus*, and *Vibrio* species are considered as major pathogens for both humans and aquatic species which enhance the effect of antimicrobial resistance through horizontal gene transfer. In some scenarios, antibiotics are routinely used as growth-promoting agents in fish and all other food animals that can enrich antibiotic resistance harboring pathogens in humans and animals (Swain et al. 2014).

Resistance feature may induce stress and diminish immune system in humans and animals (Kimera et al. 2020). As the result, extreme mass of antibiotics (~80%) consuming countries such as the United States, Canada, and the European countries have completely banned the usage of antibiotics in the animal feed (Cantas et al. 2013; Casewell et al. 2003). Previous documented report implies that the consumption of antibiotics is huge in food industries compared with hospitals (Chee-Sanford et al. 2009). Moreover, antimicrobial agents could not recognize beneficial, pathogenic, and antibiotic-resistant bacteria that eradicate all the bacteria indiscriminately and cause adverse health issue when antibiotics are used as a therapeutic agent. Therefore, unique and unconventional approach must be focused to tackle antibiotic resistance issues.

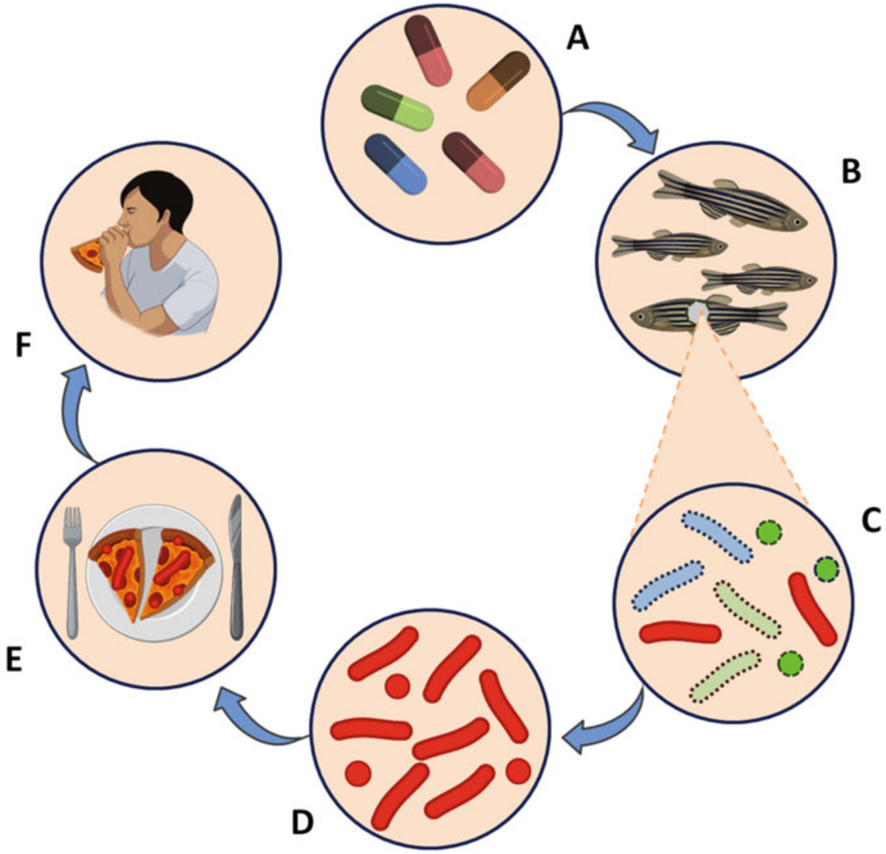

Fig. 17.1 showed the possibilities of transfer of resistance from fishes to humans. (**a**) Antibiotics for fish diseases; (**b**) fish treated with antibiotics; (**c**) antibiotics kill most of the sensitive bacteria; (**d**) antibiotic-resistant bacteria only can survive; (**e**) contaminated food; and (**f**) resistant bacteria can spread through food to humans.

17.4 Nanoparticles

Nanoparticles (NPs) exhibited high antimicrobial activity against most of the microbes such as bacteria, fungi, and viruses. With novel chemical, physical, and biological properties, nano-based approaches have been aimed as a promising choice in aquaculture, particularly as fish therapeutic agent (Das et al. 2020). Moreover, it can be utilized in drug discovery, implement nano-medicine approach and molecular diagnostics. Over the various nanoparticles, the metal group of nano-materials is believed as nano-medicine due to its wide spectrum of antibacterial properties as a consequence of the bacterial membrane interruption and formation of reactive oxygen species (ROS) that leads to ruin bacterial cells (Huo et al. 2017; Lemire et al. 2013).

17.4.1 Silver Nanoparticles

Silver nanoparticles (Ag-NPs) are considered as the most leading antibacterial agent. Ag-NPs can counteract bacteria by way of several mechanisms such as bandaging with deoxyribonucleic acid (DNA) of microbes, damaging bacterial membrane, inhibiting bacterial cell division, and the distraction with protein synthesis that can circumvent the bacterial resistance (Durán et al. 2016). In general, Ag-NPs can interact with sulfur accommodating proteins that may block cellular functions that leads to the bacterial cell death (Chen et al. 2011). Strong antibacterial activity has enumerated against Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus,* and *Pseudomonas fluorescens*, *Edwardsiella tarda, Proteus* spp., *Flavobacterium* spp., respectively. Previous report suggested that Ag-NPs have been employed to improve the quality of fish feed and water in aquaculture (Das et al. 2020).

17.4.2 Gold Nanoparticles

In biological studies, Gold nanoparticles (Au-NPs) have been successfully utilized due to its unique characteristics such as tolerable morphology with functionalized chemical surface and high water solubility (Daniel and Astruc 2004). Au-NPs have been considered for their antibacterial effects due to its three major pathways, as follows, (1) Au-NPs can modify bacterial membrane potential through interference with oxidative phosphorylation system, leads to decrease in ATP metabolism and synthesis, (2) inhibit ribosome subunits and transfer RNA binding, and (3) by means of garnishing chemotaxis process (Cui et al. 2012). In 2011, Saleh and his team used antibody-coated Au-NPs for the fish pathogen detection (*A. salmonicida*) from fish tissues. This approach has been considered as the more specific, quick, and cost-effective immunoassay (Saleh et al. 2011). In the last two decades, a lot of nanomaterial-based approaches have been implemented for both disease diagnosis and therapeutic purposes (Saleh et al. 2011). Moreover, Au-NPs have been recommended as a bactericidal agent against various fish pathogens like *Escherichia coli*, *Salmonella typhi,* and *A. salmonicida* (Lima et al. 2013; Saleh et al. 2011).

17.4.3 Zinc Oxide Nanoparticles

Zinc oxide nanoparticles (ZnO-NPs) can cause leakage of intracellular components due to the damage of bacterial cell membrane. ZnO-NPs have been found with superior bactericidal activity compared with mass ZnO against Gram-negative strains. Unlike Ag-NPs, the ZnO-NPs are responsible for most of the abnormal actions like reactive oxygen species (ROS), cell membrane disorder, formation of hydrogen peroxide, and cell damage when microbes are treated with ZnO-NPs (Sirelkhatim et al. 2015). Interestingly, ZnO-NPs can be biosynthesized with the help of *Aermonas hydrophila* strains, and active against *Aermonas hydrophila, Aspergillus flavus, Candida Albicans, Escherichia coli, Enterococcus faecalis,* and *Pseudomonas aeruginosa* (Jayaseelan et al. 2012). Das and his experiment proved that ZnO-NPs have extreme disease control aptitude caused by *Aeromonas veronii* infection and obtained least damage of ornamental fish *Xiphophorus hellerii*. Results revealed that ZnO-NPs are the most promising nano-materials for the aquaculture applications (Das et al. 2020).

17.4.4 Titanium Dioxide Nanoparticles

Titanium dioxide nanoparticles (TiO_2-NPs) have a solid bactericidal impact in fish and aquaculture environment. Hence, the usage of TiO_2-NPs has been suggested to control or reduce fish mortality rate by bacterial infections (Jovanović et al. 2015). Bactericidal effects of TiO2-NPs have been successfully examined in in vitro studies but not in case of animal experiment, due to the impact of immune response and intracellular factors of infected fish (Segal 2005). Later, it was found that TiO2-NPs may alter the function of immune system, particularly cell-medicated immune response including activation of phagocytes, T-lymphocytes, and cytokine production in aquatic animals (Jovanović and Palić 2012).

17.5 Antimicrobial Peptides

Antimicrobial peptides (AMPs) are deemed as one of the leading components of innate immunity and are distributed among microbes, plants, animals, and humans. Hence, it can provide first-line defense to the organisms (Lai and Gallo 2009). AMPs have broad spectrum of activities against broad range of bacterial pathogens, viruses, and fungi (Shabir et al. 2018). Special characteristic features of AMPs like produce in simple way and ordinary molecular structure made them more comfortable for usage. AMPs can possibly diminish the development of bacteria resistance via target bacterial cell membrane by the way of inhibiting cell wall synthesis process (Mehrnejad et al. 2008). Due to their advanced properties like rapid antimicrobial action and wide antibacterial spectrum, AMPs have been considered as most emerging agents for the development of novel antibiotics with desired properties (Aoki and Ueda 2013). Therefore, AMPs are considered as very attractive therapeutic tools for the treatment of emerging fish diseases and also act as a disinfectant to reduce the bacterial biofilms. Numerous AMPs such as cathelicidins, defensins, hepcidins, and piscidins with board antimicrobial spectrum have been effectively examined in fishes (Masso-Silva and Diamond 2014). Engineered peptides with desired efficacy and stability are further expanded to antimicrobial peptides, and also applied in therapeutic purpose in humans (Carmona-Ribeiro and de Melo Carrasco 2014).

17.6 Vaccines

Vaccines are playing a major role in case of stimulating an immune reaction that leads to raising the diseases resistance in the host. This immune system with high sensitization of pathogen has the potential to respond to pathogens and encounter shortly (Engelstad 2005). Bacterial fish vaccines are commercially available against a lot of crucial bacterial diseases causing health issues in fish farming and aquaculture industries. Although few remarkable defects like health issues and antibiotic resistance are diagnosed in antibiotic therapy, still chemotherapy is implemented for treating bacterial diseases due to lack of alternate therapies (Ma et al. 2019). To overcome these issues, vaccination has become an effective approach to inhibiting a wide spectrum of bacterial diseases in the domain of aquaculture.

Since 1940s, a lot of vaccines have been developed to protect or recover fishes from bacterial diseases. Due to the advantages of vaccines such as eco-friendly and cost-effective, antibiotics has been diminished in the therapeutic purposes. In the year of 1970s, the first bacterial vaccines have been launched in the USA, which can tackle emerging bacterial diseases like vibriosis and redmouth disease (Evelyn 1997). In the event of vaccine development particularly for fish treatment, research has focused on vaccination schemes, vaccine production, and the enhancement of protective efficiency of vaccines. There are various types of vaccines including inactivated whole-cell vaccine (Huang et al. 2014), live attenuated vaccine (Li et al. 2015), DNA vaccine (Rauta et al. 2017), and subunits vaccine (Zhang et al. 2008); these have been successfully developed as suitable vaccine candidates against several fish bacterial pathogens such as *Streptococcus iniae*, *Edwardsiella tarda*, *Aeromonas hydrophila*, and *Vibrio harveyi*, respectively, for saving or reducing mortality rate of fishes. To date, a number of inactivated whole-cell vaccines are commercially available and used in the fish farming and aquaculture industries, and other vaccines are used in live animal model clinical studies.

17.6.1 Inactivated Whole Bacterial Cell Vaccines

Inactivated whole bacterial cell (IWBC) vaccines, produced in inexpensive way, are highly recommended to control most of the bacterial fish diseases specifically in the aquaculture sectors (Mohd-Aris et al. 2019). IWBC vaccines are generated by using chemical and physical mutagenesis. Previous work reported that the combination of IWBC vaccines with adjuvants showed maximum efficiency compared with non-adjuvanted vaccines. For instance, the mixture of a formalin-killed *Streptococcus agalactiae* vaccine with 20% Freund's adjuvant has been used to recover 100%

of red tilapia fishes, but only 50% of fishes has been recovered in case of non-adjuvanted vaccines (Mohd-Aris et al. 2019). Although IWBC are harmless due to inactivation of bacterial cell, it is less immunogenic and required adjuvants for long-term immunity (Maiti et al. 2020). IWBC could provoke only humoral immune responses not cell-mediated immune (CMI) responses that may be considered as a crucial drawback of this vaccine, because CMI is required to eliminate intracellular fish bacteria such as *Edwardsiella* spp., *Piscirickettsia* sp., and *Aeromonas hydrophila* (*A. hydrophila*) (Munang'andu 2018).

17.6.2 Live Attenuated Bacteria Vaccine

Live attenuated (LA) vaccines are getting more attention due to simple vaccine development, low production cost, and the virulence factors exhibited on the surface. In addition, LA vaccines can generally induce humoral and cell-mediated immune (CMI) response that leads to adaptive immune protection in fish (Mohd-Aris et al. 2019; Wang et al. 2014). Moreover, LA vaccines can be used in very low volume, for example, few doses, and no need to add adjuvants for getting better outcome. On the other hand, the advanced genetic studies and techniques such as allelic exchange and transposon recombination have been interfered to design longer stable and decisive LA vaccines (Mohd-Aris et al. 2019). Moreover, it has the potential to enhance CMI and humoral immune responses which are essential to eradicate both intra- and extra-cellular bacteria. For instance, LA vaccine has been suggested to protect fishes from various bacterial disease in the USA and Chile (Sommerset et al. 2005).

17.6.3 Outer Membrane Protein-Based Vaccines

Advanced genetically engineered vaccines, for example, Outer Membrane Protein (OMPs) encoded carrier vectors, can induce both cell-mediated and humoral immune responses which is deemed to be a best option. Previous report revealed that the OMPs can be quickly recognized by the host immune system. For instance, the OMPs-K and OMPs-U of *V. harveyi* have demonstrated the capacity to provoke strong immune responses and their protective efficiency in fishes (Li et al. 2010; Wang et al. 2011). In other hand, the utilization of fusion proteins may improve the efficiency and strengthen the immune responses that drives to form a better efficient vaccine with wide range of protection (Liang et al. 2017).

17.6.4 Subunit Vaccines

Subunit (SU) vaccines have been using only antigenic elements for the purpose of vaccination and it may not cause any hazard to the host and nonspecific microbes. The cross-protection and standard safeties have been effectively achieved in case of the emergence of SU vaccines with conserved antigens (Chu et al. 2015). SU vaccines have depended on efficient adjuvants to provoke the suitable immune response, but in case of synthetic or recombinant antigenic components, numerous booster immunization approaches may be demanded to assure long-term immune response (Holten-Andersen et al. 2004). A lot of SU vaccines have been successfully utilized in aquaculture and produced in different expression systems. For example, salmonid alphavirus based three recombinant proteins successfully generated in Atlantic salmon fish cell line (Xu et al. 2012). SU vaccines have not been broadly produced and low successful rate without adjuvants which can induce adequate protection towards many fish pathogens, i.e., oil-adjuvant vaccine have been utilized for cold-water disease in ayu fishes (*Plecoglossus altivelis*). Moreover, the manufacturing cost of SU vaccines have been considered as more expensive when synthesized in agricultural species than animal model, because of extra protein processing techniques required to get vaccine with maximum efficiency (Ma et al. 2019).

Vaccine approach may have some obstacles like very expensive and limited in success rate even though it has the potential to block the fish susceptibility to pathogens (Das et al. 2020). Therefore some alternate approach should be focused for the protection of fishes from fish-related diseases.

17.7 Bacteriophage-Based Approaches to Eradicate Bacterial Pathogens

17.7.1 Mono-Phage Therapy

Bacteriophages, also colloquially called as phages, are auto-replicating virus that infects bacterial host with high specificity and replicates within. Phage specificity and its potential as a bio-control agent against target bacterial pathogen are considered as a vital advantage in therapeutic purposes. Unlike antibiotics, it could not ruin friendly microbes (Salmond and Fineran 2015). The bacterial cell surface receptors enclosing flagella, lipopolysaccharides, and surface proteins have been associated with phage-bacteria (host) interaction that drives to bacterial lysis process (Chaturongakul and Ounjai 2014). Phages along with remarkable characteristic features like narrow host specificity, self-replication, and quick cell-lysis process proved that phage therapy has been getting deliberation to locate and prevent the multidrug-resistant (MDR) bacteria and other zoonotic pathogens. Hence, it is essential to figure out the efficacy of phages and its bacterial lytic components responsible for fighting against antibiotic resistance. Other than that, phage therapy was encouraged for counteracting antibiotic resistance due to the deficiency of novel antibiotics.

Overall, phage therapy has been advised as an alternate to antibiotics and considered as one of the perfect choices to improve the quality of water, food, and food animals (Burrowes et al. 2011). Previous research reported that the gastrointestinal infections and human wound have been effectively treated with therapeutic phages in Soviet Union and Europe (Sulakvelidze et al. 2001; Sulakvelidze and Morris Jr. 2001). With advances, phages are available for the therapeutic applications in animals, humans, agriculture, and aquaculture. For instance, phage-related companies such as Intralytix Inc. (Baltimore, MD) and Phage Biotech Ltd., (Israel) have been developing phage treatment to protect larval oyster, clam hatcheries and shrimp from the bacterial pathogen *Vibrio harveyi* and *V. tubiashii*, respectively (Richards 2014).

17.7.2 Phage-Antibiotic Synergy

In general, the pathogenic strains may have resistance characters to their particular phages. Consequently, the joined treatment of a phage and anti-microbials has been utilized as a novel compelling device rather than a solitary treatment, as it shows a few benefits, for example, successfully enter into biofilms, expanded the suppression of bacterial development, and diminishing the possibilities of phage resistance (Comeau et al. 2007; Easwaran et al. 2020; Tagliaferri et al. 2019). In case of phage-antibiotic synergy (PAS), sub-inhibitory concentrations of antibiotics can enhance the growth of phages, increase plaque size and phage efficiency (Kamal et al. 2015). Henceforth, research on the sort of phages and antibiotics with their consolidate proportions is highly influential for advancement of successful phage treatment against multidrug-resistant microbes.

17.7.3 Various Advanced Approaches of Genetically Engineered Phages

In food safety, hospital sectors and industries, all the possible methods are employed to spot bacterial pathogens with high specificity and sensitivity (Almeida et al. 2013a; Lazcka et al. 2007). Conventional approaches are not only difficult yet in addition regularly neglect to recognize microbes even at low level samples (Almeida et al. 2013a, b). Advanced approaches in particular PCR based experiments are extremely delicate however could not distinguish among dead cells and live cells without bacterial advancement and need attention in primer design to prevent species identification failure (Lazcka et al. 2007; Lu et al. 2013). In addition, molecular approaches like antibody-based techniques do not normally achieve better for complex samples without advancement to enhance the bacterial targets (Lu et al. 2013). Current phage-based approaches with genetic engineering techniques have been suggested to defeat all the above restrictions.

Genetic designing or engineering approach has been widely employed to spot and influence the growth of bacterial infections through modified phages. Over antimicrobial action, engineered phages are designed to diagnose and control bacterial pathogens, design vaccines, antibody and anticancer drug delivery system (Pires et al. 2016). Several engineering approaches have been expanded to modify phages that can improve antibacterial activity, escalate host ranges, and diminish phage impacts on therapeutic applications.

In this case, phages can be altered to enhance the bacterial lytic action of antibiotics. For example, Lu and his team altered the lysogenic phage M13mp18 that can express repressor gene of DNA repair process at maximum range to raise antibiotic-induced killing bacteria. The engineered phages are enhancing the bactericidal efficacy of antibiotics such as the β-lactam ampicillin and the aminoglycoside gentamicin. Moreover, it can raise the lytic effect of antibiotic-resistant and persistent bacteria, and also diminish the appearance of bacterial mutant, which is related to antibiotic resistance (Lu and Collins 2009).

In another case, the engineered temperate phages have the potential to reduce the development of bacterial resistance to specific antibiotics via the deliver genes encoding antibiotic sensitiveness. For illustrative purposes, phage-mediated delivery of sensitive genes has been done in λ phages to insert gene gyrA and gene rpsL; those can reverse bacterial resistance to antibiotics and also accommodate sensitiveness to nalidixic acid and streptomycin antibiotics, respectively (Edgar et al. 2012).

In the domain of infectious diseases, bacterial biofilms are doing crucial role in case of pathogenesis. Antimicrobial treatments for biofilm are challenging to eradicate due to its resistance characteristic features. To resolve this issue, engineered phages harboring biofilm-degrading enzyme have been designed to combat every single bacterial cell in the biofilm or biofilm matrix.

17.7.3.1 Phage- Cocktail

Phage-resistant bacteria are the major drawback of mono-phage therapeutic approaches. To tackle this issue, previous research groups developed phage cocktails that can elude the emergence of phage resistance, defer the advancement of medication obstruction, and increment the proficiency of mono-phage treatment (Chan et al. 2013; Lee et al. 2014; O'Flynn et al. 2004). In addition to improving the phage specificity, various narrow host specific phages are genetically modified and combined to form a single cocktail that may have broad host spectrum efficacy. Albeit these kinds of methodology are promising, it stays hard to focus on all bacterial strains in a desired species, and the different arrangement of phages in cocktail may inadvertently target microbes outside the ideal range (Lin et al. 2012). Therefore, phage cocktails can be successfully employed to control bacterial pathogens in aqua-farming sectors.

The combinations of various phages have the potential to expand capability of phage treatment against the pathogenic microbes. Chan suggested that limited numbers (two to ten) of particular phages are the most ideal alternative for the effective therapeutic approach especially in shrimp farming systems (Chan et al. 2013). For instance, previous research proved that the hybrid phage (T3/T7) have developed with the combination of T3 and T7 phage tail fiber protein, particularly gp17 gene, and showed a wide range of host specificity and superior phage-host adsorption efficacy compared with wild type of phages (Lin et al. 2012). In another study, phage VHS1, VHM1, and VHM2 have expanded the proficiency of phage treatment against *V. harveyi* and also other *Vibrio* sp. including *V. parahaemolyticus* and *V. alginolyticus*. In any case, phage cocktail could bring a more noteworthy effect on non-target microbes, although it is still low impact compared with commercially available antibiotics (Chan et al. 2013).

17.7.3.2 Phage-Encoded Enzymes

In past few years, endolysins are phage-encoded enzymes that are getting more attention due to their unique lytic activity against Gram-negative and Gram-positive bacteria (Salmond and Fineran 2015). In general, endolysins are responsible for the releases of phage progenies through hydrolyze of bacterial cell wall, and another enzyme named holins, hydrophobic hole-forming proteins, regulates the activity of phage endolysins (Gondil et al. 2020; Wang et al. 2000). Moreover, phage genomes encoded other enzymes that could break bacterial cells, for example, virion-related peptidoglycan hydrolases and polysaccharide depolymerases (Rodríguez-Rubio et al. 2013).

Phage endolysin have been employed as a therapeutic protein, which is interfered to reduce bacterial contamination and enhance the food safety level through eradicating bacterial pathogens using their enzymatic nature (Abdelrahman et al. 2021). Among all the phage enzymes, phage endolysin has been deemed as a potential candidate instead of antibiotics. Display with some benefit highlights are profoundly species explicit, an extensive lysis range, and more averse to prompt the development of a resistant microbe. The pervasiveness of antibiotic-resistant microorganisms that cause food-borne diseases can be prevented by phage endolysins. It has been perceived as regular bio-additives and required to ensure unharmed food with maximum hygienic in food sectors. Bacterial biofilms have been totally destroyed in food and clinical conditions with the help of peptidoglycan hydrolases and endolysins of phages (Abdelrahman et al. 2021).

17.7.3.3 Phage-Encoded Anti-CRISPR System

Bacterial pathogen can block phage infection and replication in several ways such as transformation of cell surface receptors and breaking of an intracellular phage genome which are similar to the resistance mechanism of antimicrobial agents (Labrie et al. 2010). For the utilization of phage, it is mandatory to comprehend the impact of bacterial immune system or bacterial defense system, which is happening at every step of phage replication cycle. Phage infection can be initiated with attachment of phages on the host cells through particular cell surface receptors. Bacterial defense mechanisms such as modified surface receptors, bacterial inhibitors and bacterial polysaccharides have been associated with mask phage receptors, which is responsible for initial phage attachment (Dy et al. 2014; Labrie et al. 2010; Seed 2015). On the contrary, bacterial membrane-related proteins and bacterial premature cell death can prevent phage genome entering and replication process, respectively (Cumby et al. 2012; Labrie et al. 2010). Particularly, bacterial defense systems such as restriction-modification, and the Clustered regularly interspaced short palindromic repeat (CRISPR)-Cas has been considered as an active system to lysis phage genomes (Pingoud et al. 2014; Stanley and Maxwell 2018).

Bacteriophages have a notable counter effect to adapt bacterial host defense strategies. For instance, phages have the potential to alter their phage receptor-binding proteins that can assist phage-host interaction and modify bacterial genes that provoke abortive phage infection (Labrie et al. 2010; Samson et al. 2013). Due to the CRISPR-Cas systems, phage could not survive in the bacterial intracellular environment for a long time. In one way, phages can overcome bacterial CRISPR-Cas defense system by altering their genomes to evade restriction-modification system. Another way, hydroxymethylcytosine (HMC) of T4 phages are responsible for switch a gene on and off and revealed to secure T4 phage from CRISPR-mediated ruination approach, which is proved by covalent modifications in T4 DNA (Bryson et al. 2015). In other way, phage-encoded protein inhibitors of CRISPR-Cas, called as anti-CRISPRs, allow phages to escape from the bacterial CRISPR-Cas system through an active and vigorous activity (Stanley and Maxwell 2018). Overall phage-based approaches have been displayed in a schematic diagram (Fig. 17.2) to explain their technological advancement for the treatment or control of fish bacterial diseases and future pandemics in upcoming decades.

17.8 Conclusion with Future Prospects

Over the years, the prevalence or control of bacterial fish diseases could be a significant challenge due to antibiotic-resistant or uncontrollable pathogenic bacteria. Those kinds of bacterial strains can transfer virulence features from an infectious fish to humans and animals via food animals and vice versa. Hence, suitable gadgets with unique and advanced approach should be required to acknowledge and kill the

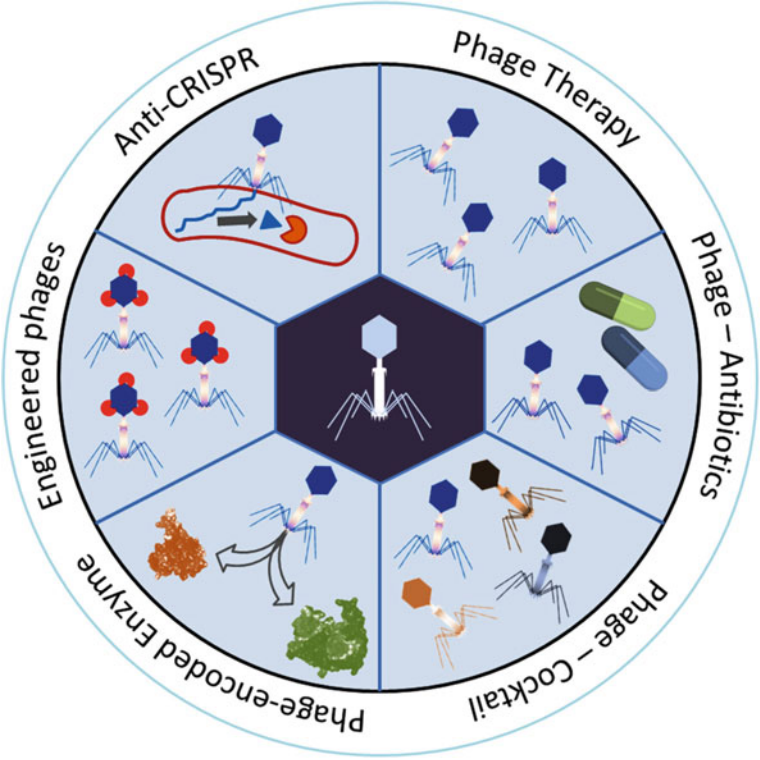

Fig. 17.2 displayed recent advanced technologies to combat multidrug-resistant fish pathogens and control bacterial resistance. (**a**) Phage therapy; (**b**) phage-antibiotic synergistic approach; (**c**) phage cocktail; (**d**) phage-encoded enzymes; (**e**) engineered phages; and (**f**) phage's anti-CRISPR defense system.

bacterial fish pathogens or multidrug-resistant fish pathogens. In the past decades, chemotherapeutic approaches have been mainly involved in the generation of microbes with resistance features. Recent years, bacteriophages, phage-derived components have been utilized for fighting against bacterial infections or reducing drug-resistant microbes and their impact on fish culture and aquaculture that leads to healthy fish and fish environment. To implement phage-based approaches in a promising way, it is mandatory to understand the interaction between bacteriophage, microbiome, and fish host. More interestingly, the efficacy of phage-based antimicrobial agents has been increased by recent advanced approaches like phage-encoded endolysins, engineered phage, and phage's anti-CRISPR defense system which would be demanded for solving the present and upcoming problem of antibiotic-resistant infections in fish world.

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Chapter 18
Herbal Biomedicines as Immunostimulants and Immunosuppressors in Fish

Nageshwari Raja, Hemalatha Karuppiah, Maheswaran Easwaran, Hyun-Jin Shin, and Juhee Ahn

Abstract In aquaculture, fish farming or pisciculture is the farming of fishes mainly for commercial food production process and plays a crucial role in socio-economic development worldwide. In last two decades, economic losses and other unseen problems aroused in the aquaculture-related production due to infectious diseases. Immunostimulants and immunosuppressors are one of the most significant approaches for improving the immune system of the aquatic animals and can rejuvenate the aquatic industry by preventing fish diseases. Various types of substances including glucans, bacterial products, lactoferrin, levamisole, and medicinal plant constituents can be used as a disease controlling agents that directly influence the innate or nonspecific and adaptive or specific immune reactions of aquatic animals. Most of the nutritional supplementations of plant constituents as immunostimulants or immunosuppressors for indirectly treating the infectious diseases in aquatic animals have high research priority among scientists. Particularly, the bioactive compounds of the plant constituents can circuitously reinforce the innate immune response of the fish reducing susceptibility to broad spectrum of

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pathogens. This chapter mainly emphasizes about the efficacy and efficiency of different medicinal plant products as immunostimulants, and also focuses on the immunological and growth performance parameters of several fish species, health, and production. Ultimately, it may shed light on the new era of herbal biomedicines as immunomodulators and immunosuppressors in the aquaculture industry and also discuss about cellular and molecular mechanisms evolved during the dietary intake of medicinal herbs in the fish species.

Keywords Immunosuppressor · Immunostimulant · Herbal medicines · Infectious disease · Immune system

18.1 Introduction

Fish and fishery based products play a major part in commercial food production, providing high nutritional value of protein and essential nutrients with increased medicinal value, contributing to marine product exports and also employing about 14.5 million people in different activities (Lauria et al. 2018). In recent years the aquaculture industry has globally encountered variety of unseen difficulties and financial losses due to contagious diseases and other factors such as poor water quality and nutrition, handling and temperature leading to immunosuppression in fish. Nowadays, infectious diseases are the major constraints for both human beings and fish farms. The strategies for controlling the diseases associated with infections in aquaculture industry are analogous to the ones followed in animal infections. Hence, the same antibiotics have been implemented for controlling the infectious diseases of fish and this largely attributing towards the antibiotic resistance which is one of the paramount health challenges to global aquaculture sustainability and also results in high mortality rate leading to high economic losses worldwide (Huttner et al. 2013).

Even though many of the antibiotics and chemotherapeutics are used in marine fish hatcheries for treating the infectious disease outbreaks, the antibiotics cause sensitization reaction and other side effects to the human beings. The increased use of antibiotics in fishes may also lead to the evolution of antibiotic-resistant strains, reduce the growth of the fish at larval stage, and also suppress the defense mechanism of the fish (Rossolini et al. 2014). Vaccination is another efficient method of treating infectious diseases and thereby advancing the immune responses of fishes against the particular pathogens. However, vaccines are very specific to the single pathogens and also expensive for treating the fish disease. At the global level, there is a need of switching over to herbal biomedicines as a promising and alternative method in aquaculture industry instead of chemotherapeutics and antibiotics for preventing or controlling the fish disease.

The medicinal herbs are not only improving the immunity but also promoting the growth performance rate of the fish and removing stress factors in fish which directly disturbs the immunological responses of the fish. The natural products used for treating the infectious diseases have tremendous advantages including eco-friendly,

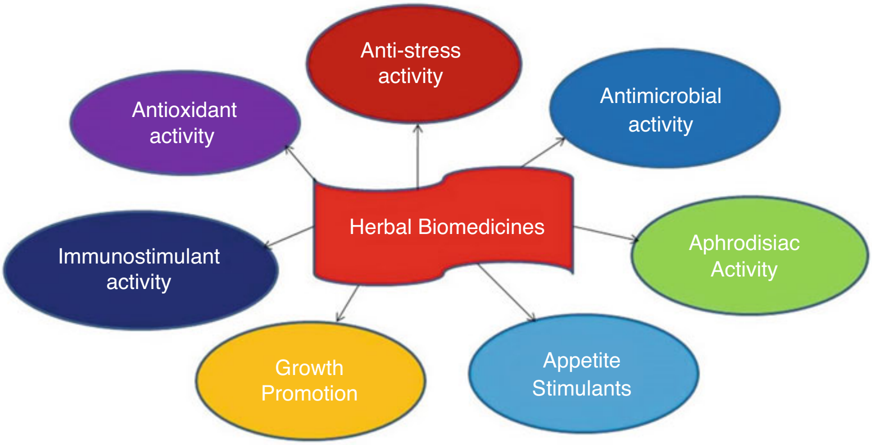

Fig. 18.1 Diversified role of herbal biomedicines in aquaculture industry

cost-effective, and minimal side effects which can also be easily fixed by them. Figure 18.1 implies the mechanism of action of herbal biomedicines as growth promoter, immunostimulant, antimicrobial activity, antioxidant activity, anti-stress activity, aphrodisiac activity, and appetite stimulants (Citarasu 2010). This chapter may shed light on the advantages of the commonly used medicinal herbs as immunostimulants in the aquaculture industry for improving feed efficiency, disease resistance, and weight gain and as immunosuppressants for inhibiting cellular and humoral immune responses for treating certain autoimmune disorders. Immunosuppressors are known to modify the immunoregulatory functions leading to increase the susceptibility of the infectious disease in fish species. This chapter mainly emphasizes about the principle behind the mechanism of immunostimulants and immunosuppressors action in fishes.

18.2 Herbal Biomedicines

Medicinal herbs and other plant-related bioactive compounds play an indispensable performance in the treatment of infections in aquaculture industry. Usually antibiotics, vaccines, and chemotherapeutic compounds are used for controlling the disease outbreaks. Diets with medicinal herbals improve both specific and nonspecific defense mechanisms such as phagocytosis in blood leukocytes, specific growth rate (SGR), extracellular and intracellular respiratory burst activities, condition factor (CF) and plasma protein level (Punitha et al. 2008) of several fish species in the global aquaculture production industry. Various plant-based derivatives and / or plant extracts play a crucial role in the disease control of aquatic animals. Herbal biomedicines have been found to be an alternative medicine to chemical therapeutics and antibiotics for treating the infectious diseases and largely contributing for the disease control in aquatic animals (Citarasu 2010). Figure 18.1 shows the mode of action and diversified function of herbal biomedicines in aquaculture industry.

The medicinal herbs could be the beneficial one in the aquaculture industry for a variety of applications including immunostimulant activity, growth-promoting factor, appetite stimulant activity, aphrodisiac activity, antimicrobial activity, anti-stress activity, and antioxidant activity due to the presence of mixture of functional bioactive compounds such as saponins, tannins, flavonoids, terpenoids, phenolic acids, carotenoids, and alkaloids. The feeds of tilapia were supplemented with Astragalus radix and Lonicera extracts for increasing nonspecific defense mechanism in fish (Yin et al. 2006). The medicinal plants for improving the fitness of the fish are pomegranate *(Punica granatum)*, Indian ginseng *(Whitania somnifera)* and garlic *(Allium sativum)*, ginger *(Zingiber officinale) and* Bermuda grass *(Cynodon dactylon)*. (Reverter et al. 2017).

18.3 Overview of Immunological Responses of Fish

The immune system is defined as a complex network of cellular and humoral compounds and soluble mediators providing protection to the body from foreign substances including microorganisms and toxins and responding to endogenous and exogenous factors by stimulating this system. Comprehensive understanding of cell functionality and morphological features of the fish may shed light on insights of fish pathologies related to the infectious diseases.

There is more similarity between the lymphocytes of mammals and human beings and speculated the hypothesis that mammalian B cells and γδT cells are evolutionarily associated with the lymphocytes of the fish (Scapigliati et al. 2018). As in all vertebrates, the fish comprises both nonspecific and specific immune responses. Both immunological parameters of the fish are mainly mediated by the soluble substances such as T cells, B cells, complement proteins, leucocytes, antimicrobial enzymes, interleukins, interferons, and other defense cells such as monocytes, granulocytes, natural killer (NK) cells, and macrophages (Biller-Takahashi and Urbinati 2014).

The innate immune system is the fundamental and first layer defense mechanism responding more quickly to pathogenic bacteria, viruses, fungus, protozoa, and parasitic infections than the specific system. The second type of immune system, adaptive or acquired immune system mainly depends on the arrival of antigens for initiating the antigen-antibody reactions and thereby increasing the circulation of antibodies and promoting the memory cells. A deeper understanding of fish immune system will definitely shed light on the development of new technologies for modulating the immune system of fish through the biologically active plant species.

The nonspecific immune system of fishes was modulated by activation of thrombocytes, lymphocytes, monocytes, and neutrophils for elimination of pathogens, increased plasma bactericidal activity, and production of oxidative radicals and

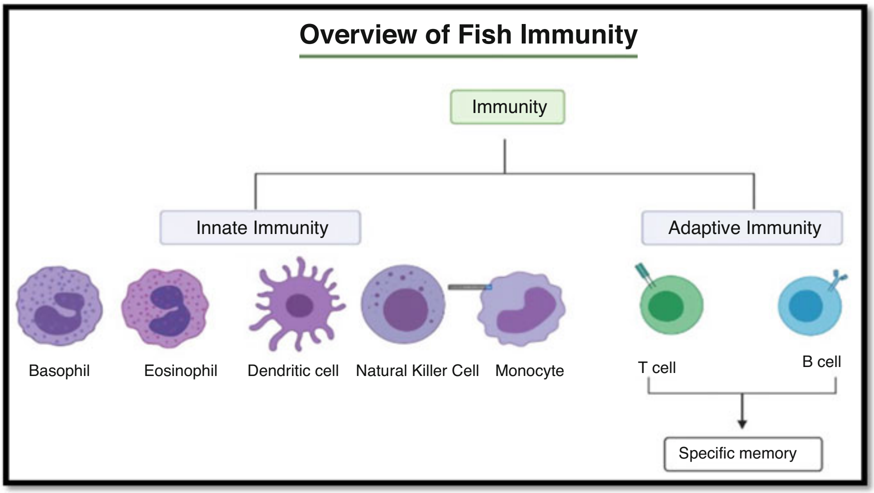

Fig. 18.2 Overview of fish immunity

peroxidase with activation of cytokines (Ainsworth et al. 1991). Innate-like lymphocytes (IIL) situated in the mucosal tissues mediate both innate and adaptive immune responses in mammals and fish and therefore maintain the gut homeostasis during the intestinal infections in fish. The innate lymphoid cells (ILC) are derived from the lymphoid progenitors and play major role in the effector and regulatory functions of innate fish immunity system. Based on the cytokines production and transcription factors, the ILC is classified into three types including ILC_1, ILC_2, and ILC_3. Fish lymphocytes have αβ- and γδ-TcR on their surface (Castro et al. 2011)

The main components of innate immunity are divided into anatomical barriers including physical and chemical barriers, cellular parameters including phagocytic cells, natural killer cells, dendritic cells, and interleukin cells, and humoral parameters including soluble mediators such as cytokines, chemokines, defensins, complement proteins, natural antibodies, and antimicrobial substances (Magnadóttir 2006). The major components of the immunity enhancing mediators are freely situated in cellular components and body fluids of fish (Fig. 18.2).

Lymphocytes have been shown to perform a prominent role in the adaptive response of fish like mammals. There are two types of lymphocytes for improving the health status as well as promoting the growth factors. Different types and subtypes of T cells are present in teleost fish (Castro et al. 2011). During the process of defense mechanism of the fish, the antigenic peptides take up dendritic cells presented to the Naive T cells through the major histocompatibility complex (MHC)–T cell receptor (TCR) interaction and hence activate their different subclasses including helper T cells 1 (Th1), Th2, follicular T cells (Tfh), Th17, and regulatory T cells (T_{reg}). The Th2 and Tfh induces the B lymphocytes to produce respective antibodies against the antigens by promoting phagocytosis, whereas the Th1 cells produces IFNγ upregulating the Th1 type adaptive immune response related proteins or activating the NK cells. The increased level of IFNγ in the fish is an indicator of immune protection against the infectious disease. The T_{reg} in turn induces the level of IL-10/TGFβ, which is a kind of cytokines produced for executing their killing functions in the fish species, thereby conferring protection against the infectious diseases. In several fish species, the T effector cells produce Th_{17} cells which induce IL-17 for neutrophil mobilization and activation and also enhance the genetic expression of tumor necrosis factor and activated macrophages. Activation of lymphocytes and phagocytes, enhancement of complement activation, and activation of lysozymes are the key indicators of immune protection mechanism in the fish species. Fish cells express CD4 and CD8-related genes which are activated in the presence of viral and bacterial pathogen and hence releasing different types of cytokines for killing of the pathogens similar to mammalian CD4 and CD8-related genes in MHC antigen presentation. The mucosal intestine can be considered as a main source for T lymphocytes in adult fish. Surprisingly, it should be noticed that mutant zebra fish for the lack of somatic recombination are still able to produce a specific protection after re-exposure of bacterial infections (Hohn and Petrie-Hanson 2012). Atlantic cod, cold-adapted teleost is deficient in MHC class II and CD4 in the genome although it is safe from bacterial and viral infections (Star et al. 2011). Likewise, Teleost fish generally produces IgM as crucial immune response but it lacks isotype switching mechanism to accumulate antibodies which are specific to the viral infections (Workenhe et al. 2010).

Conversely, there is a great interplay between the NK cells and components of the adaptive immune system. In various fish species, if any type of infection is encountered, it will ultimately modulate the genetic expressions of cytokines, thereby eliminating the pathogens from the gut and maintaining the gut homeostatic effect. The expression level of CD4 T lymphocytes was constantly maintained for 20 days after supplementation of two functional diets containing yeast extract and plant extract compared with control diet. While evaluating the immune response, the functional diets show the positive response at 20th day of trial and also upregulates the level of IFNγ in fish. On the other hand, no remarkable changes were observed on TBet, a transcription factor that controls the expression of IFNγ. In general, the herbal biomedicine helps to advance the initiation and activation of an immune response, but also hold back immune effectors activity and thereby influencing the final outcome of the immune responses in host (Andoniou et al. 2008). Furthermore, the clear understanding of fish immune system will definitely pave a new pathway for the herbal biomedicines as an alternative food additive in aquaculture.

18.4 The Use of Herbal Biomedicines as Immunostimulants

Immunostimulants are defined as natural or synthetic compounds that enhance the immune system of the aquatic animals and thereby protecting it from diseases and boosting up their growth against a wide range of infectious pathogens including bacterial, viral, fungal, and parasitic agents (Sakai et al. 1992; Meena et al. 2013) (Carbone and Faggio 2016). Herbal biomedicines are highly recommended as promising alternative to not only antibiotics and chemotherapeutics but also vaccines and other synthetic compounds due to less side effects, minimal dosage requirements, and specific mode of action towards various infectious diseases in fish species. The use of herbal plants as immunostimulants has emerged globally due to its reliable characteristics including non-toxic even at high dosage level, short withdrawal period with less tissue residues, biocompatibility, inexpensive and broad range of nonspecific immune responses against bacterial, viral, fungal, protozoal and helminth infections. The herbal biomedicines are commonly used in several forms either crude or mixed with a probiotic (Ringø et al. 2020) or with an animal product (Sattanathan et al. 2020a; Sattanathan et al. 2020b; (Zhang et al. 2009)

Several studies hypothesized that most of the medicinal herbs activate the nonspecific immune mechanism of the aquatic animals in two different methods, the first method is by stimulating the growth of favorable gastrointestinal (GI) bacteria and the second method is by directly influencing the cascade of the immune response in the fish species (Rodrigues et al. 2020). Herbal medicinal plants can be used as immunostimulants, growth promoters, antibacterial, antiviral, antifungal, and anti-stress agents, appetite stimulators, and aphrodisiac agents in aquaculture industry (Punitha et al. 2008) (Citarasu 2010).

The feeds supplemented with *Zingiber officinale* (ginger), *Viscum album* (mistletoe), and *Urtica dioica* (nettle) for fish showed increased phagocytosis and extracellular and intracellular respiratory burst activities, thereby corroborating immunomodulatory effect in fish (Dügenci et al. 2003). The aqua feeds with *Dicentrarchus labrax* (European sea bass) can improve the innate immunity of the fish by modulating the gut health of the fish species and thereby inhibiting bactericidal activity and increasing the plasma total immunoglobulin (Azeredo et al. 2017). Chinese herbs from Ganoderma (*Ganoderma lucidum*) and from Lonicera (*Lonicera japonica*) were fed with diet for strengthening the immune status of the fish by altering the intestinal morphology of the fish, elevating extracellular activity of phagocytosis, and also modulating total lysozyme activity in the fishes (Yin et al. 2006).

The ginger (*Zingiber officinale*) showed a significantly improved immunomodulatory properties, antioxidant activity, anti-inflammatory activity, growth enhancing activity, antibacterial activity, anti-parasitic activity, etc. in aquatic animals as well as in the terrestrial animals (Tan and Vanitha 2004) (Zhou et al. 2006) (Ali et al. 2008). Levy et al. reported that the monogenean parasitic *Gyrodactylus turnbulli* infection in the guppy fish can be effectively controlled by in-feed and/or bathing treatment of infected fish with ginger-based products to be an alternative and natural treatment strategies of disease control instead of the use of chemotherapeutic agent, Praziquantel for treatment of gyrodactylosis (Levy et al. 2015). The fish fed with ginger extract is reported to have rich source of protein content, increased amino acid absorption in GI tract, and also beneficial to intestine probiotic bacteria (Ali et al. 2008). Particularly the oral administration of ginger efficiently improves the disease resistance against the various disease-causing microorganisms such as *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Bacillus subtilis,* and *Proteus mirabilis* (Akintobi et al. 2013). The ginger supplemented diet in *Labeo rohita* fingerlings shows increased resistance against *A. hydrophila* infection and upregulation of antioxidant genes, anti-inflammatory cytokine-related genes, signaling molecules, and inhibitor proteins in the intestine, head-kidney, and hepatopancreas of the fish (Sukumaran et al. 2016). Recently, Lee et al. demonstrated that the feed containing the plant-originated additives including yacon, ginger, and blueberry can improve the specific growth performance rate, weight gain, and feed efficiency ratio and also greatly shows highest inhibition of *Vibrio anguillarum* infection in the black rockfish (Lee et al. 2020b).

Giri et al. suggested that *Psidium guajava L.* (guava) leaves improve the immune status of *Labeo rohita* fish species and increase weight, specific growth rate, and other important immune parameters including leukocyte phagocytic activity, alternative complement pathway activity, and lysozyme levels in the fed groups and thereby protecting it from *Aeromonas hydrophila* infection (Giri et al. 2015b). Similarly, *Labeo rohita* supplemented with banana peel flour (BPF) diets also modulate the growth parameters, anti-inflammatory immune reactions, and cytokine genes related expression in the fish species (Sib Sankar Giri et al. 2016). This study evaluated the growth performances and genetic expression of immune-related genes in the *Cyprinus carpio* fish species. Turmeric is reported as a viable alternative feed additive in aquaculture due to the increased IgM levels and lysozyme activity in the skin mucus of *Cyprinus carpio* fed with 15 g kg^{-1} and 10 or 15 g kg^{-1} of curcumin, respectively. The increased level of IgM significantly participates in pathogen elimination at infection sites (Giri et al. 2019).

Diets containing Polyherbal supplement AquaImmu fed shrimp improve growth-promoting efficiency and immunostimulant property in *Penaeus monodon* culture system. AquaImmu supplemented diet is the combination of Indian medicinal plant extracts including *Ocimum sanctum*, *Withania somnifera*, *Tinospora cordifolia,* and *Emblica officinalis* as the major compositions at optimum concentration (Chandran et al. 2014). Similarly, the AquaImmu containing diet elevates the defense system in freshwater prawn *Macrobrachium rosenbergii* (Kumari et al. 2004). *Salvia officinalis* and *Lippia citriodora* supplemented medicinal plant leaf extract in gilthead seabream (*Sparus aurata*) can be effectively used as a valuable feed additive at the concentration of 0.1% MPLE for improving growth parameters and splenocytes primary cell culture immune responses by upregulating the humoral immune response related genes of the fish species (Salomón et al. 2020).

18.5 Effect of Herbal Plants for Treating Infectious Diseases in Fish

Wang et al. investigated the effect of various plant polysaccharides such as *Schisandra chinensis* polysaccharides (SCPS), *Ficus carica* polysaccharides (FCPS), and *Radix isatidis* polysaccharides (RIPS) for preventing and controlling the bacterial infections in hydroponics and particularly recommended that FCPS has the highest activity as immunomodulatory agents and disease resistance of fish by analyzing immunological parameters including phagocytosis activity of blood leukocytes, serum lysozyme activity, detection of bactericidal activity, serum total protein, SOD activity, complement C3 and finally challenge test with *Aeromonas hydrophila* in crucian carp by intraperitoneal injection (Wang et al. 2016). Giri et al. reported that polysaccharide extracted from *Chlorophytum borivilianum* as feed additive effectively modulates immune-related cytokine genes and the innate immune reactions in *Labeo rohita* fingerlings against *Aeromonas hydrophila* infections (Giri et al. 2015a). Dietary supplementation of symbiotic diet provokes the immune-related cytokine gene regulation, antioxidant activity, and innate-adaptive immune reactions in *Labeo rohita* against *A. hydrophila* infections (Devi et al. 2019). Basal diet containing *Psidium guajava L.* (guava) leaves improves growth performance rate and increases lysozyme levels, alternative complement pathway activity, and leukocyte phagocytic activity in fish species *Labeo rohita*. The basal diet containing guava leaves does not change the plasma IgM level, but the diet upregulates immune-related genes including tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) and downregulates IL-10, transforming growth factor-beta (TGF-β), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and transcription factor nuclear factor-κB (NF-κB), thereby enhancing protection against *Aeromonas hydrophila* infections (S. S. Giri et al. 2015b). Dietary supplementation of Astragalus polysaccharides in Nile tilapia (*Oreochromis niloticus*) improves growth effect and increases bactericidal activity, phagocytic activity, respiratory burst activity, levels of plasma lysozyme, SOD, amylase, and glutathione peroxidase (GPx) activity, whereas there is no significant effect in the level of serum Malondialdehyde (MDA) or nitric oxide (NO) (Zahran et al. 2014). Recently, the expansion in the utilization of antimicrobial compounds has been accounted for the scattering of various bacterial infections (Defoirdt et al. 2011). There is a strong association between the IgM and lysozyme activity which mediates the nonspecific immune reactions after the supplementation with plant-derived immunostimulants to the fish.

18.6 Effect of Herbals for Treating Oxidative Stress in Fish

The bioaccumulation of reactive oxygen species in fish leads to the oxidative stress condition in which cells are modulated to neutralize the impacts of oxidants and to repair the redox balance by upregulation or downregulation of defensive related enzymes, structural proteins, and other transcription factors (Birben et al. 2012; Dalton et al. 1999; Scandalios 2005). The harmful impacts of ROS can be limited by enactment of non-enzymatic antioxidant defenses including glutathione, vitamins C and E, and β-carotene, and enzymatic antioxidant defenses including catalases (CAT), SOD, and peroxidases which directly scavenges the hydroxyl and superoxide radicals, thereby converting into a lesser amount of reactive species (Scandalios 2005). The excessive amount of ROS brings about oxidative stress, thereby damaging the cellular components including nucleic acids, proteins, and lipids. Disruption of structural proteins in the cell membranes might cause changes in the membrane rigidity and its mechanical strength and damages to the macromolecules resulting in modulation of cell growth, differentiation and unsaturated lipid peroxidation leading to cell death. The repeated cycles of lipid peroxidation cause the most severe damage to the cell membranes and thereby causing cell lysis (Garcia et al. 1997).

Oxidative stress is also strongly connected to the proteolysis and protein modifications among which protein carbonylation (PC) is non-enzymatic post-translational modification and an irreversible process, a reactive carbonyl group produced bound with the protein molecules leading to loss of protein functions. Hence detection of protein carbonylation levels can be used as an assay of oxidative stress by evaluating the antioxidant effects of functional diets. Diets supplemented with antioxidants in fish reduces the oxidative stress and modifies the immune-related gene expressions, conferring resistance to disease status in fish and therefore this may shed light on the era of formulation of new functional feeds in aquaculture (Smuder et al. 2010). Over the decades, various studies paying attention towards supplementation of yeast extracts by diet for salmon subjected to crowding stress, among which astaxanthin is a high carotenoid content making them as special yeast compound and conferring the reduction of lipid peroxide level in various fish species, particularly increasing the complement activity and phagocytic rate for escaping from the bacterial infections (Amar et al. 2004). Reyes-Cerpa et al. focused on the functional diet containing antioxidant activity and immunostimulant properties for the beneficial development of new functional feed additives and also for the health management strategies for aquaculture (Reyes-Cerpa et al. 2018). Ultimately, ROS, MDA, and PC are excellent indicators of oxidative damage in the Aeromonas infection (Bandeira Junior and Baldisserotto 2020).

Reyes-Cerpa et al. evaluated the effect of yeast extract *(Xanthophyllomyces dendrorhous)* and the combined plant extracts (lemon balm, common Saint John's wort and rosemary) on the antioxidant and expression of genes associated to immune response of Atlantic salmon. The yeast and plant extract diminished the level of PC and lipid peroxidation, whereas the genetic expression of membrane IgM, IFNγ, GATA3, TGF-β, and CD4 is upregulated in fish subjected to crowding stress conditions suggesting the protective role of yeast and plant extracts against oxidative damage (Reyes-Cerpa et al. 2018). The utilization of flavonoids containing diets in fish subjected to crowding stress produces better inhibitory effects towards scavenging of free radicals and lipid peroxidation and therefore can be considered as a remarkable solution to develop functional feeds with antioxidant effect (Nieto et al. 1993). Additionally, the presence of phenols present in lemon balm (*Melissa officinalis L.*) and rosemary *(Rosmarinus officinalis)* has been also found as the most prominent for their increased antioxidant activity and therapeutic properties, such as carminative, sedative, anti-inflammatory, anti-tumor effects and antispasmodic effects in various fish species (Barros et al. 2013; Peng et al. 2007). The antioxidant effect in the functional diet supplementation is directly related to the occurrence of flavonoids and phenolic compounds that can interact with reactive oxygen species and can balance between the radical producing systems and antioxidants conferring immune protection of various fish species.

There is an emergent interest in various herbal mixtures of traditional medicinal plants including Chinese herbs in animal feed for improving specific growth rate and immunity response and disease management in aquaculture. The entire medicinal plant or its parts, leaves, roots, flowers, or plant extracts can be used as immunostimulants and/or immunosuppressants in the aquaculture field. The Astragalus polysaccharide supplemented diet promotes immunomodulatory and growth performance effects in Nile tilapia (*Oreochromis niloticus*) and these findings also investigated the upregulation of the respiratory burst activities, phagocytic activity and plasma lysozyme level and also defensive related enzymes including SOD, GPx and amylase activity of cultured tilapia fish (Zahran et al. 2014).

18.7 Evaluating the Efficiency of Herbal Plants as an Immunostimulant

Two methods are generally applied for measuring the efficiency of the herbal biomedicines as immunostimulants, first method is in vitro including measurement of the expression level of immunoregulatory genes and the second method is in vivo including protection tests against the fish pathogens. The activation of cellular immune defense mechanism can be appropriately evaluated by analyzing the phagocytosis and respiratory burst activities in several fish species after supplementing the diet with herbal extracts. The immunological effect of functional diets on fish health can be measured through gene expression level of immune system-related marker genes including Th1 and Th2, GATA-3, CD4, IL-10, membrane and secretory IgM isoforms, NK cells and anti-inflammatory-related cytokine as TGF-β. Among them, the genes associated with pro-inflammatory cytokine TNF-α are one of the important immunoregulatory genes at an initial stage of bacterial infections in fish and therefore they play a key role in the immune defense mechanism against pathogens.

Phagocytosis activity of leukocytes is one of the significant fish defense elements in aquaculture and thereby indicating that the medicinal plant supplemented diet has high immune homeostatic effect.

In recent years, the accumulating scientific evidence investigated the efficiency of the functional diet on fish immune health status through various defense elements including phagocytosis activity of leukocytes in serum, respiration burst activity, lysozyme level and other expression levels of immunoregulatory genes, etc. Professional phagocytic cells perform a key role in the nonspecific immune system of fish. Phagocytes also release higher amount of reactive oxygen species during a process called respiratory burst, for eliminating the microbes from the host. The level of phagocytic activity of leucocytes in serum is increased after oral administration of immunostimulants and thereby conferring the activation of a defense mechanism in the fish immune system. Various scientific reports clearly hypothesize that medical plant extracts can promote phagocytosis in different fish species (Dügenci et al. 2003). Similarly, polysaccharides extracted from the medicinal plants significantly enhanced the phagocytic activity of leukocytes isolated from crucian carp against *A. hydrophila* infections (Wang et al. 2016). Correspondingly, Astragalus extract also increases the phagocytosis process in Nile tilapia (Ardo et al. 2008). Hence in general, the usage of herbal plant extracts in various fish has high phagocytic activity and thereby improving the outcome of health status in fish (Chakrabarti and Rao 2006; Gopalakannan and Arul 2006; Logambal et al. 2000). Concordantly, the three herbal extracts, including *Lonicera japonica*, *Cyathula officinalis,* and *Bidens pilosa,* have high phagocytic activity and phagocytic number in cobia *(Rachycentron canadum)* (Lee et al. 2020a b) upholding the previously discussed speculations in the aquaculture industry.

The effect of serum lysozyme concentrations also plays an essential defense element in the fish species. Lysozyme is one of the important humoral defense elements in the nonspecific fish immune system and the main function of lysozyme is to activate complement proteins and phagocytic cells, thereby eliminating pathogens from the host. Generally, the medicinal plant extract enhances the level of lysozyme secretion in the fish, and thereby activating the innate immune responses of various fish species. Yin et al. reported that Chinese herb Astragalus radix having rich polyphenols fed to the tilapia (*Oreochromis niloticus*) increases lysozyme activity in serum (Yin et al. 2006), and this same result observed in beluga sturgeon (*Huso huso*) fed with garlic (Akrami et al. 2015). On the other hand, Reyes-Cerpa et al. evaluated the effect of functional diet containing yeast (Xanthophyllomyces dendrorhous) and combined plant extract which produces different immunostimulatory effects with significant antioxidant activity in Atlantic salmon and thereby inferring the protection of the fish subjected to crowding stress (Reyes-Cerpa et al. 2018). Diet fed with *Salvia officinalis* and *Lippia citriodora* promotes immunomodulatory and growth effects in gilthead seabream (*Sparus aurata*) and upregulates the immune system-related genes including lysozyme, TNF-α, IgM and IL-1β, antioxidative stress enzymes, leukocyte cell surface marker and anti-inflammatory cytokines (Salomón et al. 2020).

The optimal dosage level of diet supplemented with herbs and duration of feeding also play a prominent role in growth-promoting effect and upgrading the immunologic parameters in several fish species. After 3 weeks duration, there is an increment in the phagocytosis activity in tilapia fish fed with different doses of Astragalus compared with the control during the experiment. They also suggested that there is no significant effect on the respiratory burst activities of the phagocytic cells in tilapia species (Yin et al. 2006). Concordantly, Ardo et al. recommended that diet containing Astragalus and Lonicera extracts when added to tilapia (*Oreochromis niloticus*) increases lysozyme and phagocytic activities (Ardo et al. 2008). There is an emerging interest on herbal immunostimulants due to their safety to environment, biodegradability, and rich nutritional value, thereby strengthening immune system. A diverse role of immune stimulatory functions has been revealed by medicinal herbal plants at various concentrations through different application methods including injection, immersion, oral administration, intramuscular administration, or masculinization. Conversely it is a major constraint for scientists to find out the optimal dose for improving the health status of fish and avoiding the possibility of immunosuppression. The observed mode of action in several fish species fed with functional diets containing various medicinal herbal plants is shown in Table 18.1.

Mohammadi et al. recommended that 5–10 g/kg of dietary Pistacia vera hulls polysaccharide can improve growth rate, immune response, antioxidant activity, and resistance towards *A. hydrophila* in the fish species Nile tilapia (Mohammadi et al. 2020). 3.0 g/kg cinnamon nanoparticles (CNP) at optimum dosage level can enhance digestive enzymes activity, growth performance rate, antioxidant activity, and nonspecific immunity of the Nile tilapia fish species and conferring protection against *A. hydrophila* infection. When fish is fed with 3.0–10.0 g CNP/kg diet, there is no observation of fish mortality after bacterial challenge test (Abdel-Tawwab et al. 2018b). In addition, supplementation of *Nile tilapia (Oreochromis niloticus)* with purslane diet provides improved protection against *A. hydrophila* infection and enhances the respiratory burst activity and lysozyme levels. On the other hand, dietary purslane decreases the NO levels in *Nile tilapia* over the control fish. Contrastingly, no significant changes of MDA and SOD levels were observed after 10 days of the bacterial infection, while GPX and CAT levels notably increased. Dietary purslane supplements directly reduce the fish mortality rate and protect the fish against *A. hydrophila* infection in a dose-dependent mode (Abdel-Razek et al. 2019). Yin et al. evaluated the optimal dosage effect of two Chinese herbs (*Scutellaria radix* and *Astragalus radix*) and their influence on nonspecific immune response of tilapia. The *Scutellaria radix* supplemented diet dosage level of 0.5 and 1.0% and 0.1% had respectively less inhibitory effect or no effect on phagocytic activities and lysozyme level. On the other hand, the doses of *Astragalus radix* fed at optimum range of 0.1 and 0.5% for the duration of 3 weeks improves resistance against diseases in fish (Yin et al. 2006).

Extract from Clove basil (*Ocimum gratissimum*) leaves supplemented diet fed to African catfish significantly enhances growth specific rate, innate immunity response, and also improving resistance against *Listeria monocytogenes* infections (Abdel-Tawwab et al. 2018a).

Table 18.1 Functional diets from the source of herbs with mode of action

S. no	Medicinal herbs	Fish species	Mode of Action	References
1.	Astragalus root (*Radix astragalin seu Hedysari*) and Chinese Angelica root (*Radix angelicae sinensis*)	Large yellow croaker, *Pseudosciaena crocea* (Richardson)	Elevating nonspecific immunity and disease resistance against *Vibrio alginolyticus*	Jian and Wu (2003)
2.	Cinnamon *(Cinnamomum zeylanicum)*	Nile tilapia	Growth-promoting effect and antibacterial activity to *A. hydrophila* infection	Ahmad et al. (2011)
3.	Chinese Angelica root and Astragalus root	Jian carp (Cyprinus carpio var. Jian)	Elevating nonspecific immunity	Jian and Wu (2004)
4.	Garlic *(Allium sativum)*	*Labeo rohita* fingerlings.	Stimulating immune response and disease resistance against *A. hydrophila*	Sahu et al. (2007)
5.	*Prunella vulgaris* extracts	Olive flounder (*Paralichthys olivaceus*)	Promoting innate immune response and disease resistance against *Uronema marinum*	
6.	*Lactuca indica* extract	*Epinephelus bruneus*	Enhancing nonspecific immune reaction and resistance against *Streptococcus iniae*	
7.	American ginseng *(P. quinquefolium)*	Nile tilapia *(Oreochromis niloticus)*	Growth-promoting effect and disease resistance against *A. hydrophila* infection	Mohsen Abdel-Tawwab (2012)
8.	Leaf extract from *Tinospora cordifoliaMiers*	*Oreochromis mossambicus*	Improving nonspecific immunity (neutrophil activity), specific immunity (antibody response) resistance towards *A. hydrophila*	Sudhakaran et al. (2006)
9.	*Azadirachta indica*	*Oreochromis mossambicus*	Enhanced antibody response and leukocyte count	Logambal and Michael (2000)
10.	*Bidens pilosa, Lonicera japonica* and *Cyathula officinalis*	Cobia *(Rachycentron canadum)*	Promoting growth performance rate and nonspecific immune responses	Lee et al. (2020a b)

18.8 Immunosuppressive Effects of Herbal Medicines

The application of herbal medicines as immunosuppressants shows prospective effects in aquaculture not only for treating immune mediated disorders including autoimmune diseases by altering expression level of the immunoregulatory genes and by inducing apoptosis in the T cell differentiation but also for reducing mass mortality rate in the aquaculture industry. According to the primary mode of action of the immunomodulatory agents, it can be categorized as immunostimulants and immunosuppressants, whereas the immunosuppressants can suppress the immune system by different aspects including inhibition of nucleotide synthesis, inhibition of transcription, inhibition of differentiation factors, and inhibition of growth factor signal transduction. Mostly, the immunosuppressors hinder the activation of immune defense mechanism or reduce the level of expression of its components, thus restoring normal state of immune tolerance. The immunosuppressors are of great deal in the pathogenesis of autoimmune diseases and organ transplantations in mammals (Cañas and Cañas 2012).

Fish supplemented with the powder from pomegranate (*Punica granatum*) peel has diversified effects including oxidative stress activity and immune suppressant activity when it is exposed with silver nanoparticles (AgNPs) in Nile tilapia (*Oreochromis niloticus*) (Hamed and Abdel-Tawwab 2021). There is limited data available for the effect of stressors on fish immunity. Precisely in *F. heteroclitus*, the stress factors make changes in the number of WBC cells, signal transduction pathway, or alterations in the cell products, ultimately leading to more susceptibility towards infectious diseases.

18.9 Conclusion with Future Prospects

Herbal biomedicines have been found to be an alternative approach to chemotherapeutics and antibiotics for treating infectious diseases and largely contributing for the disease control in aquatic animals. Over the past decades, the dominance of infectious diseases could be a great dealing both human as well as animals due to antibiotic resistant another side effects influencing disease in aquatic animals. Nowadays, medicinal plants have high research priority among scientists for treating the infectious diseases as well as suppressing the undesired effects caused during immune defense mechanism. These medicinal plants can be used for numerous beneficial effects such as antimicrobial activity, immunosuppressive effect, hematological and rehabilitative features, immunostimulant activity, growth-promoting factor, appetite stimulant activity, aphrodisiac activity, antimicrobial activity, anti-stress activity, and antioxidant activity. This chapter provides an innovative outlook for using herbal plants as immunostimulants and immunosuppressors supplemented with fish food for protecting from infectious diseases. Thus, if herbal plants as immunostimulants will be an emerging trend for controlling outbreaks of disease, we can reduce the maximum numbers of mortalities. Furthermore, optimal dose determination, feeding protocols, and active principle behind the use of bioactive compounds in the herbal extract as immunostimulants and immunosuppressors will shed light on the new era of herbal biomedicines in the aquaculture industry. Undoubtedly, more number of scientific evidences is needed to clarify the immunosuppressive effects of herbal plants in the aquaculture industry for making contribution seriously to aquaculture activities. Several food additives and modifiers can be used for modifying the biological activity of immunoregulatory genes in fish, particularly herbal plants can enhance the overall health status of fish species especially farmed in aquacultures.

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Chapter 19
Bacterial Fish Diseases and Treatment

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Abstract Fish food is an inevitable item in human consumption with healthy source of good quality proteins and fat. Aqua industry can help to improve food security, livelihoods for the poorest and to meet the world's food demands. But producing more seafood that is at affordable cost with rich nutrition is challenging for aqua industry. Many factors affect the productivity of aqua industry; one such an important constraint is bacterial diseases. Hence, Aqua industry, a booming business sector, immensely requires continued research with scientific and technical developments, and innovations. Study of bacterial fish disease is one such thrust area which requires intense research to understand the causes and control bacterial diseases in fish. The appearance and development of a fish disease is the result of the interaction among pathogen, host and environment. An insight into bacterial fish diseases, clinical symptoms and treatment may help to manage the bacterial diseases and so can make aqua industry a more profitable field. This chapter deals with different aspects of the most threatening bacterial diseases, occurring in farmed fishes and also in wild fishes, which are results in fish loss and economic loss worldwide. A wide range of gram positive and gram-negative bacteria causing bacterial diseases, clinical symptoms, diagnosis, treatment, vaccines and the nature of water habitat are also discussed in this chapter.

Keywords Bacterial fish diseases · Gram-positive bacteria · Gram-negative bacteria · Clinical signs · Diagnosis · Treatments

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"A disease is the sum of the abnormal phenomena displayed by a group of living organisms in association with a specified common characteristics by which they differ from the norms of their species in such a way as to place them at a biological disadvantage".

(Campbell et al. 1979)

19.1 Introduction

Water is a habitat for enumerable organisms ranging from microbes to blue whales. Many bacterial species dwell in water as saprophytes in sediments, plants, phytoplankton, and zooplankton. Some of them reside on the skin, gills, and digestive tract of fish, and they live there with mutual beneficial. Fish living in different environments like river, brackish, and marine water and which are reared in farms are prone to various communicable infections caused by a phylogenetically different group of bacterial species (Sudheesh et al. 2012). These microorganisms could also spoil and harm fish health and hence generally considered as pathogenic. In the different growth stages of fish, bacterial diseases have been frequently found in eggs, fry, and fingerlings, thus leading to heavy mortality. These microorganisms are typically harmful pathogens that invade the tissues of a fish host susceptible to infection by various stress factors (Ahmed and Kumar 2005).

Intercommunications between fish and bacteria and the infections caused by microorganisms are the field of research throughout the world, and this plays an important role in fish pathology (Pękala-Safińska 2018). Infectious bacterial diseases are one of the major limiting factors to aqua-industry and for economic and socio-economic development in India and including many other countries of the world (Bagum et al. 2013). Bacterial diseases affecting aqua-industry are mainly due to the intensification of industry without knowledge of the host, pathogen, and environmental factors (Bondad-Reantaso et al. 2005).

The outbreaks of diseases either begin with a sudden increase in an infection leading to high mortality (acute disease) or may develop slowly, with moderate severity for a longer period (chronic disease). A total of 15 different common bacterial diseases have been reported. The main diseases covered are vibriosis, pasteurellosis, enteric red mouth (ERM) disease, furunculosis and marine flexibacteriosis. Other significant diseases, with lower reports, include rainbow trout fry syndrome (RTFS), columnaris disease, motile aeromonas septicaemia, pseudomoniasis, streptococcosis, mycobacteriosis, epitheliocystis, and rainbow trout gastrointestinal syndrome (RTGS) (Toranzo 2004). There has been a constant increase in the number of bacterial species associated with fish diseases, with new pathogens regularly recognized in the scientific literature (Austin and Austin 2012).

19.1.1 Aetiology

Water ecosystems are considered to be a major habitat for aquatic animals. Various changes occurring in water ecosystems seem to be a basic factor in the development of disease, including emerging ones (Johnson and Paull 2011). The evolutionary changes that occur in fish infection mainly depend on the climatic conditions that exist in a given territory, region, or country. In addition to climatic conditions that cause fish infection, external factors such as environmental conditions, water contamination, hypoxia, stress, etc. may also lead to infection (Vijayan and Sanil 2012). Many of these bacterial pathogens are omnipresent in the water ecosystem. Stress can compromise the fish's immune system, and cause them to become more susceptible to the infection with bacteria. Stress is the major causative factor that induces infection in fish, which allows them to succumb to death more easily (Romero et al. 2012). The agents of stress are lowered water quality, overpopulation, redundant treatment and transportation, poor nutrition, and other pathogens. The disease condition depends upon the persistence of the stress varying from mild to severe. Before the onset of any clinical symptoms related to infection, there may be evident damage to the host. The isolation of bacteria from a diseased fish is taken as evidence of infection (Stevenson 1978) (Fig. 19.1).

19.1.2 Factors Affecting Aqua-Industry

Aqua-industry requires good quality of water that includes physical, chemical, and biological factors. The chemical parameters found in pond water, such as increased turbidity, temperature, salinity, pH, water conductivity, and low dissolved oxygen (FAO 2018; Jacobs and Chenia 2007; Nadirah et al. 2012), may induce changes within the water grade which greatly affect fish growth, reproduction, and survival. Acclimation helps us to know the cause clearly why healthy fish get sick under the identical condition at another time. For example, fish can remain healthy when the

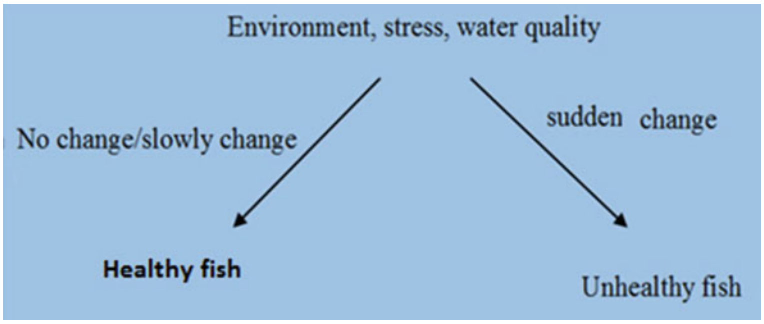

Fig. 19.1 Aetiology of bacterial diseases of fish

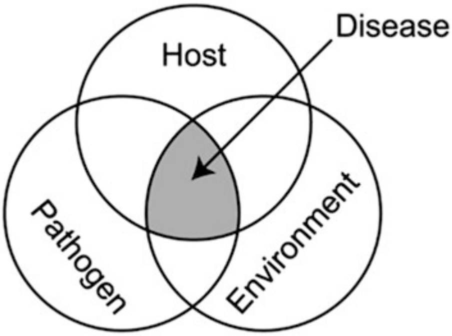

Fig. 19.2 Environmental factors and stresses contribute to outbreak of disease in fishes

pH slowly drops from 7.0 to 5.5 over several months, but at the identical time when the pH rapidly gets back to 7.0, the fish may die. The fish can survive in both acidic and basic pH and it is able to tolerate both conditions if they are introduced slowly to the standard environment.

Worldwide there are more than 13 types of bacterial genera that have been reported to cause bacterial diseases in the aqua industry (Pridgeon and Klesius 2012). The genera associated with freshwater bacterial diseases are Aeromonas, Pseudomonas, Edwardsiella, Flavobacterium, and Streptococcus. Bacteria that cause disease in marine fish are gram-negative. The most commonly associated organisms are genus Pseudomonas, Vibrio, and Myxobacteria (Fig. 19.2).

19.1.3 Types of Diseases

19.1.3.1 Fin Rot

Fin rot is a secondary bacterial infection that is caused by poor water conditions. The major etiological organisms are *Vibrio ichthyodermis or Pseudomonas ichthyodermis*. The clinical signs of this infection include frayed or reddened fins and tails. The infection may also lead to total destruction of the fins in extreme cases. In advanced cases it may become a stromal tumour of uncertain malignant potential (STUMPS). Minute haemorrhages and subsequent ulceration of the skin occurs in more serious infections.

19.1.3.2 Vibriosis

It is a type of internal infection caused by gram-negative bacteria Vibrio. The highest incidence of infection occurs in larval and juvenile stages of marine species, mainly seabass and seabream varieties (Toranzo et al. 2005). The disease is transmitted through contact with open wounds or stagnant fish, and once a fish is infected, the disease tends to progress quickly. Since vibriosis is an internal infection, fishes affected by this disease do not show any visible symptoms until the final stages of the disease. The clinical signs with some visible symptoms of this infection include red stripes on the body, red blemishes, dark swollen wounds, and cloudy eyes. Sometimes certain behavioural changes also occur with the symptoms like lethargy, loss of appetite, and difficulty in breathing. The most efficacious therapy for this infection is oral antimicrobial drugs such as kanamycin.

19.1.3.3 Tuberculosis

Mycobacterium species can infect mammals, birds, and reptiles. Some of its species will cause a localized infection, while others cause systemic disease. Mycobacterium that affects fish is called "fish tuberculosis" or wasting disease". Fish tuberculosis is more prevalent in marine fish species than in freshwater tropical fish. Fin erosion, ulceration on the body, loss of appetite, erythema of the skin, and apathy are the major clinical signs. The disease can be treated with antibacterial medicines which are effective in mild cases. Both vibriosis and fish tuberculosis can be communicated easily to humans through open injuries or sores. Careful handling is necessary while treating infected fish.

19.1.3.4 Photobacteriosis

Photobacteriosis is caused by *Photobacterium damselae sub sp. piscicida,* a halophilic member of the Vibrionaceae family. The disease was reported in several Mediterranean countries, the United States and Japan. Transmission of this disease is through the ovarian and seminal fluids from healthy brood stock. The bacterium is able to infect its host through the gills, gastrointestinal tract, and possibly through the skin. Young fish are more susceptible to the pathogenic organism (Toranzo et al. 2005). Seasonal changes that take place in water temperature are responsible for the epidemiology of the disease. At temperatures, lower than 21 °C mortality may decrease but fish become disease carriers (Magarinos et al. 2001). The infected fishes may develop surface darkening, and in several cases, a small red spot appears on the head, gills, operculum, and fin bases due to bleeding into the skin. The infection progresses quickly into acute septicaemia with splenomegaly.

Spleen is the organ of choice for isolation. Characterization and identification of pathogens is carried out using tryptic soy agar (TSA), brain heart infusion agar (BHIA), or blood agar, supplemented with 1–2% NaCl. In advanced cases, typical foci of bacterial microcolonies and severely compromised tissue appear as whitish spots and patches on the spleen surface. Histologically, multifocal necrosis is seen in spleen and liver tissues (Toranzo et al. 1991).

19.1.3.5 Edwardsiellosis

Edwardsiellosis is a systemic disease that affects freshwater and marine water fishes. The causative organism *Edwardsiella tarda* is a gram-negative, motile rod with peritrichous flagella and it belongs to the classification of Enterobacteriaceae. *Edwardsiella tarda* has a worldwide distribution and can be found in the intestines of fish and other marine animals. It is a primary opportunistic enteric bacteria that can survive in phagocytes (Srinivasa Rao et al. 2003). Clinical symptoms of edwardsiellosis differ from species to species of infected fish which may include discolouration of the skin resulting from bleeding underneath, caused by bruising. Ulceration, fin and tail erosion, and, occasionally, exophthalmia and cataracts are observed. The gills appear pale and inflamed. Haemorrhage occurs in internal organs oedematous. The kidney appears enlarged. Whitish granulomatous-like lesions, often visible on their surface, may gradually liquefy into large abscesses and spread into the surrounding musculature.

19.1.3.6 Flexi Bacteriosis

This disease is caused by *Tenacibaculum maritimum* (Flavobacteriaceae) which is an opportunistic gram-negative bacterium commonly found in seawater (Salati and Cubadda 2005) formerly known as Cytophagamarina or Flexibacter marinus or maritimus. The disease is called by various names like “gliding bacterial disease”, “eroded mouth syndrome”, and “black patch necrosis”. Any changes in climatic conditions or fluctuations in water temperature, environmental stressors may trigger the development of the disease (Toranzo et al. 2005). The infected fish develops collapsed and bleeding mouth, fins and tail appear shredded and centres of gills may decay. Several lesions may also occur in the skin. The disease can also infect internal organs of infected fish (Toranzo et al. 2005). Skin and fin abrasions (e.g. following netting or rough handling during grading procedures in nurseries, lesions as a result of feeding activities by parasitic flukes on gills or isopods on the skin) are the major clinical signs that occur quickly.

19.1.4 Classification of Bacterial Fish Diseases

The flowchart (Fig. 19.3) illustrates the classification of fish diseases based on the type of bacteria and nature of water habitat.

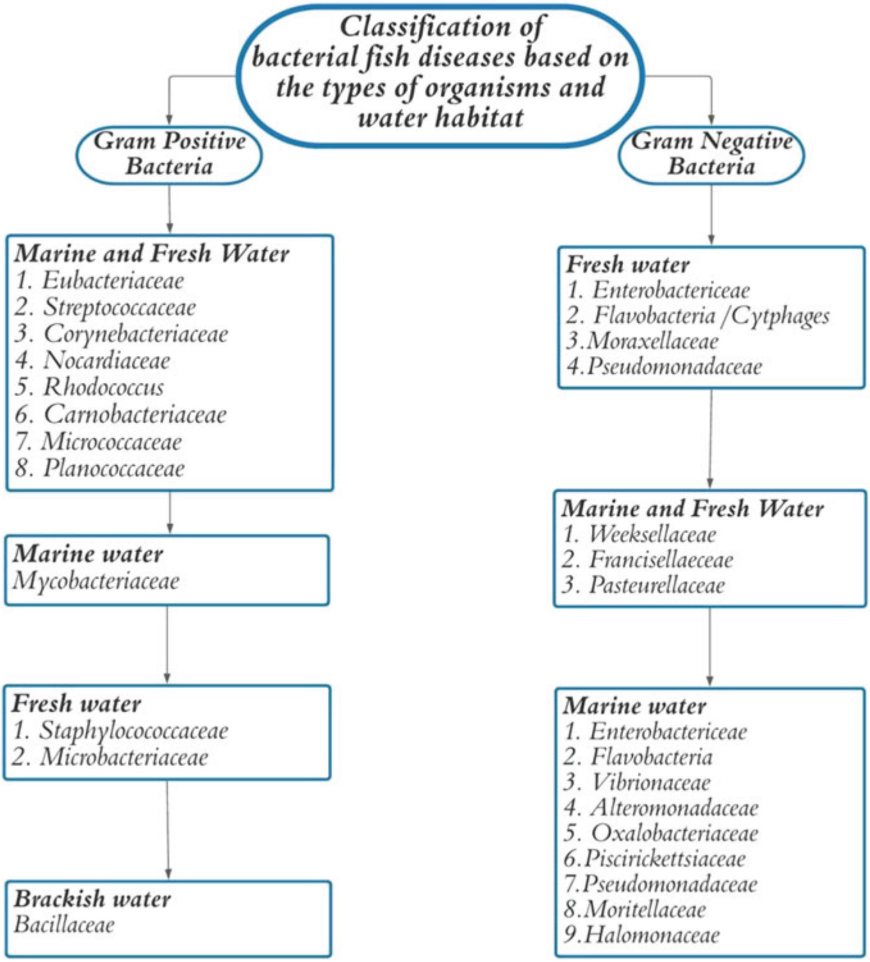

Fig. 19.3 Classification of bacterial diseases based on organism and nature of water habitat

19.2 Gram-Positive Bacteria

19.2.1 Bacterial Diseases of Marine and Freshwater Fishes

19.2.1.1 Eubacteriaceae Representative

Eubacterium tarantellae species has been classified as the anaerobic gram-positive bacteria which have been found to cause a neurological disease known as "Eubacterial meningitis" in marine and river (freshwater) fishes (Austin 2011).

Clinical Signs

Fishes affected by *Eubacterium tarantellae* show twirling in water, darkened pigmentation, uncoordinated swimming movements, and inability to orient themselves which may lead to death of fish. Gram-positive, filamentous anaerobic bacteria are identified by examining the brain tissue smears from the infected fish. Fish deaths due to meningitis caused by gram-positive anaerobic bacteria have been observed in several species of fishes like striped mullet (*Mugil cephalus*), snook (*Centropomus undecimalis*), and gulf flounder (*paralicthys albigutta*) (Austin 2011).

Diagnosis

Gram-positive, filamentous anaerobic bacteria identified by examining the brain tissue smears from the infected fish. To confirm the infection of filamentous and gram-positive anaerobic bacteria, the brain tissue of the fish is examined under a microscope. For diagnostic purposes, isolation and analysis of the causative agent or a direct fluorescent antibody test is carried out. The organism is further characterized by the production of haemolysins and lecithinase and also detecting the presence of lactate acetate in the fermented products.

Treatment

Treatment involves chloramphenicol, erythromycin, novobiocin, penicillin, and tetracycline which is given orally at a dosage of 100 mg/kg per day for 5 days.

19.2.1.2 Streptococcaceae Representative

The gram-positive cocci belonging to the genera *Streptococcus* and *lactococcus* are considered as the important fish pathogens all over the world. There are several different species of gram-positive cocci, including *Lactococcus garvieae, Streptococcus difficilis, Streptococcus iniae, Streptococcus. parauberis, Streptococcus phocae, Lactococcus piscium, Vagococcus salmoninarum,* and *Carnobacterium piscicola.* The disease caused by *Enterococcus seriolicida/Lactococcus garvieae* is called lactococcus or enterococcal infection or enterococcus or streptococcosis (Mata et al. 2004).

Streptococcosis is a “septicaemic disease” that infects both freshwater and seawater fishes as well as warm- and cold-water fishes. The disease is also known as red boil disease which has been first reported in rainbow trout (*Salmo gairdneri*). Red boil disease mainly affects Golden shiners, Japanese eels, Rainbow trout, Yellow tails, Striped mullet (*Mugil cephalus)* and Hardhead catfish (*Arius felis*). *Lactococcus garvieae does* not affect the internal organs of Golden shiners but produces skin lesions on the body surface. Severe damage is found in the spleen, intestine, and liver with the accumulation of ascitic fluid in the peritoneal cavity (Mata et al. 2004).

Clinical Signs

The signs of the disease include lethargy, inappetence, swollen abdomen, stomach and intestine filled with yellowish fluid, and in some fish slight haemorrhaging in the eye in *Streptococcus difficilis* infection. Septicaemia along with the brain damage including meningitis occurs in *Streptococcus iniae* infection. Discolouration, loss of orientation, corneal opacity, and internal and surface haemorrhaging lead to death by *Streptococcus iniae* serotype II infection. During summer, weight loss, haemorrhage on the anal, abdomen and pectoral part, pale liver, and congested kidney develop in *Streptococcus parauberis* infection. *Enterococcus* and *Lactococcus* are characterized by haemorrhagic septicaemia. Diseased fish show typical signs of lethargy, anorexia, loss of orientation, and bilateral exophthalmos. Other signs are petechiae on the inside wall of the operculum and congestions of blood vessels in the pectoral and caudal fin (Mishra et al. 2018).

Diagnosis

Enterococcus and *Lactococcus* species are diagnosed by gross appearance which includes haemorrhage of liver, kidney, brain, intestine and spleen. The haematocrit values are significantly lower than that of healthy fish. The above characteristic signs and symptoms indicate the presence of gram-positive cocci in the internal organs and are further confirmed by isolation and determination of the cultural characteristics of *Streptococcus.*

Treatment

The infection can be treated by using antibiotic sodium nifurstyrenate 50 mg/kg/ day which can control streptococcal infection in yellow tails. Oxytetracycline and chloramphenicol at 50 mg/gallon can treat streptococcal infection in golden shiners. Erythromycin at the rate of 25 mg/kg of fish for 4–7 days is used to control the streptococcal infections in yellow tails, which will give better results than daily treatments of Oxytetracycline or ampicillin. Oral administration of peptidoglycan seems to increase the resistance of disease in yellow tails. Recently the use of probiotic bacteria has shown good results in controlling *Enterococcus seriolicida* and *Lactococcus garvieae* infection in rainbow trout.

19.2.1.3 Corynebacteriaceae Representative

Corynebacterium is responsible for “bacterial kidney disease (BKD)” which is a systemic infection that causes high mortality among feral and farmed salmonid fish dwelling in river water and marine water. The disease is chronic but sometimes there may be acute outbreaks. The disease was first reported in the United States by Belding and Merrill in 1935. The pathogen is small, non-motile, a sporogenous gram-positive diplobacillus. Based on morphology, the organism was classified under *Corynebacterium or Renibacterium salmoninarum*. BKD infects progressively because the pathogen is very slow-growing. It is also called Dee disease, White boil disease, Salmonid kidney disease, or Corynebacterial kidney disease (Eissa et al. 2006).

Clinical Signs

The pathogen can be transferred from one fish to another fish and also from adults to their progeny via eggs. Infected fish take a few months to show the signs of disease, so BKD is one of the most difficult diseases to treat. Exophthalmia and small closed blebs or open lateral lesions are typical external signs. The lesions may form large shallow ulcers. Internally, the kidney is the most affected organ by this disease and it also has a swollen abdomen. In some cases, haematopoietic, excretory functions are affected and the kidney is also destroyed.

Diagnosis

The typical signs of the disease and gram-positive Diplobacilli infected tissue which constitutes the presumptive diagnosis. Acute and rapid serological diagnosis is through immunodiffusion-specific antisera and definitive diagnosis is through the lesions in the kidney of the infected fish. In addition, Coagulation test, Indirect and direct fluorescent antibody tests have been developed for rapid diagnosis. Diagnosis can be made within 10 min by direct and within 2 h by indirect fluorescent antibody tests.

Treatment

Erythromycin is given orally at the rate of 9–10 g/kg/day for 3 weeks which will provide partial control over the disease. Sulphonamides fed at 4.2 g/100 kg/day have been successfully used for prophylaxis in salmon. Since BKD is a transmitted disease, avoidance is the most preventive way to treat the fish and prevent transmission by way of the eggs (Hirvela-Koski 2005).

19.2.1.4 Nocardiaceae Representatives

Nocardia Species

Nocardia species are gram-positive, catalase-positive and rod-shaped bacteria that infect river and marine fishes to cause Nocardiosis. It is a long-term and problematic bacterial infection of warm-water fish. The disease finally acts as a major weakening factor in many types of fish. The infected fish often have other secondary infections also. Nocardiosis embarks as a silent infection, which can be unidentifiable for months in fry or juvenile fish. The infection is chronic and the species multiply slowly within fish tissues before any visual symptoms. In some cases lethargy and the death rates also increase. The symptoms of nocardiosis are similar to mycobacteriosis (Wilson 2012).

Clinical Signs

Fishes of all age groups may be infected by this disease with symptoms like lesions, exhibited as small white spots, present in the dermis, muscle, gills, and internal organs. The visual symptoms of this disease are diverse. The visible lesions are found in skin nodules and skin, whereas the opercular lesions are found as fleshy white masses at the base of the gills. As nocardiosis is similar to mycobacteriosis, the internal pathology diagnostic procedures are easily confused especially if mixed infections exist.

Diagnosis

Nocardiosis may be misinterpreted as mycobacteriosis, because of the correlation between the clinical symptoms and pathology linked with the two diseases. Isolation, identification, and histological sections of the infected organs are selected for diagnostic purposes. Nocardioform bacteria can grow on similar kinds of media, with an incubation period ranging from 2 to 14 days.

For the preliminary assessment a thorough visual examination of the fish is needed. The skin and body wall was examined for lumps and ulcers. The presence of dry, grey-yellow, inspissated pus under the skin nodules is one of the basic diagnostic confirmations. Also, pale gills will be seen under the operculum and irregular whitish lumps at the base of the filaments. The colony of nocardia appears as folded, granular, or powdery, pinkish-white to yellow-orange or light brown colonies with aerial mycelium around the edges. For diagnosis Gill, kidney, or spleen imprints or stamps collected (in duplicate), dried, and stained using Grams or an acid-fast stain. 5 mm sections of the infected tissues are used for histological diagnosis and must be preserved in 10% buffered formalin.

Nocardial granulomas without the epithelioid cells in the earliest stages of development are confused easily with piscine mycobacteriosis. It can be confirmed furthermore by the presence of acid-fast organisms in the *nocardia* lesion which show a positive reaction for *nocardia* with Fite-faraco acid-fast stain. There are different types of specific and sensitive methods that have been applied recently for diagnosis, including antibody-based and DNA-based methods (Wilson 2012).

Treatment

Antibiotics such as streptomycin and sulphisoxazole have been used for treating Nocardiosis. Treatment with trimethoprim and sulphamethoxazole at 40 mg/l mixed in feed along with the addition of benzalkonium chloride at 2 mg/l in water decrease the mortality rates of the infected fish. Antibiotic doses need to be high and the duration of treatment must be extended for a prolonged period. The application of protective therapies applied to asymptomatic young fish may be beneficial. Restriction through husbandry and good management methods is the best way to prevent nocardial infections.

19.2.1.5 Rhodococcus Representatives

Rhodococcus Erythropolis

Rhodococcus is a genus of aerobic, non-sporulating, non-motile, gram-positive bacteria which is closely related to Mycobacterium and Corynebacterium. Low level mortalities are recorded in fishes infected by Rhodococcus species. Chinook salmon are a type of anadromous fish (they hatch in freshwater streams and rivers then migrate out to the saltwater environment of the ocean to feed and grow) (Olsen et al. 2006a, 2006b).

Clinical Signs

In Canadian farmed chinook salmon, there is a development of melanosis and ocular oedema, leading to a break in the cornea. There was no other evidence of involvement of any internal organs. With Atlantic salmon, the presence of granulomas in the kidneys is found to be apparent (Olsen et al. 2006a, 2006b).

Diagnosis

Characterization and Identification of Pathogens

Two colonies with denser growth are obtained from diseased tissue following inoculation with blood agar, MacConkey agar, and TSA with incubating at 19 °C for 14 days. Both the colonies are from the organs (Kidney and spleen) of chinook salmon with ocular lesions.

Treatment

Glycopeptide antibiotics (vancomycin, teicoplanin), rifampicin, quinolones, aminoglycosides, carbapenems, and macrolides are effective for this infection.

19.2.1.6 Carnobacteriaceae Representative

Carnobacterium Piscicola

Carnobacterium belongs to gram-positive bacteria. Pseudokidney disease is provoked by Carnobacterium piscicola. This organism can be isolated from rainbow trout, cutthroat trout, coho, and salmon. All river and marine water fishes are susceptive to stress conditions. Lactic acid bacteria alone function as probiotics to fishes. Others are pathogenic to fish (Leisner et al. 2007).

Clinical Signs

Visible signs of infection outside the body are abdominal distension, redness at the base of fins, and subdermal blood sores, and within the body, the liver, spleen, and nephrons are enlarged. Accumulation of ascitic fluid in the peritoneal cavity is common. There are also haemorrhages in the reproductive system of the male, intestinal tract, and muscle. A grey pseudomembrane may be present which resembles BKD infections. Other visible signs of infection outside the body like fin rot were absent. Yet, muscle haemorrhage and hyperaemic air bladder are seen. The heart and gills appeared normal. The disease affects fishes that are greater than a year old and have undergone pressure, during handling and spawning. The general signs and symptoms are diverse which comprise septicaemia, the abdomen distended with ascitic fluid, muscle abscesses, blood blisters below the skin, and internal haemorrhaging.

Diagnosis

Characterization and Identification of Pathogens

Diagnosis is by isolating and characterization of the causative organism. Preliminary isolation is from kidney or lesion using tryptone soya agar (TSA) or Brain heart infusion agar (BHIA) grown aerobically at 15–24 °C for 24–72 h.

Presumptive Diagnosis

Colonies appear pinpoint, opaque, entire, circular, and non-pigmented when grown on TSA. The organism is a non-motile, non-spore-forming, non-acid fast, facultatively anaerobic, gram-positive rods or coccobacillus. The size is 1.1 to 1.4 × 0.5 to 0.6 μm. Other phenotypic analysis showed negative results for biochemical analysis like urease, oxidase, catalase, H_2S, nitrite reduction, and lactose and xylose fermentation. The organism shows positive reactions for arginine dihydrolase and lactic acid production from glucose (no gas), maltose, mannitol, and sucrose.

Confirmatory Diagnosis *Carnobacterium piscicola* can be differentiated from the other *Carnobacterium species* by the fermentation patterns using inulin and mannitol (Leisner et al. 2007).

Treatment

Erythromycin is given as treatment for Lactobacillus-Carnobacterium strains (Michel et al. 1986; Baya et al. 1992).

19.2.1.7 Micrococcaceae Representative

Micrococcus leuteus

Micrococcus luteus is a gram-positive, non-motile, coccus, tetrad in arrangement, pigmented, a saprotrophic bacteria in the family Micrococcaceae. Signs and symptoms are related to Rainbow Trout Fry Syndrome.

Clinical Signs

Rainbow trout (marine fish) and brown trout (freshwater fish) fishes commonly are infected by *Micrococcus leuteus.*

Exophthalmia, enlargement of the abdomen, darkening of the skin, petechiae, and focal lesions are the signs seen in moribund fish.

In many fish inflammation of the intestine, liver obstruction, and bleeding in the tail part of the muscles will be present (Pękala et al. 2018; Austin and Stobie 1992).

Diagnosis

The organisms were cultured by using scrubbed medium (kidney, liver, spleen) pasteurized milk agar at 25 °C for 48–72 h (Austin and Stobie 1992). In some rainbow trout, a disease called micrococcosis infects the fish but the recognition of the etymological agent of this disease is undetermined (Austin and Stobie 1992).

Treatment

Antimicrobial complexes, i.e. streptomycin, chloramphenicol, and tetracycline, are used for the treatment. This complex is used to suppress the development of infections in the rainbow trout griddle (Austin and Stobie 1992).

19.2.1.8 Planococcaceae Representative

Planococcus Species

Planococcus is a gram-positive or gram-variable, cocci or short rod-shaped bacteria which infects marine and freshwater fishes. Motile gram-positive cocci, planococcus, causes rainbow trout fry syndrome (RTFS) or rainbow trout fry anaemia. The organisms have been connected with a compact off-white 12.4 mm in diameter, disk-like concave patch on the heads of Atlantic salmon (Austin 1985).

Clinical Signs

Large rainbow trout fishes weighing 500 g show symptoms like the accumulation of fluid in the kidney and little quantity of ascitic fluid in the abdominal peritoneal cavity. The fishes also show symptoms of anaemia, enlarged kidney, liver and broadened spleen (Austin and Stobie 1992).

Diagnosis

Characterization and Identification of Pathogens

By using BHIA, pasteurized milk agar and TSA, the bacterium can be isolated from kidney scrub and incubated at 25 °C for 7 days. After 48 h of incubation the organism produces off-white to yellow hoist glassy colonies (1–2 mm in diameter) (Austin et al. 1993).

Treatment

Using antimicrobial compounds: The compounds such as carbenecillin, penicillin G, erythromycin and tetracycline are subtle to the organism which may be virtual for chemotherapy (Austin et al. 1993; Austin and Stobie 1992).

19.2.2 Bacterial Diseases of Marine Water Fishes

19.2.2.1 Mycobacteriaceae Representative

Mycobacteria gram-positive, non-motile, acid-fast rods which are extensively spread in nature. They are copious in the soil, on the skin of vertebrates, on the surface of plants, in foods consumed by humans, and mammals. Mycobacterium have so many species, i.e. *Mycobacterium abscessus, Mycobacterium marinum, Mycobacterium neoaurum, Mycobacterium montefiorense, Mycobacterium shottsii, Mycobacterium gordonae*, and *Mycobacterium pseudoshottsi.* Some species of mycobacteria are extremely infectious and cause diseases such as tuberculosis and leprosy in humans. Mycobacteria that cause ailments in fishes differ from the causative organism for animals. Mycobacterial infections in fishes are called “mycobacteriosis of fishes”.

Mycobacteriosis is uncommon in freshwater food and recreation fishes. It is more persistent in wild marine fishes. Mycobacteriosis is a major disease in marine water aquarium fishes. It causes a chronic infection. Mycobacterium genus is a member of the order Actinomycetales and family Mycobacteriaceae; fish mycobacteria can grow in several types of media. Identification can be made using several criteria, including acid-fastness, growth rate, pigment production, colonial morphology.

19.2.2.2 Clinical Signs

The disease shows some physical and extreme changes like non-curing, slight to intense deep skin ulcers, corneal ulcers, discolouration, boniness, white nodules on viscera, loss of appetite, and exophthalmos. The internal signs are small greyish tubercles, or nodules in the liver, kidney, and spleen. Outbreaks occur in fishes of freshwater families like Anabantidae, Characidae, and Zebrafish. Previously it affected only salmonids but recently affects other species of cultured food fish such as tilapia, European sea bass, and striped bass. The initial Mycobacteriosis was noted in carp (*Cyprine carpio*). Increased infection is seen in goldfish (*Carassius auratus*).

19.2.2.3 Diagnosis

Characterization and Identification of Pathogens

Diagnosis of mycobacterium disease is relatively easy by using an acid-fast staining method. Lesions caused by these bacteria are rod-shaped, gram-positive bacilli used for routine diagnosis. Tissues can be smeared and stained, but less preferable. To isolate the organism it takes up to 30 days, for confirmation of the organism it takes up to 90 days. In some cases, mixed infection occurs due to several mycobacterium species being involved.

Treatment

Kanamycin is blended with food to effectively cure the disease. It is absorbed from water by fishes. The dosage of the drug is 0.01%. Other antibiotics such as erythromycin, streptomycin, and rifampicin are effective under experimental conditions.

19.2.3 Bacterial Diseases of Freshwater Fishes

19.2.3.1 Staphylococcaceae Representative

The species in the family of staphylococcaceae are *Staphylococcus aureus, Staphylococcus epidermidis*, and *Staphylococcus warmeri* (gram-positive).

Clinical Signs

The typical signs are corneal redness caused by the formation of new blood vessels which leads to opaqueness as in eye disease. Because of this disease tissues become degenerated, forming a hollow cup. The optic nerves will be affected and in turn the fishes show lethargicness and become dark. The internal organs are not infected. But the infection causes jaundice in African sharp-catfish (*Clarius gariepinus*). Infectious varieties of *Staphylococcus* sp. were first proclaimed from Japan, in yellow tail (*Seriola quinqueradiata*) and red sea bream (*Chrysophrys major*). The signs and symptoms are exophthalmia, congestion, and ulceration on the tail. The infected fish showed haemorrhage on the fins, gills, and opercula, with distended abdomen with ascitic fluid accumulation, and anaemic liver. The species was also isolated from moribund cultural grass carp and juvenile gilthead sea bream. Mortality is around 12% per day at normal water temperature. The species *Staphylococcus warneri* affects the rainbow trout in Turkey with exophthalmia and ulcerations on the fins. Clinical signs are that the abdomen was swollen due to accumulation of ascitic fluid.

The kidney showed normal architecture while the liver showed change in colouration.

Diagnosis

For diagnosis the eye and brain tissue can be examined. The external change in the fish shows that the fishes are affected by the infection.

Treatment

Treatment with erythromycin at a dosage of 20 mg/kg BW/day for 10 days showed good therapeutic function. Drugs like amoxicillin, erythromycin, and trimethoprim sulphamethoxazole are effective in in vivo experiments.

19.2.3.2 Microbacteriaceae Representative

Microbacterium Paraoxydans

Microbacterium belongs to the family Microbacteriaceae. It contains 96 species. From these 96 species, *Microbacterium paraoxydans* is one of the species which cause disease in fish. This bacteria promotes plant growth. The family microbacteriaceae encompasses a large number of aerobic gram-positive bacteria which have high G + C content. This character can be used to differentiate it from other actinobacteria which have both unusual B group cell wall peptidoglycan and unsaturated respiratory menaquinones.

The bacteria show variation in cell morphology like elongation of coccoid shape, rods are small and irregular and show branched fragmenting hyphae (Park et al. 1993; Stackebrandt et al. 1997). Microbacterium species are yellow pigmented; it is found in various environments like soil and water. The organism can be recovered from Nile Tilapia.

Clinical Signs

Lethargy, erratic swimming, melanosis, red or opaque eyes, scale loss, blindness, and exophthalmia are the symptoms included in this disease.

19.2.4 Bacterial Diseases of Brackish Water Fishes

19.2.4.1 Bacillaceae Representatives

Bacillus Species

The initial outbreak of *Bacillus* species disease led to mortality of farmed populations of *Clarias carpis, Clarias gariepinis, Clarias nigrodigitatus, Heteroclarias,* and *Heterobanchus bidorsalis.*

Clinical Signs

Diseased fish showed symptoms of dizziness, apathy, abnormally thin or weak and necrotizing dermal inflammation, death occurring in a few days. The peritoneal cavity consists of blood tinged fluid. Organs like liver and kidney showed evidence of infection like petechiae and focal necrosis. Other signs include splenomegaly with softness and friability. Myocardium also showed softness and flabbiness. The stomach shows an excess of blood supply in its blood vessels. Bacillary necrosis can be seen in farmed populations of catfish (*Panagasius hypophthalmus*). The signs of the disease are presence of white necrotizing and granulomatous areas (1-3 mm diameter) in the kidney, liver, spleen, and other visceral organs.

Bacillus Cereus

Bacillus cereus is an occasional fish pathogen causing bronchial necrosis in common carp and striped bass.

Bacillus Mycoides

Fishes, which are infected with *Bacillus mycoides,* appear darker in colour, lacked appetite, showed ulcers on the dorsal part of the fish, and focal necrosis is seen in the epaxial muscle.

Bacillus Subtilis

Bacillus subtilis is also a fish pathogen, causing bronchial necrosis in common carp.

19.2.4.2 Diagnosis

Characterization and Identification of Pathogens

Isolation of Bacillus species can be carried using nutrient agar and incubation can be at high temperature of 37 °C for an unspecified period. Sample for isolation can be taken from tissues with ulcers, tissues from brain, kidney, liver, and necrotic muscle.

It will be inoculated into a range of different media, like sheep blood in agar base, MH agar, and BHIA with incubation at different temperatures for an unspecified duration. The rhizoidal colonies with filamentous, swirling patterns will be seen.

The colonies are 1 mm in diameter, and are cream in colour on TSA medium after 24 hours of incubation at 28 °C. The organisms are gram-variable, slender and motile.

The biochemical characterization showed oxidase-positives, but non-reactive to sugars, and they favour growth at 15 °C to 37 °C, produce positive for hydrogen sulphide and break down gelatin.

19.2.4.3 Treatment

Bacillus species are sensitive to tetracycline but not penicillin. *Bacillus mycoides* show sensitivity to erythromycin, nalidixic acid, nitrofurazone, novobiocin, and oxytetracycline but not to ormetoprim-sulphadimethoxine.

19.3 Gram-Negative Bacteria

19.3.1 Bacterial Diseases of Freshwater Fishes

19.3.1.1 Enterobacteriaceae Representatives

Edwardsiella Ictaluri

Enteric septicaemia is seen among young catfish when the temperature is lower, which is during the month of September and October. *Edwardsiella ictaluri* infection mainly affects river water fish Pangasius hypophthalmus. The infected fish may swing enervated at a vertical position at the water surface just right before death (Crumlish et al. 2002).

Clinical Signs

Exterior signs are haemorrhages on the throat and mouth, exophthalmia and open sores on the head, especially on the frontal bone of the skull (Hawke 1979). This disease is also called as Bacillary necrosis of Pangasius because of the abrasion on the kidney, liver, and spleen (Crumlish et al. 2002).

19.3.1.2 Diagnosis

Characterization and Identification of Pathogens

From the infected fish kidney, liver, spleen, intestine brain, or muscle abrasion are isolated, then inoculated into Brain-Heart Infusion Agar. Then incubated at 26 °C for 48 h. The colonies are circular (2 mm), convex without pigmentation (Hawke 1979). The clear diagnostic approach is attained by the plasmid profiles for *Edwardsiella ictaluri* (Lobb and Rhoades 1987; Speyerer and Boyle 1987).

The serological identification of *Edwardsiella ictaluri* can be achieved by monoclonal antibodies of IiFAT (indirect immunofluorescence antibody test) as an immunoassay enzyme (Rogers 1981; Ainsworth et al. 1986). The use of rapid iFAT (immunofluorescence antibody test) at the same time can recognize two pathogens, i.e. *Flavobacterium columnare* and *Edwardsiella ictaluri* using two fluorochromes which have two different spectra properties (Alexa Fluor 488 discharge green while 594 discharge red fluorescence).

19.3.1.3 Treatment

Vaccination with multivalent *Edwardsiella ictaluri* antigens showed development of sensitive and resistant antibodies and T lymphocytes in channel catfish (Camp et al. 2000).

19.3.1.4 Preparation of Vaccine

Lipopolysaccharide (LPS) extract indicates secured action followed by injection (Saeed and Plump 1987). The defence action is consulted by multiple injections by using FCA with balanced LPC (liver progenitor cell) antigenicity. The systematic vaccine was achieved by engrossment of channel catfish at limited death rates (Plumb and Vinitnantharat 1993). The systematic vaccine results in 96.7% mortality in the control group, while the immerse vaccination decreases mortality to 6.7% and the immerse oral vaccination decreases to 3%.

19.3.1.5 Flavobacteria/Cytophages Representatives

Flavobacterium Species

Flavobacterium is a gram-negative, non-motile rod-shaped bacteria. *Flavobacteria* are found in soil and fresh water. Several species are known to cause disease in freshwater fish.

19.3.1.6 Clinical Signs

Epithelial swelling in gills is noticed and distal tip of adjacent Gill lamellae is fusioned. In some cases *Flavobacterium* sp. is associated with hyperplasia (Kudo and Kimura 1983a, 1983b).

19.3.1.7 Diagnosis

Characterization and Identification of Pathogens

The organism can be isolated using TSA supplements with Sodium chloride (0.5%-3.0%). Yellow-orange colonies will be formed after 24 h. They are gram-negative, motile, and pleomorphic; arginine dehydrolase and oxidase will be produced. But H_2S, indole and ß-galactosidase will not be produced. The Voges Proskauer results are positive (Acuigrup 1980). Several acids are produced by Arabinose, Inositol, Mannitol, and Melibiose.

Diagnosis

Flavobacterium has been diagnosed by the API20E Rapid Identification System (Acuigrup 1980).

Treatment

Oxytetracyclines are considered for the treatment of infected fish (Acuigrup 1980).

19.3.1.8 Flavobacterium Columnare

Columnare was first identified in a wide variety of freshwater fish comprising black bullheads, chub, eel, bass, goldfish, rainbow trout, salmon, sheath fish, white grappie, Chinook salmon and white suckers. Diseases caused by *Flavobacterium* *columnare* are seen in various stages. In young fish, there is an inconsequential pathology that occurs in the gills, which causes obstruction of blood vessels decoupled on the epithelium of lamellae. In adult fish, it causes abrasion in the gills or in musculature. On the body, at the end of the dorsal and pelvic fin, small abrasions begin, due to discolouration. The infected areas grow in size up to 3–4 cm in diameter and more than 25% of the total surface area of fish. For the identification of *Flavobacterium columnare* pathogen, Bootsma and Clerx's medium and cytophaga agar are prepared and used. As a result, the pathogen gives rise to yellow-orange pigmented colonies.

Diagnosis

Chemotaxonomic methods consist of whole cell body acids and commercialized design with dominant fatty acids is used for diagnosis.

19.3.1.9 Treatment

Vaccine Development

When heat-destroyed cells are used for vaccination to juvenile coho salmon via food, it leads to the production of antibodies (Fujihara and Nakatani (1971). In channel catfish, agglutinating antibodies are used as vaccines (Schachte 1978).

Management Techniques

To prevent the fishes from this disease is to keep the water temperature as cool as possible because at high temperature the condition of the columnaris is more severe. There was a high level of expansion and demolition at high virulent culture resulting in the destruction of gill tissue towards the base, which frequently occurs in Carp and Rainbow Trout.

Antimicrobial Compounds

NaCl (sodium chloride) baths are used for treating infections (Farkas et al. 1980). Other than sodium chloride, arsenic, cadmium, selenium mixture, copper, and lead at 1–3 µg/l for 1 day are also used (Macfarlane et al. 1986).

19.3.1.10 Flavobacterium Johnsoniae (Cytophaga Johnsoniae)

This disease was first identified among the farmed freshwater barramundi and Lattes Calcarifer in Queensland, Australia. Over a 2-week period 2–5% of moralities were noted at 27–28 °C. The affected fish expresses the signs of superficial erosion of skin and pectoral fins in the lower jaws (Carson et al. 1993). *Flavobacterium johnsoniae* reacquired from a variety of infected fish like, Skin ulcers, Gill necrosis, and systemic disease (Flemming et al. 2007).

19.3.1.11 Diagnosis

Characterization and Identification of Pathogens

The pathogen can be isolated on Cytophaga agar medium with the incubation period for 7 days at 27 °C (Carson et al. 1993). From phenotypic experiments *Flavobacterium johnsoniae* can be identified (Rintamäki-Kinnunen et al. 1997). These are gram-negative bacteria with gliding filaments with yellow colour colonies at 10–30 °C. These colonies produce ß-galactosidase, catalase, and oxidase. Nitrogen is utilized from asparagine, potassium nitrate, and ammonium, and acids are produced from glucose.

19.3.1.12 Treatment

Vaccine Development

From the Streptomycin resistance strain, a vaccine was developed and used for the treatment. Grass carp is protected by this vaccine method. Accordingly, I.P used for 28 days gives the best results in diseased fish (Li et al. 2015).

Antimicrobial Compounds

Effective compounds used for the treatment are acriflavine and oxolinic acid (Carson et al. 1993).

19.3.1.13 Moraxellaceae Representative

Acinetobacter Species

Acinetobacter is a gram-negative bacteria pertaining to Gammaproteobacteria. They give oxidase-negative, show convulsive movement and are present in pairs.

Schizothorax is a cyprinid fish found in south and central Asia. They are primarily found in rivers, streams and lakes. *Acinetobacter lwoffii* infection has been identified among the Atlantic Salmon and cyprinid fish in a group of 60 sexually matured in which the temperature was between 8 and 11 °C through the autumn 1978. Through the event of this disease, the fish which was 5–12 kg in weight were wild reserves from Surma and Norway. After the fifth week of disease, the aggregated transience numbered 92% of the total population.

Clinical Signs

Through, the clinical symptoms were visible only among 40% of the animals consisting of hyperaemia of blood vessels, haemorrhaging with oedema expanded from the base of the fins. Due to this, ulcers were developed, abrasions seen in kidney and liver, and little haemorrhages present in the air bladder (Roald and Hastein 1980).

19.3.1.14 Treatment

Antimicrobial Drugs

Oxytetracycline seems to be implied for chemotherapy (100 mg/fish), when delivered by intramuscular injection.

19.3.1.15 Moraxella Species

Moraxella is a gram-negative bacteria. The transience of this disease resulted in the Potomac River, Maryland, USA, among the juvenile, Morone saxatilis, through winter 1987.

Clinical Signs

This disease causes the gills of the fish to be damaged with Trichodina and Ergasilus. The affected fish shows symptoms like huge haemorrhagic abrasions and misplaced scales on the dorsal surface. The liver became bulge, pale and blotched in emergence. Haemorrhages were found to be present in the swim bladder. The layer substances emerge to attach the body wall with the liver (Baya et al. 1992).

Diagnosis

Characterization and Identification of Pathogens

Growth of round, elevated, luminous and sludge colonies grow at 22 °C for 48 h isolated from kidney, pancreas and liver on TSA medium (Baya et al. 1992). Considering that comparison to Moraxella bacteria was distinguished (Juni and Bøvre 2005), the organisms heavily look like the Acinetobacter. (Roald and Hastein 1980).

Treatment

Therapeutic Drugs for Microbes

The drugs Chloramphenicol, oxolinic acid, tetracycline, nitrofurantoin, and penicillin were effective against this bacterial species (Baya et al. 1992).

19.3.1.16 Pseudomonadaceae Representatives

Pseudomonas fluorescens

Pseudomonas fluorescens was first identified in the fresh water. This organism is identified in fish spoilages (Shewan et al. 1960). And also this organism primarily causes the damages in fish tissues (Otte 1963). However, the pathogenic capability was inferior. Many wide range of fish species get infected by this organism, including goldfish (Carassius auratus) (Bullock 1965), grass carp and black carp (Bauer et al. 1973), rainbow trout (Sakai et al. 1989), tench (Ahne et al. 1982), silver carp and bighead. The infected places were found to be eroded and this organism is mainly associated with tail rot or fin rot (Schäperclaus 1979). It has been reported to 90% of high mortalities recorded in tench fry.

Clinical Signs

Clinical signs have been visualized at the base of the skin, haemorrhagic lesions were noted. In the peritoneal cavity, ascitic fluid was accumulated. In gills, liver, kidney, and lumen and submucosa of the gut, petechial haemorrhages were noticed. Silver carp and bighead have shown the signs of stress and they were triggered by disease (Markovic et al. 1996). Ulcers and haemorrhage were reported in rainbow trout on the gills and fins (Sakai et al. 1989).

19.3.1.17 Diagnosis

Characterization and Identification of Pathogens

These organisms are gram-negative cultures which consist of oxidative rods that are produced by catalases and oxidases. And it exhibits motility by polar flagella. From thiositol, maltose, sorbital, xylose, arabinose, mannitol and sucrose, trehalose acids were produced. Citrate has become utilized. The Voges Proskauer test results were negative. Cultures were grown at 4 °C with the fluorescent pigments.

19.3.1.18 Treatment

On examination, isolates displayed organisms vulnerable to nalidixic acid (quinolone synthetic antibiotics), tetracycline, and kanamycin. These antibiotics prevent the bacterial cells from dividing and repairing, thereby killing them (Sakai et al. 1989). The next examination described extensive vulnerability to neomycin, gentamycin, kanamycin, amikacin, oxytetracycline, complete version of penicillin, erythromycin, chloramphenicol, and sulpha drugs (Markovic et al. 1996).

19.3.2 Bacterial Diseases of Freshwater and Marine Water Fishes

19.3.2.1 Weeksellaceae Representatives

19.3.2.2 Chryseobacterium Scophthalmum (Flavobacterium Scophthalmum)

These are gram-negative, uniformly rod-shaped organisms which form orange-pigmented colonies. Both freshwater and marine water turbot kind of fish are infected by this disease.

Clinical Signs

The disease causes gill hyperplasia, haemorrhaging in gills and lethargy and extensive internal haemorrhaging and distended abdomen in Turbot (Mudarris and Austin 1989, 1992).

Diagnosis

Characterization and Identification of Pathogens

The pathogenic bacteria was isolated from infected organs using K media (Mudarris and Austin 1989). The bacterial colonies produce oxidase and catalase. From cellobiose and glucose, acids are produced. The Voges Proskauer test shows negative. Casein, tributyrin, aesculin, and gelatin have been deteriorated. However, chitin or starch was not degraded. Thin sections of the colonies exhibit the presence of thick cell envelopes (Mudarris et al. 1994).

Treatment

Antimicrobial Compounds

Mortalities were controlled by using 50 mg/l immersion for 30 min daily for 10 days or furazolidone was used by i.p. injections with an average dosage of 50 mg/kg.

19.3.2.3 Pasteurellaceae Representative

Pasteurella Skyensis

The Pasteurellosis disease is caused by *Pasteurella skyensis*, a gram-negative, non-motile, rod-shaped, and pleomorphic bacteria which were isolated by culture on seawater blood agar. Pasteurellosis in Atlandic salmon can cause symptoms such as boils in skin, at the pectoral fin and internal organs and in some cases so-called "blood eye". The reproduction of Atlantic salmon occurs in rivers and then on maturation migrate to ocean.

19.3.2.4 Diagnosis

Characterization and Identification of Pathogens

Cultures were isolated from the kidney of infected fishes by using TSA culture medium supplemented with 15% of sea salts and 5% with defibrinated horse blood and incubated for 48 h at 20 ° C. Small grey colonies were formed (Birkbeck et al. 2002). Esterase, indole, lysine, oxidase (weakly), leucine arylamidase, ornithine decarboxylase, and naphthol-ASBI-phosphohydrolase are produced by gram-negative catalase-negative rods. Several acids are produced from carbohydrates but not from sucrose, galactose, raffinose, and rhamnose. It also has a weak haemolytic activity. The analysis of 16S rRNA sequencing of pathogen phenotypically, associated with *Pasteurella phocoenarum* family with 97% of homology. It clearly confirmed that it is closely related as phylogenetically (Birkbeck et al. 2002).

Treatment

Good sanitation and management procedures should be used to avoid overcrowding and other stresses that may predispose fish to disease. Prophylactic chemotherapy with sulphonamides, nitrofurans, or antibiotics has been employed successfully. Sulphonamides at 200–400 mg per kilogram of body weight per day or chloramphenicol at 20–40 mg per kilogram of body weight per day, both fed for a minimum of 6 days, are used to control outbreaks (Matsusato 1975). In vitro studies by Kusuda and Inoue (1976) showed that the antibacterial activity of ampicillin was 8–16 times that of chloramphenicol. They suggested that ampicillin would be useful in treatment of Pasteurella septicaemia. Vaccine is under trial and proposed to be completed in the later of year 2020.

19.3.2.5 Francisellaeceae Representative

Francisellosis is a disease caused by bacterial species belonging to genus Francisella infecting a wide range of animals especially fishes. Francisellosis in fish is characterized by the development of non-specific granulomas in spleen and kidney. This granulomatous infection is mainly caused by *Francisella noatunensis* and *Francisella noatunensis subsp. orientalis*. These are facultative intracellular gram-negative pathogens. TM agar (Soto et al. 2014) and modified cysteine heart agar are used for isolation (Lewisch et al. 2014). During the isolation process the cell culture was grown at 20 °C. Small opaque colonies were formed which then extended in diameter to 2–3 mm (Olsen et al. 2006a, 2006b).

19.3.2.6 Francisella Noatunensis

This is aerobic, non-motile, coccobacilli gram-negative bacteria. *F. noatunensis* bacteria infect fish and results in bacterial francisellosis disease. This bacteria is generally present in freshwater and saltwater ecosystems. The disease was first found in tilapia fry with the homology sequence of pathogens. *Francisella noatunensis* infection was first identified in wild and farmed Atlantic cod in Norway but piscine host range keeps on increasing, so Francisella culture should be given priority in routine diagnosis.

Clinical Signs

The infected fish will swim inactive and inappetance and also emaciated. Many fish showed exposed dermal haemorrhagic nodules and corneal opacity. The intestinal mucosa becomes thickened in infected fish. White nodular formations were diagnosed in renal organs, heart, spleen and hepatic tissues along with bloody ascites (Olsen et al. 2006a, 2006b). Chronic inflammatory granulomatous infected fish consists of a huge number of intracellular gram-negative cocco -bacilli which belong to the Francisella family that is associated with Francisellosis (Zerihun et al. 2011). The white granulomas mainly occurred in marine species of three-line grunts in Japan. By using PCR the small subunits of rRNA were amplified and sequenced. Francisella-like bacteria were found in freshwater reared in hybrid striped bass with the water temperature at 20–29 ° C. Bacterial affected fishes behaved lethargically, skin haemorrhage occurred over the abdomen and darker pigmentation was observed. Enlargement of the kidney and spleen, often exophthalmia and interstitial granulomas was noted (Olsen et al. 2006a, 2006b). 50–60% of farmed tilapia has died in Costa Rica. Also has the signs as loss of appetite, abnormal swimming patterns, anaemia and Exophthalmia (Soto et al. 2009).

Diagnosis

Characterization and Identification of Pathogens

Cultures comprise the non-motile, weakly gram-negative and negatively oxidase. These cultures were incubated at 22 °C on Cystine Glucose Blood agar medium and demonstrated enzyme activities like esterase (C4) and esterase lipase (C8).

Molecular Methods

Cultures depended on 16SrRNA sequencing which was close to *Francisella philomiragia*. So, *Francisella noatunensis subsp. orientalis* and *Francisella noatunensis* (Ottem et al. 2009) were identical to *Francisella philomiragia.*

Treatment

Infected Atlantic cod and three lined grunts were vaccinated by florfenicol, oxolinic acid, erythromycin, streptomycin, rifampin, and flumequine which resulted in control of the disease.

19.3.2.7 *Francisella noatunensis* subsp. *orientalis*

Clinical Signs

Those infected fry expressed exophthalmia; they become lethargic and also have pale gills. In addition, the spleen and kidney becomes enlarged and it has emptied intestines. Enlargement of gallbladder was also noticed (Jeffery et al. 2010).Nile tilapia and blue tilapia and Mozambique tilapia were recovered from granulomatous disease. Also infection with this organism associated with wild caught Caesar grunt and French grunt. Spleen and kidney were infected by granulomas. Infected fishes died off (Soto et al. 2012).

Diagnosis

Characterization and Identification of Pathogens

Outbreaks of infections were caused by Rickettsia-like microbes (Chern and Chao 1994). These colonies formed have size more than 1 mm diameter and lateral development occurred by incubation process at 22 °C for 3 days. They are aerobic weak gram-negative bacteria, with shape intermediate between spherical and rod-shape (cocco-bacilli) which liberate hydrogen sulphide when incubated on medium containing cysteine at temperature 10–30 ° C. Replication of organisms from invading head kidney macrophages after infection causes apoptosis and cytotoxicity within 24–36 h. By using the (PQ1104) strain for hybridization of DNA, 60.3% of homology was associated with *Francisella noatunensis* (Mikalsen and Colquhoun 2009). For *Francisella noatunensis subsp. orientalis*, specialized quantitative methods and the real-time PCR has been used for identification.

Treatment

Vaccine Development

Tilapia was protected by immersion vaccination with bacterial suspension of 10^7CFU/ml for 30 min to 180 min by immersion challenge with wild type (Soto et al. 2011). Antibacterial antibiotics like Florfenicol can be given orally for the treatment of tilapia for 10 days, at an average dosage of 15 mg/kg of fish.

19.3.3 Bacterial Diseases of Marine Water Fishes

19.3.3.1 Enterobacteriaceae Representatives

Edwardsiella tarda

Edwardsiella tarda is a facultative anaerobic, motile, gram-negative rod-shaped flagellated bacteria. This bacteria infects channel catfish, eels, carp, tilapia, chinook salmon and flounder and leads to edwardsiellosis disease (red disease) (Han et al. 2004).

Clinical Signs

The exterior signs are circular abrasion on the postero-lateral part of the body, which then leads to the development of ulcers spreading into hair-filled regions. From the side, these are converse bulge areas and if ruptured, release foul odour. The disease affects fish when the fish is transported to fish processing plants. The scanning of disease affecting tilapia shows the symptoms of loss of pigmentation, blurred of the eyes, abdomen filled with ascitic fluid.

Edwardsiella tarda was identified from the spleen and renal tissue of seawater flounder.

Diagnosis

To determine the presence of *Edwardsiella tarda* in affected fish tissue, fluorescent antibody technique (FAT) has been used for diagnosis (Amandi et al. 1982). Lindstrom et al. (2009) examined the most effective field for diagnosis by using FAT. The monoclonal antibodies of FAT are best suited for diagnosis as being an enzyme for immune assay. ELISA is also used for diagnosis of 44 kd outer membrane proteins.

Treatment

Vaccine Development and Immunostimulants

Vaccination of prophylaxis has been a favourable outcome with oral immune stimulants, namely β-glucan and vitamin C and E for protection. Yano et al. (1989) reported that injection of beta-1, 3 glucans at 2-10 mg/kg of fish inflates resistance for *Edwardsiella tarda*.

Probiotics. *Bacillus subtilis, Enterococcus faecium* are those fermented by citrus by-products when nourished to young olive flounder for 70 days gives protection against pathogenic culture as immunostimulation.

19.3.3.2 Flavobacteriaceae Representatives

Tenacibaculum maritimum (=*Flexibacter maritimus* = *Cytophaga marina*)

Tenacibaculum maritimum is a gram-negative, gliding bacteria. *Tenacibaculum maritimum* infection in fishes leads to a disease named as tenacibaculosis, which is one of the most devastating diseases to marine fish community. *Tenacibaculum maritimum* can infect the large population of fish and drastically impact fishing industries all over the world.

Clinical Signs

The infected fish species exhibits clinical signs of fin rot, skin lesions, and paleness of internal organs. This bacteria dwells in host-associated environments, especially in fish renal tissues, and leads to tenacibaculosis, which exhibits clinical signs of lesions and necrosis nearly on all external surfaces of the infected fish (Avendaño-Herrera et al. 2004).

Specifically in juvenile fish, the external changes include mouth erosions, gill erosion and tail rot (Handlinger et al. 1997). Initially, on the fins, head and trunk injury develops as grey-white cutaneous in older animals. This condition leads to ulcers from these lesions being depraved (Wakabayashi et al. 1984). Acute and chronic, these are two stages of disease which develop in Salmonids. The acute tenacibaculosis developed 48–72 h after infection with more than $1 \times 10^3 \times 10^3 \times 10^2$cells/ml epithelial abrasion. In chronic tenacibaculosis tiny blisters appear on the epidermal layer which leads to ulceration and exposing the musculature beneath. Gill necrosis occurred due to lesions formed on the fins and jaw. This bacterial infection results in ulcers in wedge sole (López et al. 2009). Black patch necrosis of Dover sole and bacterial stomatitis of Atlantic salmon (Ostland et al. 1999).

Diagnosis

Characterization and Identification of Pathogens

Tenacibaculum maritimum can be isolated from infected tissues of fish by using Cytophaga agar, mixed with 70% marine water and tryptone-yeast extract-cystine agar medium (Hikida et al. 1979). Cultures containing a very homogeneous group of thin flat layers of colonies which appear pale yellow in colour was identified and is composed of aerobic gram-negative rod-shaped bacteria with size 2–30 × 0.5 μm, which sometimes have filaments up to 100 μm long (Bernardet et al. 1990). In older cultures, microcysts do not occur but cells become spherically shaped with a diameter of ~0.5 μm. The gliding motility is the typical attribute of all isolated bacteria and a non-gliding strain also has been isolated from infected puffer fish (*Takifugu rubripes*) (Tanvir et al. 2014). The growth of pathogens is noticed at a temperature range from 14.6 to 34.3 °C and at pH range from neutral to mild alkaline.

Serology

To detect the pathogen *Tenacibaculum maritimum* whole cell agglutination is effective (Wakabayashi et al. 1984), and also for the identification in tissues of fish FAT can be used (Baxa et al. 1986).

Molecular Methods

16S rRNA sequence amplified with specific PCR primers can be used to identify *Flavobacterium branchiophilum, Flavobacterium columnare* and *Tenacibaculum maritimum* (Yeh et al. 2006); however, conventional PCR cannot detect the DNA of the cells because freezing-thawing destroys the cells (Suomalainen et al. 2006a, 2006b) but nested-PCR detected nearly 1–250 cells per PCR cycle in gill, skin and mucus samples of infected fishes. Some asymptomatic fishes show the presence of ten bacterial cells/cycle by nested-PCR (Bader et al. 2003). Mostly, 89% in the tissue blocks from fish detected the DNA from the pathogen, which did not have clear signs of gill disease and in 95% of the tissue blocks from fish with mild-to-severe gill erosion detected the presence of DNA from pathogen. Hence, for the detection of *Flavobacterium psychrophilum and Tenacibaculum maritimum, q*PCR technology has been found to be more reliable than culturing techniques (Fringuelli et al. 2012).

Treatment

Vaccine Development

Freund's incomplete adjuvant (FIA) can be injected as immunostimulant to Atlantic salmon (0.1 ml via i.p.) followed by a formulated suspension of *Tenacibaculum maritimum* containing ~$1.79 \times 10^1 \times 10^3 \times 10^3 \times 10^3$ cells/ml with relative percentage survival of 79.6% and without relative percentage survival of 27.7% (Van Gelderen et al. 2010). Besides the success of the adjuvanted version of vaccine at reducing mortalities, side effects of the adjuvant are also very likely. Prominent side effects are the development of black/brown pigmentation by melanin formation on the stomach with inflammation in the form of granulomas and cysts (Van Gelderen et al. 2009).

Plant-Based Immunostimulants

1.0–2.0 mg of aqueous or ethanol extracts of an Asian plant *Liriope platyphylla* which is commonly known as big blue lilyturf is used as an immunostimulant for olive flounder (*Paralichthys olivaceus*). Plant-based immunostimulation methods enhance the RBC count with increased haemoglobin concentration, and also enhanced the lymphocyte and monocyte counts and thus complement the phagocytic functions (Harikrishnan et al. 2012a, 2012b, 2012c).

Antimicrobial Compounds

Tenacibaculum maritimum exhibits moderately sensitive to chloramphenicol, doxycycline, oleandomycin, oxytetracycline, sulphamonomethoxine and thiamphenicol, highly sensitive to ampicillin, erythromycin, josamycin, nifurpirinol, penicillin G and sodium nifurstyrenate, and weakly sensitive to nalidixic acid, oxolinic acid, spiramycin and sulphisoxazole (Baxa et al. 1988). Trimethoprim and amoxicillin dosage have been recommended at 80 mg/kg body for the treatment (Soltani et al. 1996).

19.3.3.3 Vibrionaceae Representatives

Vibrio alginolyticus

Vibrio alginolyticus is gram-negative sea bacteria. Most mortality that occurred in farmed sea bream is by *Vibrio alginolyticus* infection that leads to bacterial septicaemia.

Clinical Signs

This organism causes anaemia. Infected fishes expressed sluggishness and developed ulcer, skin darkening and blockages in air bladder, liver and capillaries in the intestinal wall. Enlargement of intestine and gallbladder are present. Low levels of mortality noted in turbot with gill disease in recirculating aquariums (Austin et al. 1993). This organism causes corneal opaqueness and exophthalmos, their signs correlated with *Vibrio harveyi. Vibrio alginolyticus* is also present in ulcerous fish. High mortality occurred in silver sea bream by *Vibrio alginolyticus* (Ye et al. 2008). Also this bacteria is the causative organisms for disease in gilt-head sea bream. Black sea bream fry is associated with *Vibrio alginolyticus* and red spot caused by *Vibrio alginolyticus* in sea mullet fish (Burke and Rodgers 1981). Diseased fish have sluggish swimming and also suffered by exophthalmia which leads to death (Rameshkumar et al. 2017). These organisms are present in damaged tissues.

Diagnosis

Characterization and Identification of Pathogens

The organism can be isolated from blood by using TSA, TCBC with seawater agar. An incubation period is at 15–25 °C for 2–7 days (Colorni et al. 1981). From moribund eels the isolates of *Vibrio alginolyticus* was successfully done by differential medium of *Vibrio alginolyticus* agar which has been combined with high concentration of sodium chloride and bile salts at 37 °C of incubation. Green-yellow colonies will be produced, which comprise motile, gram-negative rod-shaped organism producing hydrogen sulphide, lysine, indole, catalase and ornithine but β-galactosidase or arginine dihydrolase will not be produced. Nitrates are deduced. The Voges Proskauer (VP) test and methyl red (MR) test results are positive. Sensitivity of vibriostatic agent O′129 has been recorded. From maltose, mannose, glycerol, sucrose and salicin the acids will be produced.

Molecular Methods

In diseased marine fish, the diagnosis of the disease is achieved by loop assisted isothermal multiplication with gyrB target gene. The limitation of 3.7 × 102 CFU/ml is used for rapid detection.

Treatment

Vaccine Development

Culture of *Vibrio alginolyticus* containing flagellin *fla*A genes given as treatment in red snapper by i.m.(8 μg/fish). The antigen will be accumulated in the liver, spleen and kidney, with the expressions of *fla*A genes corresponding to 88% of relative percentage survival (RPS) after 7–28 days, and 84% of the RPS by recombinant *fla*C genes. Infectious spleen and kidney necrosis virus (ISKNV) evaluation is carried out by combination vaccine with *Vibrio alginolyticus*, with 80% of RPS on orange-spotted grouper. Large-yellow croakers can be protected against the challenge by i.p. injection with 100 μg protein per fish. The booster dose can be given 2 weeks after first vaccination (Qian et al. 2008).

Plant-Based Immunostimulant Agents

In Rockfish, *Aloe vera* has increased the resistance to *Vibrio alginolyticus* for 6 weeks after feeding at 5 g *Aloe vera*/kg of diet. Using chitosan and chitin to kelp grouper has increased the immunostimulation. Also, globin, haemoglobin, albumin, leucocyte, and erythrocyte counts are enhanced. Chitosan gives more protection compared to chitin in challenge (Harikrishnan et al. 2012a, 2012b, 2012c). In Clownfish, white mangrove aqueous leaf extract fed at 1–8%, developed survival after challenge and also enhanced immunostimulation. 70%, 80% and 85% of survival is increased while using extracts at 1, 4, and 8% (Dhayanithi et al. 2015). Clownfish is examined with another *Rhizophora apiculata* (Mangrove) extracts fed at 5% and 10% showed enhanced immunostimulation. Immunostimulation and growth is improved in *Amphiprion sebae* with 5% of mangrove extract and enhancing 85% of survival rates. In another experiment, grouper juveniles fish is tested with *Sauropus androgynus* extracts for 30 days fed at 1.0 and 2.5 g/kg resulted in improved immunostimulation as well as susceptible to the challenge (Samad et al. 2014).

Antibacterial Compounds

Antibacterial compounds such as chloramphenicol have been used for this disease. It was first successfully achieved by Colorni et al. (1981). 50 mg of drug was given per kg body weight of fish per day. Also nitrofurantoin dosed at 50 mg/l of water/1 h. Both antimicrobial compounds can reduce the fatality rates.

Vibrio Anguillarum (*Bacterium anguillarum)*

Canestrini 1893 was the first to discover a bacterial fish pathogen of marine species known as *Bacterium anguillarum* (later named as *Vibrio anguillarum*). *Vibrio anguillarum* is rod-shaped polar flagellated gram-negative bacteria. It causes red pest disease in eel fishes. It is damaging to the economy of aqua industries worldwide.

Clinical Signs

The external symptoms of the disease are skin discolouration, red necrotic abrasion in abdominal muscle and erythema surrounding the vent and within the mouth. The gut filled with viscous fluid. This infection was also noticed in Pacific salmon fingerlings. The pathological abnormalities include abnormalities in the blood, connective tissue, gills, renal organ, liver and posterior gastrointestinal track and splenomegaly. Fin rot is another state as cribbed to *Vibrio anguillarum.*

Diagnosis

Characterization and Identification of Pathogens

The pathogen is retrieved from infected tissue, innoculated on BHIA and nutrient agar additive at 0.5–3.5% (w/v) with NaCl, TCBS and seawater at 15–25 °C and incubated for 1 week. The taxonomy of the pathogenic bacteria divided these species into three biotypes on the basis of biochemical reactions.

Type A called as *Vibrio anguillarum* forms typical colonies, giving rise to indole and acid formation from mannitol and saccharose.

Type B called as *Vibrio anguillarum* forms an anguillicide and does not bring about indole or acid formation from saccharose and mannitol.

Type C called as *Vibrio anguillarum* forms an ophthalmia and generates acid from saccharose and mannitol, but does not give rise to indole.

A clarified diagnostic test has been described for *Vibrio anguillarum*, requiring “glucose motility deeps”. Another necessity of GMD is an oxidation fermentation test medium containing dextrose 1.0%(w/v), yeast extract 0.3%(w/v), phenol red broth base (difco) 1.6% w/v, agar 0.3%(w/v).

Treatment

Management Techniques

Infected eels can be moved to well-aerated water and added to reduce complications with vibriosis.

Disease Resistant Fish

The genetically modified disease-resistant fish strain has an opposite confirmation at bestow on resistance to vibriosis. Among various transferrin genes of Coho salmon and Steelhead trout, Winter et al. (1980) stubbornly maintained that there was no difference in resistance to vibriosis. Lately, in the major histocompatibility complex (MHC) 11 α genes in Japanese finders an association has been made between polymorphic and *V. anguillarum*, and in turn three alleles paol DAA*1301, paol DAA*1401, paol DAA*2201 were related notably with resistance (Xu et al. 2011).

Vaccine Development

Johnson and Amend 1983 integrated a vaccine into gelatin and put in oral and anal ingestion to subdue digestion and intestinal symptoms. The results showed the best decrease in mortality.

Vibrio ordalii

Vibrio ordalii is a gram-negative bacillus. In seawater fish vibriosis is the fundamental reason. In Japan, the *Vibrio ordalii* was reported by Muroga et al. (1986a, 1986b). This disorder was recognized as haemorrhagic septicaemia. In gill tissue, skeletal muscle, cardiac muscle, and inside the anterior and posterior of the GI tract, there has been a tendency for the formation of micro colonies among pacific salmon. Contrarily, while in comparison with bacteraemia caused by *Vibrio anguillarum* evolved substantially later in the disease cycle. By the way, the use of seawater and thiosulphate-citrate-bile salts-sucrose agar (TCBS) *Vibrio ordalii* incubated at 25°c for 7 days (Ransom 1978; Ransom et al. 1984). By means of plasmid describing, *Vibrio ordalii* became analogous, ribotyping and serotyping aid lipopolysaccharide (LPS) class and changed into miscellaneous by Biolog-Gen fingerprints. In *Vibrio ordalii*, cynicism plasmid has no longer been determined (Crosa 1980). However, without DNA polymerase I, a 30 kb enigmatic plasmid delegated as PMI101 copied without recreating a single-stranded mean has been set up in all isolation of *Vibrio ordalii* (Bidinost et al. 1999). However, proteases have no longer been entrenched (Kodama et al. 1984).

Diagnosis

Molecular Techniques

The gene for haemolysin, a 112 base pair fragment which is commensurate by using PCR and RT-PCR. The RT-PCR gave 1 h faster copies than the PCR, and it discovered from 5.27×10^2 to 4.13×10^3 CFU/ml or in other words 62–145 versions of voh B gene in spleen and kidney of Atlantic salmon with bacterium.

Treatment

Probiotics

A segregate of *Vibrio alginolyticus* before used as probiotic in Ecuadorian cipher rookery has been practically at commanding disease provoked by *Vibrio ordalii* (Austin et al. 1995).

19.3.3.4 Alteromonadaceae Representatives

Pseudoalteromonas piscicida

Pseudoalteromonas piscicida is a gram-negative gammaproteobacterium present in marine environments. It was reported among the eggs of damselfish, *Amphiprion clarkii* and *Amblyglyphidodon curacao*, characterized by the whitening of the eggs resulting in death within 24 h. (Nelson and Ghiorse 1999). Using with marine agar, the infected eggs were cultivated at 28 °C for 2 days. This culture was experimented among the Damsel fish eggs resulting in balanced mortality which is in contrast to uninfected controls (Nelson and Ghiorse 1999). From *Amblyglyphidodon curacao* eggs were recognized by identifying 16S rDNA sequencing called *Pseudoalteromonas piscicida.*

Shewanella putrefaciens

Shewanella putrefaciens occurs in various distinct forms and is a gram-negative bacterium. It has been extracted from both marine environments and anaerobic sandstone in the Morrison Formation in New Mexico. This disease affects the rabbit fish, *Siganus rivulatus* with high transience occurred mostly in Red sea cages. Gram-negative bacteria was recovered which was effective in affecting the healthy fish mostly during the spring season. This disease has not been reported in any other species. From the API 20 E rapid identification system, a taxon from *Alteromonas putrefaciens* has been rechanneled as *Shewanella putrefaciens*. This disease affects fish that have the external signs such as inertia, exophthalmia, haemorrhaging around the mouth and fin damage. At the same time, the internal signs were not identified. In Poland, the fish was affected with necrotized gills and necrotized abrasion on the skin among the common carp and rainbow trout particularly during 2007–2012. They have the interior signs such as swollen kidney, enlarged spleen, etc. From the infected spleen, kidney and liver, the bacterium was separated; in turn it was incubated with sodium chloride 3%(w/v) in addition to Brain Heart Infusion agar (BHIA) for an concealed period of time (Saeed et al. 1987).

Treatment

Vaccine Development: The formalin killed vaccine gives clear evidence in limited death rates when administered by i.p. injection (Saeed et al. 1987). When compared to unvaccinated conditions, these two injections reported 40% decreased mortalities. Engrossment vaccination ends in field results.

19.3.3.5 Oxalobacteraceae Representative

Janthinobacterium lividum

The gram-negative rod-shaped bacteria were related with transience which was purple pigmented affecting marine fishes in farms in Scotland and Northern Ireland. The moribund rainbow trout in Scotland was recognized with Rainbow Trout Fry Syndrome. The rainbow trout in Northern Ireland was faced with high death in 2–3 weeks.

Clinical Signs

During January 1992, furthermore purple pigmented bacteria was identified among the large rainbow trout (100-200 g in weight) resulting in the skin abrasions, which were enervating with epiretinal membrane. Small fish exhibit inactive movements, increased skin complexion, skin abrasions, pale gills and abdomen expanded. In the interior, the kidney and spleen become enlarged and serous fluids are filled in the peritoneal cavity. The sockdolager, jumbo, and buster was affected with external scrape, the skin removed off along the loin to tail and showed muscular abnormality (Austin et al. 1992).

Diagnosis

Characterization and Identification of Pathogens

The diversity of media comprises blood agar (5%v/v bovine blood in Gibb blood agar base), cytophaga agar, Histone lysine demethylase 2 (KDM2), Lactose

Fermenters (L-F) medium and Trypticase soy agar or tryptone soya agar (TSA) in which the standardized specimen of whole fish (liver, spleen, kidney and substances from surface abrasions) was grown on the surface of various media at 22 °C for 14 days. After 3 days, the purple pigmented population was identified.

Treatment

Antimicrobial Compounds

The organisms are conscious of oxolinic acid, potentiated sulphonamides and furazolidone (Austin et al. 1992). Accordingly, it is suspected that these chemicals would be convenient for chemotherapy.

19.3.3.6 Piscirickettsiaceae Representatives

Piscirickettsia salmonis

Debased or constrainly parasitic *bacilli* such as Rickettsias and Chlamydias have been habitual bacterium of invertebrates and occasionally referred to attach with fish disease which is termed as Huito disease (Schafer et al. 1990). Salmonid rickettsial septicaemia (Cvitanich et al. 1991) were seen among Quinnat Salmon, Silver Salmon and Rainbow Trout, extended to Atlantic Salmon in Norway (Olsen et al. 1997) and White Bass in California (Arkush et al. 2005). Though the bacterium was at first analogous with Salmon, later it outstretch to other groups integrated to sea dace (McCarthy et al. 2005; Arkush et al. 2005).

Clinical Signs

The infected fish shows inactive and were repleteness. Exterior symptoms include melanosis, gills were paled and anaemia. At first the disease was not observed to take place during fresh water of fish culture. The fish from fresh water was moved into seawater after 6–12 weeks; the death has been recorded (Fryer and Hedrick 2003).

Diagnosis

Characterization and Identification of Pathogens

Isolation of this bacterium was achieved by kidney disease which affects the fish in Salmon egg cells cytoplasm for 5–6 days at 12–21 °C (Fryer et al. 1990). But the growth did not take place in blood agar, BHIA, plasma medium and Loffler medium.

Serology

Isolating of tissue culture is achieved by using iFAT, the spotting in acridine orange stained smears was recommended (Lannan et al. 1991).

Treatment

By Microwave Radiation

For *Piscirickettsia salmonis*, the microwave radiation at 700w was proposed (Larenas et al. 1996a, 1996b).

Vaccine Development

Formalized cells (106.7 TCID50/ml) were delivered showing growth of good preservation in a field experiment with Coho Salmon (Smith et al. 1999). The process of using oral vaccination every 3 days at 6 mg for 30 days called "micromatrix" has been reported and revealed promise.

19.3.3.7 Pseudomonadaceae Representatives

Pseudomonas alcaligenes

These gram-negative rod-shaped organisms of ~2.4 × 0.8 μm mainly infect Moribund fish of fresh and marine water.

Clinical Signs

Infected fishes express signs like pale gills with the swollen and they are affected by anaemic and they also have swollen spleen, haemorrhaging in their kidney and gonad and ascites were also present. Blood spots were developed around the oral cavity. The hybrid sturgeon was taken for further experiments, 33% of mortalities have been recorded with 2×10^6 CFU/Fish after 5 days of infections.

Diagnosis

Characterization and Identification of Pathogens

Using the kidney and liver of Moribund fish, the isolation process was developed with BHIA for an incubation period of 10 days at 28 °C. They are translucent, white in colour and circular; it may be present in individual or in pairs. Colonies are motile and they have unique polar flagella. It is classified as phylogenetic by Biolog -Gn, which utilize L-alanine, L-arginine, L-malic acid and pyruvate. 16S rRNA sequencing corresponds to 99% of homology with *Pseudomonas alcaligenes* (Xu et al. 2015).

19.3.3.8 Moritellaceae Representatives

Moritella marina (=Vibrio Marinus)

Moritella marina is a halophilic psychrophilic facultative anaerobe gram-negative with curved or straight rods, motile polar flagella that generate PUFAs (polyunsaturated fatty acids) and DHA (docosahexaenoic acid). At low temperature these organisms were correlated with facial skin abrasions of *Salmo salar* orchard in Iceland specifically ~10 °C. In a different part of cold marine environments, *Moritella marina* has been seen from the ocean floor to the intestinal tract of marine organisms. Many of the *Moritella* species are thought to live in collaboration with marine organisms.

Moritella viscosa

In recent times in Scotland, an unspecified cause of ulcer seems on the fringe of *Salmo salar* in seawater (Salte et al. 1994; Lunder et al. 1995; Benediktsdottir et al. 1998). Consequently, *Moritella viscosa* are retrieved from two diseased farmstead Atlantic cod in Norway. In some cold-water fish, *Moritella viscosa* has been found to cause skin ulcers (Urakawa et al. 1998).

Diagnosis

Molecular Methods

The *Moritella viscosa* has been determined conveniently by using PCR which is fixed at the limit of $6.09 \times 10^{1} \times 10^{13}$ g of DNA, and is identical to ten bacterial genomes (Grove et al. 2008).

Treatment

Vaccine development: *Salmo salar* which were vaccinated through intraperitoneal injection with an assisting entire cell, authorized interruption carrying *Moritella viscosa* were stored against ensuring summons to attain an RPS of 97% (Greger and Goodrich 1999).

19.3.3.9 Halomonadaceae Representatives

Halomonas cupida

Halomonas are gram-negative, motile, rod-shaped cells. These organisms usually occur in unpigmented or yellow-tinted colour. Halomonas bacteria strains require NaCl for growth. They are mostly found in water resources with elevated levels of salinity and usually inhabit deep-sea sediment and deep-sea waters (Okamoto et al. 2004). This bacteria infects *black seabream* and *Acanthopagrus schlegelii* (Kusuda et al. 1986).

Diagnosis

Characterization and Identification of Pathogen

Bacteria obtain through intestinal microflora and in addition to infected tissue. Homogenate cultures were inoculated by BHIA with the incubation period of 24 hours at 25 °C (Kusuda et al. 1986). An oxidative-fermentation test was inactive. Several enzymes and amino acids are produced by catalase, arginine dehydrolase and ornithine dehydrolase and lysine. Hydrogen sulphide and indole are not produced, nitrates became deduced. However, Voges Proskauer reactions and Methyl red test have come to be negative. Haemolysis occurred in eel erythrocytes. Acids are generated by galactose, maltose, adonitol, mannitol, L-rhamnose, D-sorbitol, trehalose, salicin and sucrose (weak). However, acids are futile by inulin and fructose. Above all, these manners clearly resemble the relationship between *Alcaligenes cupidus*. But dependent on 16s rRNA sequencing (Dobson and Franz Mann 1996), this toxon was changed to *Halomonas cupida* (Baumann et al. 1983).

19.4 Summary of Bacterial Fish Diseases

The overview of bacterial fish diseases is given in Table 19.1.

19.4.1 Bacterial Fish Disease During Hatching of Eggs and Larvae

In the course of the meticulous hatching of eggs and raising of marine larvae, extraordinary kinds of interactions between microorganisms and organic surfaces can also occur. This ends in the initiation of an autochthonous microflora or be the start of infection. Among habitats and hosts, microorganisms may be effortlessly travelled, inside the aquatic surroundings. Egg surface reflects the bacterial

Table 19.1 Major bacterial fish diseases, causative agents and its host.

Gram-positive bacteria		
Fish diseases	Causative agent	Host range
Eubacteriaceae representative Eubacterial meningitis	*Eubacterium tarantellae*	Striped mullet, snook, gulf flounder
Streptococcaceae representatives Streptococcosis Lactococcosis (septicaemic disease)	*Lactococcus garvieae, Streptococcus difficilis, Str.Iniae, Str.Parauberis, Str, phocae*	Rainbow trout, Golden shiners, Japanese eels, yellow tails, striped mullet, hardhead catfish
Corynebacteriaceae representative Bacterial kidney disease	*Renibacterium salmoninarum*	Salmonid fish
Mycobacteriaceae representatives Mycobacteriosis	*Myc.abscessus, Myc.marinum, Myc.neoaurum, Myc.gordonae*	Zebra fish, Salmonids, European sea bass, Tilapia, Striped bass, Carp
Staphylococcaceae representatives Eye disease	*Sta.aureus, Sta.epidermidis, Sta.warneri*	Silver carp, African sharp catfish, Yellow tail, Red sea bream, Gilthead sea bream
Carnobacteriaceae representative Lactobacillosis, Pseudokidney disease	*Carnobacterium piscicola*	Salmonids
Nocardiaceae representatives Nocardiosis, ocular oedema	*Nocardia spp. (Noc. Asteroides, Noc. Salmonicida; Noc. Seriolae), Rhodococcus sp., Rhodococcus erythropolis.*	Most fish species, *Oncorhynchus tshawytscha, Salmo salar*
Bacillaceae representatives Septicaemia, bacillary necrosis, branchionecrosis, ulceration	*Bacillus spp., Bacillus cereus, Bacillus mycoides, Bacillus subtilis*	Different varieties of freshwater fish species including catfish (*Pangasius hypophthalmus, carp* (*Cyprinus sp.*), striped bass (*Morone saxatilis*), channel catfish (*Ictalurus punctatus*)
Microbacteriaceae representative	*Microbacterium paraoxydans*	Nile tilapia
Micrococcaceae representative Micrococcosis	*Micrococcus luteus*	Rainbow trout
Planococcaceae representative	*Planococcus* sp.	Salmonids
Gram-negative bacteria		
Enterobacteriaceae representative Enteric septicaemia of catfish	*Edw.ictaluri*	*Amieurus nebulosus*, *Pangasius hypophthalmus*, *Danio devario*, *Pangasius hypophthalmus*
Red pest, edwardsiellosis, emphysematous putrefactive disease of catfish	*Edw.tarda (Paracolobactrum anguillimortiferum, Edw. anguillimortifera)*	Various freshwater species

(continued)

Table 19.1 (continued)

Gram-positive bacteria		
Fish diseases	Causative agent	Host range
Flavobacteria and cytophages Columnaris, saddleback disease	*Flavobacterium columnare*	Many freshwater fish species
Gill disease, skin disease	*Flavobacterium johnsoniae* (= *Cytophaga johnsonae*)	Barramundi (Lates calcarifer), koi carp, rainbow trout, longfin eel (Anguilla mossambica)
Gill disease; generalized septicaemia	*Chryseobacterium scophthalmum (= Flavobacterium scophthalmum)*	Turbot
Bacterial stomatitis, gill disease, black patch necrosis	*Tenacibaculum maritimum (=Flexibacter maritimus)*	Various marine fish species
Vibrionaceae representatives Eye disease, septicaemia	*Vibrio alginolyticus*	Cobia (Rachycentron canadum), gilt-head sea bream, grouper (Epinephelus malabanicus), sea bream (Sparus aurata)
Vibriosis	*V. ordali*	Various marine fish species
Alteromonadaceae representatives Egg disease	*Pseudoalteromonas piscicida*	Damselfish
Septicaemia	*Shewanella putrefaciens*	Rabbit fish (*Siganus rivulatus*)
Oxalobacteriaceae representative Anaemia	*Janthinobacterium lividum*	Rainbow trout
Moraxellaceae representative Acinetobacter disease	*Acinetobacter sp.*	*Salmo salar*
	Moraxella sp.	Striped bass
Piscirickettsiaceae representative Coho salmon syndrome, salmonid rickettsial septicaemia	*Piscirickettsia salmonis*	Salmon, sea bass (Atractoscion nobilis)
Halomonadaceae representative	*Halomonas (=Deleya) cupida*	Black sea bream (*Acanthopagrus schlegeli*)
Francisellaceae representative Francisellosis, visceral granulomatosis	*Francisella noatunensis*	Atlantic cod, *Salmo salar*, three-line grunt, striped bass, tilapia
Francisellosis	*Francisella noatunensis subsp. Orientalis*	Tilapia, French grunt, Caesar Grunt
Pasteurellaceae representative	*Pasteurella skyensis*	*Salmo salar*

composition of the aquatic surroundings, due to the diverse plant life that in the end develops at the egg floor. Representatives of mucilaginous microflora may damage developing eggs. The microflora of marine invertebrates and plankton can be ruled via probably pathogenic *vibrios* at certain times of the 12 months. End result of including meals at excessive awareness of water is a brilliant medium for the boom of heterotrophic or opportunistic microorganisms. The opportunistic microorganism can cause many fish illnesses. Fish decreased from resistance towards contamination due to pressure in handling or transport of fish. Aquatic invertebrates are natural meal resources for fish larvae and are also co-inhabitants of larval ecosystems. This correlation will suggest that the inception of a larval microflora may also be stimulated by means of the primordial microflora of invertebrates, whether they are food organisms or co-population of larval ecosystems or rearing centers. The fish may also take in invertebrates as meals, the fish may be inflamed through a particular pathogen, which the invertebrates have contained. The pathogen vibrio group comprises microorganisms with a dynamic position in the marine and brackish environments. A particular courting exists among some marine vibrios and their invertebrate hosts. This interaction can be specific, for the reason that bivalves may additionally have lectins that bind vibrios, which include *Vibrio Cholerae*, *Vibrio Vulnificus* which might be pathogenic to fish.

Larval production system have the problems by poor egg quality it leads to heavy mortalities. Eggs are overgrown through incubation techniques. Indigenous microflora may affected by bacteria. And also hatching and hamper egg maturing affected by bacteria. In farmed fish, their short and long period of health has been affected by larval incubators and microflora on eggs. Microflora has been controlled by using antibiotics. Various techniques are used to remove the adherent microflora and during transportation the eggs are have been preventing against the bacterial pathogens in aquaculture. Ozonization, antibiotics, UV-irradiations and membrane filtration methods are used to larviculture. These methods are furtherly interrupt the growth of opportunistic bacteria. Overgrowth of bacteria has led to hypoxia or delayed hatching. Exo proteolytic enzymes were released by adherent bacterial epiflora, it affects the chorion and also zona radiata were destroyed. An intra ovum, infections may transferred by pathogens.

The research reported that in grownup fish the proof encouraged that restrained placenta and excreting immunity is chief in protecting in opposition to bacterial ailment. The regular excretion and discharge of sludge push to goblet cells in the dermis, gills, and membrane of the gastrointestinal vicinity restrict microbial contamination. Excretion of interleukin-like factors recommends that goblet cells may have immunostimulatory functions (Sigel et al. 1986). Relevantly small is familiar about ontogenesis of immunity in fish. It has been deduced that the “resistant ability” in fish larvae is not completely enlarged up to they are many weeks old (Chantanachookhin et al. 1991). Still, this may fluctuate among the fish genus. Ig advantageous cells are confirmed which are observed within a few weeks.

19.4.2 Bacterial Fish Disease During Transportation and Storage

Transportation and storage structures have an effect on the behaviour of fish. The fish becomes vulnerable because of a lack of oxygen supply during transportation and the safety of Betok fish is depleted. Any other aspect, which could affect the prevalence of abnormalities in Betok fish, is advertised within the fish market of Segiri. It is because of the transportation procedure which makes use of pickup and excessive density. The fishes tend to get friction between a fish and another fish. Furthermore, it is also because of the consignment field, so the condition of fish turns weaker.

The fishes had been transported in an easy etching with wet structures that have a tendency to have red spots, reducing, losing scales on the surface of the frame, operculum, and head. The fishes were also discovered to have eminent eyes (exophthalmia) and fingertips. Pathologies can be caused by friction between a fish with any other fish. It is investigated that these fishes have scales and fins which are rigid and sharp, which is able to induce damage to another fish when it is in the contagious container. The length of time also influences the transport of intensity/frequency of fish rubbing. This allows greater harm for the sale procedure. There had been additional triggers for the incidence of accidents to the outside organs of the fish. It results from reconciliation with excessive density without aeration and with a small quantity of water.

It is recognized that there are five kinds of bacteria, specifically: *Aeromonas, Pseudomonas, Staphylococcus, Streptococcus,* and *Bacillus*. The fifth type of bacteria is a bacterium that is generally determined in freshwater fish and numerous human beings reported the infection of fish farming (Austin and Austin 2007). The presence of microorganisms tremendously depends on environmental conditions and the condition of the fish itself. The transportation and storage complex of fish plays a function within the rise of intensity and incidence of bacterial infections in Betok fish.

19.5 Conclusion

The growth of organic matter such as fecal material or uneaten fish food leads to the microbial growth in the water. This greatly affects the fish's health. As well as the dead fish in the pond or a tank for a longer period results in the growth of the microorganisms. Some disease in fish leads to economic losses. Dietary supplements also play a major role in fish health. When the fish are fed with adequate diets like vitamins, natural plant products, and immunostimulants, it leads to better growth and reproduction. Even some bacterial pathogens are capable of creating disease in human beings also when they consume infected fish.

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