Oomycetes: Fungal-Like Menace in Cold-Water Aquaculture

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Abstract

One of the emerging oomycete pathogens of rainbow trout and other salmonids is designated as *Saprolegnia* spp. Rainbow trout and other cold-water fish are most susceptible to *Saprolegnia* infections in early and advanced life stages. *Saprolegnia* has a complicated and well-characterized life cycle that includes both sexual and asexual stages. It has been traditionally identified and distinguished, using patterns of asexual and sexual characteristics, while the sexual characteristics, oospore and lipid droplet position in the oospore, and the asexual characteristics, such as mycelium and germinating cyst, have been most frequently used in identifications. This chapter highlights the morphological and molecular identification of *Saprolegnia* spp., symptoms of *Saprolegnia* infections and control measures, including biocontrol methods.

Keywords

Saprolegniasis · Morphological identification · Molecular characterization · Control measures

16.1 Introduction

Since the 1990s, the main species captured and cultured in the Indian Himalayan regions have been *Oncorhynchus mykiss*, *Schizothorax richardsonii*, *Tor putitora*, *Labeo dero* and *Labeo dyocheilus* (Sarma et al. 2018). Rainbow trout (*O. mykiss*) is

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the most cultured cold-water exotic species in more than 100 countries, including India (FAO 2020). The primary producers of rainbow trout are Iran, Turkey, Chile, Norway, Peru and other European Union countries. In India, trout farming has advanced steadily, and the cold-water aquaculture industry in Himalayan states and union territories has grown exponentially over the past 15 years. Due to intensification, improved feed, infrastructure facilities and trout production in upland states have attained increasing attention (Sarma et al. 2018). Significant impediments to the development of intensive trout aquaculture in India are various health disorders, climate change, old rainbow trout stocks and effluent discharges (Singh 2020; Barat et al. 2015).

Rainbow trout and other salmonids are most susceptible to *Saprolegnia* infections in early and advanced life stages (Mehrabi et al. 2020). *Saprolegnia* is generally considered a secondary infectious agent that causes severe economic losses in cultured freshwater fish, affecting skin, gills and other organs (Van West 2006). Saprolegniasis in fish is mainly caused by *Saprolegnia parasitica* and *S. australis* (Sandoval-Sierra and Diéguez-Uribeondo 2015). *S. parasitica* can cause devastating impacts, leading to 'winter kill' in catfish and accounting for losses up to £24 million, representing 10–50% of farmed fish (Bruno et al. 2011). The losses can even exceed 30–50% annually because of *S. parasitica* infection in coho salmon farming in Japan (Hatai et al. 1990; Bruno et al. 2011). Saprolegniales are responsible for the weakening of fish immune mechanism by haemodilution and production of effector proteins (Van West 2006; Walker and van West 2007; Romansic et al. 2009; Rezinciuc et al. 2014; Van Den Berg et al. 2013; Masigol et al. 2019, 2020).

In India, major bottleneck in rainbow trout farming in flow-through system is the saprolegniasis infection by oomycetes. The fluctuations in water temperature, water currents and poor management practices in farms and hatcheries are the key risk factors for the saprolegniasis. Changes in temperature regime weaken the fish's immune system and provide an environment for developing infections in culture conditions (Sarowar et al. 2014). Further, prolonged incubation period in hatcheries produces oomycetes spores from the dead to healthy eggs, causing significant economic loss at the early stages of fish production.

16.2 Oomycetes: Fungal-Like Organism

Oomycetes or water moulds or fungal-like organisms are eukaryotic microbes different from true fungi with distinct phylogenetic, physiology and biochemical properties (Judelson and Blanco 2005). They have evolved either pathogenic or saprophytic lifestyles and have fungal-like characters such as filamentous hyphae (Kamoun 2003). It causes the most destructive disease in animals, plants and fishes, which results in significant economic losses (Derevnina et al. 2016). They are stramenopile and superficially resemble true fungi with their appearance, ecological niches and physical characters (Verret et al. 2010).

Although fungi and the animal kingdom share a common evolutionary ancestor, oomycetes are more closely connected to golden-brown algae (Table 16.1). The

Table 16.1 Major Oomycetes (*Saprolegnia* sp.) abstracted from India and abroad affecting rainbow trout and other important fish

Species	Host	Region/ country	References
S. parasitica	*Schizothorax richardsonii*	Bhimtal, India	Tandel et al. (2021)
S. parasitica and *S. australis*	Rainbow trout (*Oncorhynchus mykiss*)	Bhimtal, India	Tandel et al. (2021)
S. parasitica	Golden mahseer (*Tor putitora*)	Bhimtal, India	Shah et al. (2021)
Saprolegnia diclina	Tilapia (*Oreochromis niloticus*)	Riyadh, Saudi Arabia	Mostafa et al. (2020)
Saprolegnia parasitica	Stripped catfish (*Pangasianodon hypophthalmus)*	UP, India	Kumar et al. (2022)
S. parasitica	Atlantic salmon (*Salmo salar* L.), ova, fry or brood stock	Canada	Elameen et al. (2021)
S. parasitica, *S. diclina*, *S. salmonis*	Sockeye salmon (*Oncorhynchus nerka*) and Coho salmon (*Oncorhynchus kisutch*)	Japan	
S. parasitica, *S. ferax*, *S. diclina*	Brown trout (*Salmo trutta*) and one strain from common whitefish (*Coregonus lavaretus*)	Norway	
S. diclina *S. ferax* *S. Hypogyana* *S. parasitica* *Achlya americana*	*Channa gachua*, *C. striatus* *C. batrachus* *C. gachua* *H. fossilis*, *Mystus* sp., *C. punctatus* *H. fossilis*, *Labeo rohita*	Andhra Pradesh, India	Mastan (2015)
Saprolegnia parasitica	Rainbow trout (*Oncorhynchus mykiss*)	Iran	Mehrabi et al. (2019)
Saprolegnia parasitica	Pike (*Esox lucius*)	Netherlands	Minor et al. (2014)
Saprolegnia species	Rainbow trout, *Oncorhynchus mykiss*	Tehran, Iran	Johari et al. (2014)
Saprolegnia diclina, *S. ferax* and *S. parasitica*	*C. catla*, *C. mrigala* and *L. rohita*	Bhopal, India	Chauhan et al. (2012)
Saprolegnia diclina	*Clarias batrachus*	Bhopal, India	Chauhan et al. (2012)
S. parasitica	*Tor putitora*	Bhimtal, India	Singh et al. (2013)
Saprolegnia spp.	Nile tilapia (*O. niloticus*)	Egypt	Mahboub and Shaheen (2021)
Saprolegnia australis	Salmonid eggs, *Salmo trutta*	Spain	Rezinciuc et al. (2014)
Aphanomyces invadans	*Labeo rohita*, Tilapia (*Oreochromis niloticus*)	Bangladesh	Sarowar et al. (2019)

(continued)

Table 16.1 (continued)

Species	Host	Region/ country	References
Saprolegnia parasitica	Gilt-head seabream (*Sparus aurata*)	Turkey	Dinçtürk et al. (2019)
Saprolegnia spp.	Rainbow trout	Iran	Tour Savad Kouhi et al. (2021)
Saprolegnia parasitica and *S. australis*	Female crayfish, *Pacifastacus leniusculus*	Sweden	Edsman et al. (2015)
Saprolegnia parasitica and *Saprolegnia ferax*	*Cyprinus carpio var. communis* and *specularis*	India	Magray et al. (2021)
S. delica, *S. ferax*	Rainbow trout (*Oncorhynchus mykiss*)	India	Magray et al. (2021)
Saprolegnia spp.	African catfish (*Clarias gariepinus*) eggs and adults	Ethiopia	Melaku et al. (2017)
Saprolegnia parasitica	White fish (*Rutilus frisii kutum*) eggs	Iran	Kalatehjari et al. (2015)
Saprolegnia spp.	Common carp, *Cyprinus carpio* L.	Iraq	Salih and Mustafa (2017)
Saprolegnia parasitica	Rainbow trout (*Oncorhynchus mykiss*) eggs	Iran	Salehi et al. (2015)
Saprolegnia parasitica	*O. niloticus* and *C. gariepinus*	Egypt	Younis et al. (2020)
S. diclina	*Salmo salar* *Salvelinus alpinus* *Salmo trutta* *Coregonus lavaretus* *Lampetra fluviatilis* *Esox lucius* *Perca fluviatilis*	England	Pickering et al. (1979)
S. parasitica (CBS 223.6) *S. parasitica (ITT 320/15/20)* *Saprolegnia delica (ITT 290/15/15)*	*Esox lucius* *Salmo trutta fario* *Oncorhynchus mykiss*	Italy	Tedesco et al. (2019)
Saprolegnia parasitica	*Oncorhynchus mykiss*	Iran	Khosravi et al. (2012)
Saprolegnia parasitica	*Oncorhynchus mykiss*	Korea	Shin et al. (2017)
Saprolegnia diclina *S. parasitica* *S. hypogyna* *S. ferax*	*Salmo salar*	Norway	Thoen et al. (2015)
Saprolegnia sp.	*Oncorhynchus mykiss* eggs	Finland	Hoskonen et al. (2015)

(continued)

Table 16.1 (continued)

Species	Host	Region/ country	References
Saprolegnia parasitica	*A. leptodactylus*	Sweden	Diéguez-Uribeondo et al. (1994a, b)
Saprolegnia diclina	Pejerrey, *Odontesthes bonariensis*	Japan	Kitancharoen et al. (1995)
Saprolegnia parasitica	*Salmo trutta*	Sweden	Diéguez-Uribeondo et al. (1996)
Saprolegnia parasitica	*Oncorhynchus mykiss*	UK	Pottinger and Day (1999)
Saprolegnia parasitica, *S. diclina*, *S. salmonis*	Rainbow trout, all freshwater species, incubator eggs	Egypt	Hussein and Hatai (2002)
Saprolegnia parasitica, *S. diclina*, *S. salmonis*	Rainbow trout, all freshwater species, incubator eggs	USA	Torto-Alalibo et al. (2005)
Saprolegnia parasitica	*Oncorhynchus mykiss*	Iran	Mirmazloomi et al. (2022)
Saprolegnia parasitica, *S. australis* and *S. ferax*	*Oncorhynchus mykiss*, *Salmo trutta*	Italy	Pavić et al. (2021)
Saprolegnia spp.		Japan	Hatai et al. (1990)
Saprolegnia parasitica	Brown trout, *Salmo trutta fario*	Spain	Diéguez-Uribeondo et al. (1994a, b)

composition of the cell wall, genome size, ploidy structure, many cytoplasmic gene sequences and mitochondrial morphology can all be used to distinguish between biflagellated water moulds and true fungi (Bruno et al. 2011). Plant pathogenic oomycetes, *Phytophthora infestans*, cause late potato blight, which losses UK £3 billion annually (Phillips et al. 2008). Sudden oak death caused by *P. ramorum* and the soybean pathogen *P. sojae* continues to cause serious economic losses to the plant industry (Beakes et al. 2012).

Among the animal pathogenic oomycetes, crustacean pathogen, *Aphanomyces astaci*, has caused high mortalities, virtually wiping out freshwater crayfish from UK rivers (Edgerton et al. 2004). *Haliphthoros milfordensis* and *Homarus americanus* cause disease in lobsters and penaeid shrimp, respectively (Cawthorn 2011; Hatai 1992). Oomycetes, *S. parasitica*, *A. invadens*, *S. australis* and *S. diclina*, are causing cotton wool and ulcers in fishes, such as salmonids and carps (Van West 2006; Phillips et al. 2008).

16.3 Saprolegniasis

One of the emerging oomycetes pathogens of wild and cultivated freshwater and brackish water fish, crustaceans, and plants is designated as *Saprolegnia* spp. (Sarowar et al. 2019). It causes financial losses in food fish, ornamental fish, and overall fish industries (Eissa et al. 2013). Saprolegniasis ordinarily is viewed as cottony white, dark, earthy-coloured, red or greenish masses on the skin or gills of freshwater or salty fish (Yanong 2003). *S. parasitica* causes infections by producing long 'hooked shape boat' second cysts for attachment to fish and then producing hyphae growth to germinate in fish skin/eggs to establish infections finally. It can suppress the host immunity by the production of effector-specific proteins into the host (Sarowar et al. 2019). *Saprolegnia* is responsible for causing infection in living and dead fish and their eggs (Cao et al. 2014).

Mortalities related to *Saprolegnia* are therefore limited to the late autumn, winter and early spring seasons due to the mass mortality, caused by saprolegniosis being especially devastating at lower water temperatures (Eissa et al. 2013). Salmon farming is mostly affected by *S. diclina* and *S. parasitica*, which are generally prevalent in temperate climates like Northwest Europe, Chile, Japan and Canada (Van Den Berg et al. 2013). The emergence of white or greyish cotton-like structures on eggs, gills and skin in the early stages of *Saprolegnia* infections is the first unpleasant symptom. The illness spreads swiftly and frequently results in mortality, which causes massive losses of fish and ova (Howe and Stehly 1998; Stueland et al. 2005a). Loss of equilibrium, lethargy and rubbing infected areas to the borders of tanks or ponds are a few examples of abnormal behaviour (Van West 2006). Oomycete infections spread via a host colonization process that involves destroying the epidermis with hyphae and the development of effectors that target the host (Wawra et al. 2012), respiratory failures, impaired osmoregulations (Van West 2006) and degrading enzymes (Jiang et al. 2013).

16.4 A Typical Life Cycle of *Saprolegnia* sp.

Saprolegnia, an oomycete pathogen, has a complicated and well-characterized life cycle that includes both sexual and asexual stages (Robertson et al. 2009). While sexual reproduction enables survival in unfavourable settings until they are more favourable for germination and further colonization, asexual reproduction is mostly used to acquire new hosts (Van Den Berg et al. 2013). In contrast to true fungi, Saprolegniaceae has life stages like mycelium, primary cyst, secondary cyst and germination cyst (Srivastava et al. 2018). They produce aseptate mycelium for growth and development and are filamentous and coenocyte. Zoospores are formed in sporangia and are unicellular, terminal, biflagellate and separated from hyphal filaments by basal septa (Bruno et al. 2011). The *Saprolegnia* zoospore is dimorphic and diplanetic, consisting of a pyriform primary form with flagellum insertion at the tip and a reniform secondary spore with flagellum insertion at the lateral edge

(Beakes and Ford 1983). The term 'encysted zoospore' or 'cystospores' refers to a thin-walled cyst formed when a zoospore encysts (Bruno et al. 2011).

16.4.1 Asexual Reproduction

Gemmae (or chlamydospores) are produced during asexual reproduction, and the mycelium's hyphal cells process sporangia at their tips to release motile primary zoospores (Bruno et al. 2011). *Saprolegnia* mycelium is distinguished by having comparatively broad and thick hyphae (Diéguez-Uribeondo et al. 2007). The hyphae are coenocytic and aseptate. Rarely, septa are created in sporangia or infrequently in germlings, which are sexual structures (Liu et al. 2014). The zoosporangium then produces motile primary zoospores, which encyst to form primary cysts in less than a minute (de la Bastide et al. 2015). Prior to encysting, germinating, and releasing a secondary zoospore, primary zoospores are active for a short period of time (Robertson et al. 2009). The two flagella, tinsel and whiplash, are shed during encystment, and a cell wall is also formed (Minor et al. 2014). A secondary zoospore, or new primary cyst, can emerge from the ensuing primary cyst (Bruno et al. 2011). Primary cysts have tufts or solitary, unbranched tubular hairs between 0.5 and 1.5 μm in length, extending over them (Ali et al. 2013).

Compared to primary zoospores, secondary zoospores are motile for a longer time. The most infectious stages after encyst, secondary zoospores, cause secondary cysts to develop, which are distinguished by the appearance of long, hooked hairs (Söderhäll et al. 1991). Finding a suitable host (a fish or an egg), secondary zoospores develop into hyphal cells and mycelium in the host tissue, which starts the infection process (Van Den Berg et al. 2013). To locate a suitable host, these zoospores display chemotaxis, pH-taxis, geotaxis and electrotaxis (Masigol et al. 2020). Wide, coenocytic germ tubes loaded with cytoplasm are characteristics of direct germination. Following the period of rest, germination occurs; once germination has taken place, the life cycle may be shortened (Willoughby and Hasenjäger 1987). Production of effector host-targeting proteins, impaired osmoregulations, respiratory failures (Wawra et al. 2012; Van West et al. 2010), impaired osmoregulation, epidermis destruction by hyphae, destruction of the epidermis by hyphae (Belmonte et al. 2014), respiratory failures (Van West 2006) and degrading enzymes, such as glycosyl hydrolases, are all part of the oomycetes (Jiang et al. 2013). According to Rezinciuc et al. (2018), *S. parasitica* cysts feature long, hooked hair bundles that are involved in pathogen attachment and are approximately three times stronger than other *Saprolegnia* spp. that have shorter or no hooked hairs.

16.4.2 Sexual Reproduction

Gametangia, the male antheridium and female oogonium, are produced during sexual reproduction and merge to produce oospores (Van West 2006). Large oospores or eggs are produced by the oogonium, which varies in size, shape and

number depending on the species. To conserve food reserves, saprolegniales' oospores feature a core ooplasm, a granular appearance and significant amounts of lipid (Diéguez-Uribeondo et al. 2009). Half as many chromosomes as those seen in the nuclei of hyphae are present in a male haploid (n) nucleus (Bruno et al. 2011). The only morphological identification source for species categorization and characterization is the characteristics of oospores, antheridium and oogonia. However, it can be difficult to maintain sexual reproductive stages in a lab environment, and many *S. parasitica* isolates do not engage in sexual reproduction (Fugelstad et al. 2009). After 4–6 weeks of suboptimal temperature, several isolates of *S. parasitica* frequently reproduce sexually (Bulone et al. 2019).

16.5 Morphological Identification

The basic steps for diagnosing *Saprolegnia* infections include morphological characteristics (Table 16.2) such as oogonium ornamentation, antheridium origin and oospore forms; nevertheless, confirmation research using multiple molecular methods is necessary (Tandel et al. 2021). *Saprolegnia* has been traditionally identified and distinguished using patterns of asexual and sexual characteristics (Bangyeekhun et al. 2001). While the sexual characteristics, oospore and lipid droplet position in the oospore, have been used to differentiate species, the asexual characteristics, such as mycelium and germinating cyst, have been most frequently used in identifications (Maurya et al. 2009). The length of this bundle of long, hooked hairs produced by *Saprolegnia* spp. on secondary zoospore cysts varies depending on the strain of oomycetes (Hatai 1994). On its secondary zoospore cyst, *S. hypogyna* may generate a single, long, straight hair. It also has an esterase isoenzyme similar to that of *S. parasitica* (Hatai and Hoshiai 1993). *S. parasitica* does, however, have a single short hair slightly wider than *S. diclina* (Hussein and Hatai 2002). By examining the production of hypogynous antheridia in the latter phase, isolates of *S. ferax* and *S. hypogyna* were distinguished from one another (Hatai and Hoshiai 1992). However, maintaining the sexual stages in lab settings is difficult.

16.5.1 *Saprolegnia parasitica*

Coker (1923) described *S. parasitica* from the fish hatchery. Kanouse (1932) explained the reproductive structures of *S. parasitica* Neish (1977) and reported that *S. parasitica* isolates producing reproductive structures may be assigned to *S. diclina* (Humphrey 1893). As a result, the *S. diclina-S. parasitica* complex developed, which included the five species *S. parasitica*, *S. diclina*, *S. australis*, *S. shikotsuensis* and *S. Kauffmania*. Willoughby et al. (1983) divided the complex into three subgroups based on the following: S. diclina Type I contained fungi that parasitized salmonid fishes, Type 2 was a parasite of coarse fish and Type 3 was saprophytic (Hatai 1994). The presence of long hairs on the secondary cysts, the

Table 16.2 Morphometric characteristics of *Saprolegnia* sp.

Life stages	*Saprolegnia parasitica*	*S. australis*	*S. diclina*	*S. ferax*	*S. salmonis*	*S. hypogyna*
Mycelium	Branched, with average width of 35–39 μm	Dense and diffuse	Moderately branched with average width of 35 μm	Sparingly branched with (average width of 42–44 μm		
Sporangia	Usually variable bent and irregular with average width of 325 μm	Renewed internally, cylindrical; divides into larger primary with 250 × 32 μm long, secondary up to 600 μm long	Curved cylindrical, with 420–480 μm long	Irregular, spherical or cylindrical with average 352–951 μm long		
Spore	Dimorphic with 9–11.5 μm long	Dimorphic; discharge	Dimorphic, encysted spores with diameter of 9–12 μm	Same as diclina		
Cyst	Average diameter 10–11 μm	Average diameter 11 μm	Average diameter 11 μm, spherical gemmae	Average diameter 9–11 μm		
Oogonia	Lateral or terminal with diameter between 86 and 110 μm	Lateral or terminal or intercalary with diameter between 59 and 80 μm	Required higher incubation period to culture, when grows to intercalary or terminal, lateral or with diameter of 50–70 μm	Lateral or terminal or intercalary with diameter between 60 and 80 μm in	Abundant, spherical shaped and elongated with antheridial	
Oospore	Lateral or terminal but rarely catenulate, with 86–110 μm diameter	Rarely mature to sub-centric; with 4–12 per oogonium, 22–27 μm diameter	Centric or spherical with 6–26 per oogonium and diameter between 14 and 28 μm	Centric or spherical with 14–28 μm per oogonium, 15–63 μm in diameter		
Antheridia	Antheridia diclinous	Branched or tubular	Simple	Generally tubular		Without antheridial branch
References	Vega-Ramírez et al. (2013) and Refai et al. (2016)	Refai et al. (2016)	Refai et al. (2016) and Vega-Ramírez et al. (2013)	Masigol et al. (2020)	Masigol et al. (2020)	Vega-Ramírez et al. (2013)

degree of indirect germination and isolation were used to identify the asexual *S. parasitica* species (Willoughby 1985; Beakes and Ford 1983). Long, hooked hairs arranged in bundles on the secondary cysts and indirect germination in a liquid media with little nutrition are hallmarks of *S. parasitica* (Bruno et al. 2011; Lone and Manohar 2018). According to Diéguez-Uribeondo et al. (1994a, b), *S. parasitica* was characterized based on the ornamentation of the cysts and the pattern of germination from brown trout; physiological traits such as sporulation, zoospore mobility and repeated zoospore emergence are not used to identify the species but may be used to determine pathogenicity and host specificity.

The majority of *Saprolegnia*, isolated from salmonids, exhibit *S. parasitica*-like traits. Nevertheless, even if they have these distinct physical traits, the toxicity of *Saprolegnia* strains reported from challenge trials may vary considerably (Yuasa and Hatai 1996; Fregeneda Grandes et al. 2001). Then, many authors disagree with these descriptions, and molecular markers have been used to corroborate the identification further. This can be done by employing phase-contrast microscopy to examine the intricate structure of the secondary zoospore cyst of *S. parasitica* (Inaba and Tokumasu 2002; Bangyeekhun et al. 2003). Diéguez-Uribeondo et al. (2007) showed that isolates representing varied geographical and morphological are a component of the same genetically homogenous lineage. Vega-Ramírez et al. (2013) described *S. parasitica* with characters such as abundant gemmae with irregular shape and size, often in the chain and terminating and intercalary hyphae. Irregular, bent sporangia contain 9–11.5 μm zoospore and the bundle of long, hooked hair and germination pattern. Ali et al. (2013) reported that *S. parasitica* isolates could co-develop biofilm communities where they could grow and breed. They also investigated *S. parasitica*'s ability to create biofilms for survival, reproduction and resistance against various drugs, used to control it. According to physical characteristics such as aseptic hyphae, clavate zoosporangiums, saprolegnoid zoospore discharge and the absence of sexual features, Kim et al. (2013) identified *S. parasitica* from wild brook lamprey with morphological characters showed aseptic hyphae, clavate zoosporangium, saprolegnoid zoospore discharge, whereas sexual characters were absent. A secreted serine protease from *S. parasitica* called SpSsp1 has been identified by Minor et al. (2014) as a possible vaccine target. SpSsp1 appears to be recognized by antibodies in trout serum. The function of *S. parasitica*'s bundles of long, hooked hairs as attachment structures is supported by microscopic, physiological and bioinformatics pieces of evidence. The structures are either made of N-glycosylated proteins, or they may help spread the cyst extracellular matrix on the host surface (Rezinciuc et al. 2014, 2018).

Saprolegnia species and their close oomycete relatives invade epidermal tissues of a wide range of freshwater unusually cold-water fishes and infest moribund eggs (Wilson 1976; Neish and Hughes 1980; Willoughby et al. 1983). More saprophytic species, like *S. diclina*, are typically isolated from water and sporadically from fish or eggs that have already contracted the disease. However, these isolates lack the long hairs on secondary cysts and typically cannot kill fish that have been intentionally challenged (Hatai 1994). The morphological characteristics of *S. diclina* include sub-centric oospores, and lack of centric oospores differs it from *S. parasitica*. The

water moulds are mycelium-forming microfungi spread by spores, conidia or hyphal fragments. It has been found that it is challenging to specify species only based on morphology (Chukanhom and Hatai 2004). Fregeneda Grandes et al. (2001) opined that many bundles per cyst could be pathogenic isolates despite features like bundles of long and variable hair termination under transmission electron microscopes. In support of this, Stueland et al. (2005a) also suggested pathogenicity indicators, an initial growth rate of germinating cyst in pure water and long, hooked hairs on the secondary cyst of *S. parasitica* in Atlantic salmon. Particular morphological characters of *S. parasitica* are long, hooked hairs in the bundle of the secondary cyst and indirect germination in a low nutrient liquid medium. However, *S. diclina* lacks such type of characters and cannot produce any mortality in challenge trials. The complicacy in overlapping morphological characters and the development of sexual characters in lab condition for particular species are the critical issues in morphology-based species identification method (Tandel et al. 2021).

16.5.2 *Saprolegnia australis*

S. australis is closely related to *S. parasitica* and *S. diclina* and is often overlooked due to its high morphologically and phylogenetically closet to other species of *Saprolegnia* (Johnson et al. 2002). Sexual reproduction characters in culture within a short period of incubation, such as pitted oogonia with variable shapes, predominantly obpyriform with immature and mature oospores, are morphologically closet with the characters of *S. diclina* and *S. australis* (Tandel et al. 2021). *S. australis* has been isolated from infected eggs of salmonids, crucian carp in Southern China and Nile tilapia in Egypt (Liu et al. 2015; Zahran et al. 2017), and the species is reported to be pathogenic in opportunistic condition (Sandoval-Sierra et al. 2014). Vega-Ramírez et al. (2013) opined that long-stalked pitted obpyriform, elongated or spherical oogonia with partially or nearly filling sub-centric oospores and predominantly diclinous antheridial branches are the distinguished features of *S. australis* and *S. diclina*. Similar results were reported in sequence analysis of *Saprolegnia* from crucian carp eggs in Southern China and Nile tilapia (El-Ashram et al. 2007; Zahran et al. 2017).

Morphological characters of *S. australis* described briefly by Vega-Ramírez et al. (2013) include dense to diffuse mycelium; slender hyphae and cylindrical sporangia dimorphic spores; abundant gemmae; and clavate, single terminal or intercalary, pitted and smooth wall with terminal oogonia. Oogonial stalks in length: straight, curved, twisted or irregular and unbranched. Sexual characters include oospores that may or may not mature or may abort; when mature, sub-centric; spherical to sub-spherical; 4–12 per oogonium, but usually not filling it; 22–27 μm in diameter; germination not observed—antheridial branches, predominantly diclinous, monoclinous or androgynous. Antheridial cells branched or straightforward, persisting; tubular or attached in a digitated fashion; fertilization tubes present or absent, not persisting.

16.5.3 *Saprolegnia diclina*

S. diclina belongs to the order Saprolegniales (water moulds), causing saprolegniasis. *S. diclina* and *S. parasitica* are considered the extremely serious fungal diseases affecting freshwater fishes, leading to high mortality of fish and significant fin ancial losses to aqua hatcheries (Thoen et al. 2011; Van Den Berg et al. 2013; Songe et al. 2016).

The presence of abundant diclinous antheridial branches of this heteroecious species, which completely or partially envelop the oogonia, makes it easily identifiable. Both centric and sub-centric oospores are produced by *S. diclina*, and the two can coexist in the same oogonia. Milanez (cited by Johnson et al. 2002) observed sub-centric oospores in the oogonia in several of his specimens of Humphrey's species. Three variants of *Saprolegnia diclina* (parasitic forms from salmonids and perch and exclusively saprophytic ones) have been discovered by Willoughby et al. (1984) based on the length-to-diameter ratio of the oogonium. The occurrence of species has been reported from various regions such as in Canada, Czechoslovakia, Denmark, France, Finland, Germany, Iceland, India, Iraq, Japan Mexico, Belgium, the British Isles, Latvia, Middle Europe, Switzerland, the USA, the West Indies Nepal, Poland, Portugal, the Republic of China, Romania and South America (Johnson et al. 2002).

S. diclina is generally saprophytic, feeding on dead plant and animal tissues. However, it is also competent in a parasitic extant, making them facultative necrotrophs. *S. diclina* are primarily thought of as opportunistic secondary pathogens usually common in freshwater environments attacking the host in distress conditions (for instance, when they are infected by other pathogens, they suffer injuries or are exposed to environmental circumstances that are unfavourable) (Songe et al. 2016). The primary disseminative and means of infection in the life cycle of fungus are thought to be the countless sporangia that are produced by each expanding colony and released in enormous quantities as motile zoospores. The rate of spore release from infected fish might exceed 190,000 per minute (Willoughby and Hasenjäger 1987).

Effects of hyphal infection, which spreads swiftly among neighbouring eggs, lead to the destruction of aquaculture hatcheries (Smith et al. 1984). According to several studies (Cao et al. 2014; Rand and Munden 1993), *S. diclina* primarily infects fish eggs, and it has been hypothesized that this species has altered to specialize in egg invasion (Diéguez-Uribeondo et al. 2007).

S. diclina infection caused substantial alterations in the eggs of females, including an almost entirely destroyed and somewhat invisible chorion (Songe et al. 2016). According to Rand and Munden, incursion of live fish eggs by *Saprolegnia* strains may be favoured/eased by both mechanical pressure and their mycelia's enzymatic activity. They discovered enzymes on *S. diclina*-infected brook char eggs and hypothesized that these enzymes may have changed the chorionic membrane's integrity by dissolving structural polymers and allowing hyphae penetration.

16.5.4 *Saprolegnia ferax*

S. ferax, a member of Saprolegniaceae, is also considered an important pathogen causing saprolegniosis in embryonic stages of fish and amphibians (Cao et al. 2014; Fernández-Benéitez et al. 2011; Sarowar et al. 2014). These species are ubiquitous in freshwater ecosystems, more often seen as parasites than as saprophytes, and under some circumstance opportunistic pathogens as well, multiplying on fish that have physical wounds, are under stress or have infections (Pickering and Willoughby 1982). *S. ferax* species is known to cause ulcerative cutaneous necrosis (Kaminskyj and Heath 1996). Wani et al. (2017) reported the discovery of *S. ferax* for the first time in India in the waterbodies of Pachmarhi, Hoshangabad, India.

Oospores which can be central or sub-centric and spherical or elliptical and which are 10–18 in number nearly fill the oogonium, measuring 22–28 m in diameter, with gemmae having varying size and orientation, making the monoecious *S. ferax* distinguishable. Ordinarily, it can be distinguished by a combination of prevalent characteristics, such as broad, sparsely or conspicuously pitted oogonia, oospores which may be centric and sub-centric (at times within the same oogonium), discharged sporangia exhibiting oogonial sporadic development as well as the prevalence of monoclinous antheridial branches or androgynous.

The *S. ferax* mitochondrion's 47 kb compact circular genome, which codes for rRNA genes, 18 respiratory chain proteins, 37 protein, 16 ribosomal proteins, 25 tRNA genes, the import protein secY as well as large and small ribosomal subunits, has been discovered through the process of sequencing, and the division of genome into two single-copy sections is attributed to 8618 kb inverted repeat (Grayburn et al. 2004).

Asia, Australia, Belgium, British Isles, Canada, Czechoslovakia, Denmark, France, Germany, India, Iraq, Japan, Lapland, Latvia, Middle Europe, Nepal, the Netherlands, Poland and the United States all have reported *S. ferax* in their regions (Johnson et al. 2002).

16.5.5 *Saprolegnia delica*

It is one of the members of water moulds, characterized by the presence of white growth that resembles cottony wool and is common to all oomycetes. The species isolate is found to own fibrous, elongated, tapering hyphae containing zoospores within circular ends as well as a robust, non-septate mycelium growing in coenocytic hyphae at tips.

S. delica possess various life cycle stages, including cylindrical tapering zoosporangium and a mono-hyphae with multinucleated cytoplasm. Zoospores and oospores, found in the sexual and asexual structures such as the zoosporangium and oogonium, could be clearly seen when viewed under the microscope. In addition, many smooth-walled (pitted or unpitted) oogonia, each containing 5 to 22 sub-centric oospores, are believed to be present, measuring 12 to 35 μm in diameter (Magray et al. 2021). Variable antheridial cells (monoclinous or

androgynous) either branched or unbranched have been found apically connected to the oogonia.

S. delica is a pervasive opportunist pathogen that may infect both rainbow trout and carp fingerlings. Several earlier investigations support the development of necrotrophic and facultative nature (deriving food from both living and dead tissues) of the *S. delica* species, thereby infecting both rainbow trout eggs and fingerlings of carps (Fregeneda-Grandes et al. 2007; Songe et al. 2016). From the investigation conducted by Rezinciuc et al. (2018), it was concluded that prolonged mortality of farmed *Salmo trutta* eggs was caused by *S. australis*, which is known to show relatedness to *S. delica*, and designated oomycetes as fundamental diseases of fish and their embryos.

Moreover, *S. delica* and other *Saprolegnia* species are considered dangerous to certain other fish species of freshwater, their embryos/young ones and even other aquatic organisms due to their intrusive infection in Atlantic salmon and salmon eggs (Phillips et al. 2008; Chukanhom and Hatai 2004). *Saprolegnia* species actively inhibits the host immunity, while the main infestation is ongoing, paving the way for it to enhance infections; therefore, it is probable that *S. delica* together with *S. ferax* and *S. parasitica* will arise as the main disease of fish and other aquatic life.

The histopathological evidence of saprolegniasis, caused by *S. delica* to fish fingerlings, whereby necrosis, skin infection, lesions, degeneration of scales and rotting of fins occurred, is corroborated by earlier studies making it a potential risk to the viability/profitability of aquaculture sector (Margay et al. 2021).

16.6 Symptoms of *Saprolegnia* Infections

Fish infected by *Saprolegnia* sp. exhibits white patches of mycelium on their skin, gills and fins. According to Pickering and Willoughby (1982), oomycete patches may contain one or more *Saprolegnia* species and turn grey due to the presence of bacteria and detritus (Bruno and Wood 1999). According to Noga and Dykstra (1986), oomycetes differ from fungal infections in fish, and it typically causes superficial infections that spread from the skin to internal organs and produce a mass of mycelium resembling a cotton ball. Oomycetes also elicits a very mild mononuclear inflammatory response. The mycelium is in charge of creating and dispersing motile zoospores, which can germinate when attached to a new host and form new mycelial mats. On the fish skin, particularly around the head, dorsal and caudal fins, gills, muscular layer and internal organs, the disease's gross symptom often appears as a relatively superficial, cotton wool-like, white proliferation of mycelia (Hussein et al. 2001). In *Anguilla anguilla*, *Saprolegnia diclina* infections led to clinical signs and histological abnormalities such as epithelium loss, ulcerations, oedema and myofibrillar degeneration, according to Pickering and Willoughby (1982). *Saprolegnia* can function as primary or secondary ectoparasite. The fish may become more susceptible to saprolegniosis as a result of stressors such as handling, other infections, mechanical injury, sexual maturity, temperature changes, inadequate hygiene or social interactions, according to Diéguez-Uribeondo

et al. (2007). Low water temperature, handling, spawning times and injury were reported as predisposing factors for *Saprolegnia parasitica* in Nile tilapia. Zahran et al. (2017) also described clinical signs, including cotton wool-like white to dark grey growth due to *S. parasitica*. Osmoregulatory dysfunction and electrolyte homeostasis disruption are the main causes of death (Thoen et al. 2011). The loss of protective mucus from the epidermis caused by a sudden drop in water temperature makes it easier for zoospores to connect to the skin and cause infection (Fregeneda-Grandes et al. 2007).

Histology provides in-depth insight into the health of a fish's whole environment and aids in the pathogen identification process (Aranguren and Figueras 2016). Several publications have documented the histological changes, caused by *Saprolegnia* species infections in fish eggs and other organs (Hussein et al. 2001; Songe et al. 2016; Shin et al. 2017). *S. parasitica* causes osmoregulatory issues by destroying the fish's epidermis and dermis layers (Pickering and Willoughby 1982). However, hyphae destroy the chorionic membrane, which controls the osmosis of embryo, in fish eggs (Liu et al. 2014). According to microscopic histopathology study, *Saprolegnia* hyphae enter epidermal tissues, causing cellular necrosis and penetrating the muscle and blood vessels of the infected fish (Shin et al. 2017). *S. parasitica* infections result in epidermal and dermal alterations, such as spongiosis in the epidermis, haemorrhagic foci or mononuclear inflammation between the thick layer of connective tissues (Gieseker et al. 2006). However, *S. parasitica* harms the eggs by piercing the intact chorion with hyphae, but *S. diclina* is finished with the chorion (Songe et al. 2016). Bly et al. (1992) described histological alterations brought on by saprolegniasis and looked at the destruction of mucus-secreting cells, the absence of leucocytes and the presence of fungal hyphae at the lesion's degraded dermal surface. In *S. parasitica*-infected *Ctenopharyngodon idella*, gill histology revealed severe necrosis, disappearance of branchial epithelium and loss of epithelial interlayer with secondary lamellae (de Freitas Souza et al. 2019).

16.7 Molecular Description of *Saprolegnia* spp.

DNA fingerprinting, genetic diversity by random amplification of polymorphic DNA (RAPD-PCR), internal transcribed spacer (ITS) regions of ribosomal DNA genes and nuclear ribosomal DNA (nrDNA) are used in the molecular identification and characterization of *Saprolegnia* spp. (Sandoval-Sierra and Diéguez-Uribeondo 2015). *Saprolegnia* spp. are responsible for the disease in the wild as well as cultured aquatic animals. Therefore, there is an increasing interest in the identification and characterization of pathogenic *Saprolegnia* isolates. It has not been possible to distinguish the species based on sexual reproductive characters due to ambiguity, which often fails to produce sexual and asexual characters of the genus *Saprolegnia* (Johnson et al. 2002). To resolve the taxonomic identification ambiguities in *Saprolegnia*, different molecular tools have facilitated species identification through sequencing of internal transcribed spacer (ITS) regions of ribosomal DNA genes, the nrDNAs (White et al. 1990; Diéguez-Uribeondo et al. 2007).

Additionally, *Saprolegnia* typing techniques like random amplified microsatellite polymorphism (RAMP) and random amplification of polymorphic DNA (RAPD-PCR) allow differentiation between genotypes of *Saprolegnia* isolates (Bangyeekhun et al. 2003; Naumann 2014). The fish pathogenic *Saprolegnia* genome has been analysed, using restriction fragment length polymorphisms (RFLPs) and Random amplification of polymorphic DNA (RAPD). DNA polymerase chain reaction (RAPD-PCR) methods offer a sensitive and quick assay for determining the genetic distance between various *Saprolegnia* isolates. Random amplified polymorphic DNA (RAPD)-PCR has been used within the oomycetes to distinguish different strains and species.

To distinguish between numerous *Saprolegnia* strains and species more effectively, it would be highly desired to develop fingerprinting techniques like multilocus sequence typing (MLST), microsatellites or one of several polymorphism techniques (Molina et al. 1995). Molecular characterization of fish pathogenic *Saprolegnia* is helpful for advancing epidemiological investigations into the point of infection, how the disease spreads and how to control it. Saraiva et al. (2014) characterized the tyrosine gene encoding the mono-oxygenase enzyme that catalyses the O-diphenols to quinines from *S. parasitica* for melanin formation and suggested that the application of gene silencing can be used to characterize gene functionally.

16.8 Control of *Saprolegnia* sp. in Aquaculture

Due to mutagenicity, teratogenicity and carcinogenicity, the use of malachite green, n-methylated diaminodiphenylmethane as fungicides or ecto-parasiticide has been prohibited since 2002 (Srivastava et al. 2004). Moreover, permitted chemical formulations and treatments, such as formalin and hydrogen peroxide, pose a significant risk to people and wildlife. Therefore, they will probably be outlawed soon. Therefore, attempts have been made to find out alternative antimycotic medicines that are suitable for fish of all the life stages and are safe and efficacious (Shah et al., 2021). Formalin, copper sulphate, hydrogen peroxide, boric acid, ozone, iodophor, sodium chloride and peracetic acid are among the alternatives to malachite green. Biocontrol agents like bacteria and some of the bioactive ingredients like curcumin, cinnamaldehyde and eugenol are still under investigation for fish.

To prevent fungus infections in aquaculture, formalin (an aqueous solution of 37% formaldehyde) is frequently used (Gieseker et al. 2006). A practical and safe dose of 150–300 mg/L formalin gives protection against *Saprolegnia* in rainbow and brown trout (Seymour 1970). However, Bailey and Jeffrey recorded effective control of fungal outbreaks in rainbow trout, following a 60-min exposure at 250 ppm. Bly et al. (1996) reported 12.5 mg/L formalin for zoospore inhibition and being suppressive at 7.5 mg/L for channel catfish. Gieseker et al. (2006) said 100 mg/L was very useful and reduced 29% mortality in rainbow trout. Willoughby (1985) reported that acriflavine at a dose of 750 mg/L improves tilapia eggs' hatchability. US Food and Drug Administration, USFDA, in 2018, has approved formalin for egg treatment in

aquaculture. Still, formalin has been banned or discouraged in several countries because of its harmful effects (Romansic et al. 2006).

Furthermore, the use of formalin in fish is uneconomical and undesirable as fish are destined to human consumption. Boric acid or boracic or borax is a weak acid with antifungal properties which have been demonstrated against *Candida glabrata* (Ray et al. 2007), *C. vaginitis* (De Seta et al. 2009) and against *Saprolegnia* sp. (Ali et al. 2014). The mechanism of action as an antifungal is the disruption of fungal cell wall (Lilley and Roberts 1997), mitochondrial degeneration or disruption and inhibition of oxidative metabolism or enzymes that are toxic to *Saprolegnia* sporulation and germinations (Ali et al. 2014, 2019). Boric acid is a hydrate of boric oxide: the weak conjugate acid of a dihydrogen borate, which is reported as antiseptic, anti-oomycetes, antifungal and anti-viral. It is used to control *Saprolegnia* infection in eggs and yolk-sac fry. Ali et al. (2014) studied the in vitro efficacy of boric acid, which decreased *Saprolegnia* spore activity, and mycelial growth at a concentration of above 0.2 g/L. The complete inhibition of germination growth was observed at a concentration of 0.8 g/L. Peracetic acid is a mixture of acetic acid and hydrogen peroxide, which disintegrates to hydrogen peroxide and acetic acid when dissolved in water (Straus et al. 2012). Peracetic acid degradation products are non-toxic and can easily dissolve in water.

Copper sulphate acts as an anti-oomycete by disturbing energy biogenesis, protein synthesis and developing internal oxidative stress (Hu et al. 2016). Copper sulphate is approved as algicide, herbicide and molluscicide by US Environmental Protection Agency, and also it is listed in Allowed Synthetic Substances by USDA National Organic Program (NOP) for use in organic livestock production. It is demonstrated as an anti-parasiticide, i.e. *ichthyophthiriasis* (Noga 2010), and acts against saprolegniasis in channel catfish (Straus et al. 2012). The recommended dose is 10 mg/L for channel catfish, for *Ictalurus punctatus* eggs and also for North African catfish, *Clarias gariepinus* fry and yolk-sac stages (Ataguba et al. 2013), 0.5 mg/L for the inhibition of *S. parasitica* mycelium and 1 mg/L for reduction of primary zoospore in grass carp, *Ctenopharyngodon idella* (Sun et al. 2014).

The efficacy of sodium chloride (NaCl) as an antifungal and environment-friendly agent has been reported by many authors in freshwater finfish aquaculture (Khodabandeh and Abtahi 2006). NaCl acts by changing osmotic gradient, thereby increasing osmoregulation (Stockwell et al. 2012). Rasowo et al. (2007) reported 1000 ppm NaCl concentration with 30-min exposure to effectively control saprolegniasis during egg incubation in *C. gariepinus*. However, Pérez et al. (2003) suggested 2500–5000 ppm for crayfish, and Ali et al. (2011) advised 8000 ppm and 12,000 ppm for *Saprolegnia diclina* and *Aphanomyces,* respectively. Iodine has been applied in the hatchery production of rainbow trout for routine disinfection of eggs once they reach the eyed stage. The recommended dose is 50 mg/L for 5 min (Stueland et al. 2005b) and 60 mg/L as bath up to 30 min (Eissa et al. 2013).

16.8.1 Environment-Friendly Control Measures of Oomycetes Infections in Fishes

As alternatives to teratogenic agents, Frenken et al. (2019) proposed seven biological concepts for protecting aquatic organisms, amphibians and plankton against zoosporic diseases, which may be useful, less harmful and more sustainable than the available chemical methods. Which includes prevent or reduce transmission (control of distribution pathways) and vectors, increase the diversity of host species, vaccination and immunization, stimulate defence and production of antifungal peptides by the host, use probiotics hyperparasitism, and use parasite eaters.

There has been a significant achievement in controlling saprolegniasis in aquaculture, which includes the use of immunostimulants in feed such as pyridoxine (Saha et al. 2016), fluconazole (Saha et al. 2017) and miconazole nitrate (Singh et al. 2018), for *Labeo rohita*. The aloe vera (*Aloe barbadensis*) (Mehrabi et al. 2019), dietary nettle (*Urtica dioica*) (Mehrabi et al. 2020), muli bamboo (*Melocanna baccifera*) (Khan et al. 2018) leaves ethanolic extract, and 1,3; 1–6- β-D- glucans has also been tried to control saprolegniasis (Hamad and Mustafa 2018). The inhibitory activity of antibacterial metals such as silver zeolite (Nemati et al. 2019) and copper nanoparticles was used to control saprolegniasis in white fish, *Rutilus frisii kutum* eggs (Kalatehjari et al. 2015). Recently, commercial formulations like Vikron-S (Rahman and Choi 2018); addition of bacterium (*Aeromonas* media strain A199), *Burkholderia* sp. HD05 (Zhang et al. 2019), quellenin from *Aspergillus* sp. (Takahashi et al. 2018) and cladomarine from *Penicillium coralligerum* YK-247 (Takahashi et al. 2017); as well as the use of antimicrobial peptides (antifungal peptide from *Pseudomonas* protegens XL03) (Wang et al. 2011) have been used for the control of saprolegniasis in aquaculture.

16.9 Conclusion

Oomycetes, which resemble fungi, are many of the pathogens that cause devastating diseases in cold-water aquaculture. Around the world, saprolegniasis significantly reduces the production and profitability of trout farms and hatcheries. Despite these hurdles, the use of hazardous chemicals and the lack of suitable therapies make existing fungal management strategies in cold-water aquaculture ineffective and unsustainable. In order to address the issue, new techniques to control/treat infectious diseases may be examined, including the use of plant extract, novel drug delivery vehicles and natural-origin substrates/compounds or essential oils.

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