



# Fish Prey, Food Habits, and Interspecific Relationships

# 6

Xinjun Chen, Bilin Liu, and Yunkai Li

## Abstract

The communities of fishery resources and other aquatic organisms are linked by food chains and food webs. The number of a certain group not only is related to the number of its predators and food but also depends to a large extent on the food security of fish; the quantity, quality, and availability of food; the length of feeding season; and the quantity and quality of fish species in the waters which constitute the main contents of the study of feeding habits in fishery resources. Fish feeding ecology is an important part of fish ecology, though feeding ecology research can provide basic data for further analysis and understanding of population dynamics. Based on the analysis of stomach contents, fish feeding ecology can be divided into three levels: individual level, population level, and community level. In recent years, with the development of science and technology and the remarkable progress of element analysis technology, new techniques such as stable isotope method and fatty acid labeling method have been applied in the study of fish feeding ecology. This chapter focuses on the relationship between fish and food chain, the types and

characteristics of fish feeding, and the research methods and explains how fish ensure their food supply, and at the same time, the conception and research method of fatness and fat content were also introduced. As a result of the development of marine fishery, the practice of fishery production and management has put forward higher requirements for us, that is, not only the static research but also the dynamic understanding of feeding habits, that is, the changing law with time and space, and the quantitative relationship between predation and predation among populations, these results will provide a mathematical model for resource assessment, in particular, the establishment and improvement of ecosystem-based models for fishery resources assessment and management provide valuable basic information.

## Keywords

Fish prey · Food habits · Feeding ecology

## Abbreviations

$s_i$  the standard deviation of the rows  
 $\Gamma_i$  the proportion of prey organisms in the stomach contents of fish; the percentage of a component in the food

X. Chen (✉) · B. Liu · Y. Li  
College of marine sciences, Shanghai Ocean University,  
Shanghai, China  
e-mail: [xjchen@shou.edu.cn](mailto:xjchen@shou.edu.cn); [Bl-liu@shou.edu.cn](mailto:Bl-liu@shou.edu.cn);  
[Ykli@shou.edu.cn](mailto:Ykli@shou.edu.cn)

$d_{ij}$	the Euclidean distance (specimen point distance) between each fish species
$I$	the selection index
$I_i$	the selection index of fish for prey $i$
$L$	Body length
NGS	next-generation sequencing
$p_i$	the proportion of the same prey organism as that in the environmental; the percentage of the same component in the food base; Specific prey abundance
$Q$	fullness
$r_i$	the proportion of prey organisms in the stomach contents of fish; the percentage of
$S_i$	the content (volume, weight, and quantity) of prey $i$ in the stomach contents; the standard deviation of the rows
$S_{ti}$	the stomach content of the ingesting fish with prey $i$ in the stomach
$W$	body weight
$^{13}C/^{12}C$	the carbon stable isotope ratio
$^{15}N/^{14}N$	the nitrogen stable isotope ratio
$^{18}O/^{16}O$	the oxygen stable isotope ratio
$\delta^{15}N$	nitrogen stable isotope ratios
$\delta^{13}C$	carbon stable isotope ratios

## 6.1 Feeding Relationships and Food Chains Among Fish

### 6.1.1 Composition of Fish Feeding

As one of the most important living conditions for fish, prey constitutes the first link in interspecific relationships. The state of fish prey security regulates the growth, development, and reproduction of fish and influences the population dynamics and even the abundance of fisheries.

In general, fish diets are very broad and complex and include aquatic plant groups, ranging from lower unicellular algae to macroalgae and aquatic vascular plants; aquatic animal groups, involving almost every phylum of invertebrates to vertebrate fish; and humic substances, which

are also important prey for some bottom-feeding fish.

The composition of prey varies greatly from species to species, with some feeding on plankton in the pelagic zone and others becoming aggressive carnivores feeding on shrimp, crabs, cephalopods, and even their own juveniles, while others prefer organic detritus on the bottom, becoming detritus consumers. Fish species differ greatly among the broad spectrum of their prey. This is the result of the adaptation and evolution of fish over time.

### 6.1.2 Food Chains, Food Webs, and Their Ecoefficiency

#### 6.1.2.1 Food Chains and Food Webs

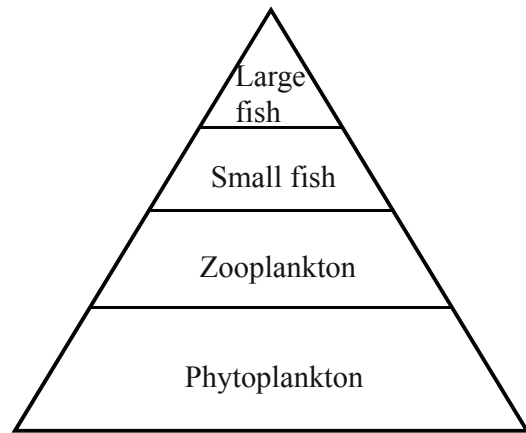
The longitudinal and interspecific food relationships among various organisms in aquatic ecosystems, mainly in the form of predation, prey, and competition, form food chains; multiple food chains form a complex weblike structure, collectively known as a food web.

The food chain refers to the food relationship between fish and prey organisms and predators. The relationships are food-primary consumer-subconsumer-higher trophic levels. Several links in a multilevel trophic relationship also have complex intertrophic relationships, such as small animals feeding on smaller animals or plants being preyed upon by larger animals, i.e., lower-level consumers providing food for higher-level consumers. Thus, one link is interlocked with another in a chain-like fashion.

A single animal often feeds on a variety of organisms, and a variety of other organisms are similarly interdependent and nutritionally linked. Therefore, all kinds of organisms in a whole watershed are interconnected and mutually constrained, forming a complex grid-like network called a food web. Food webs are formed gradually during the long-term development of ecosystems and play an important role in maintaining the stability and balance of ecosystems. Each link in a food chain is called a trophic level, which indicates the trophic position of animals in a food web.

The lowest link in the food chain in lakes and oceans is the primary producers, i.e., phytoplankton (unicellular algae and autotrophic bacteria) and macrobenthic algae (including higher vascular plants); the second link involves the animals that feed on the plants, and they are primary consumers, i.e., phytophagous animals (herbivores); then, the third link involves the animals that prey on these animals, i.e., carnivores, and they are secondary consumers, which are secondary predators. This chain continues, and finally, there are heterotrophic bacteria, also known as decomposers (decomposers). They can break down and reduce plant and animal carcasses and debris in lakes and oceans into the nutrient salts needed by the primary producers to grow and reproduce. In this way, nutrient salts are transported through a series of links, forming a closed loop, also known as the food chain. The existence of this food chain is not only a condition for the survival of lakes and marine life but is also an important structure for maintaining the transformation of matter and the flow of energy throughout a watershed. These relationships are of great importance for the development of living resources in lakes or oceans.

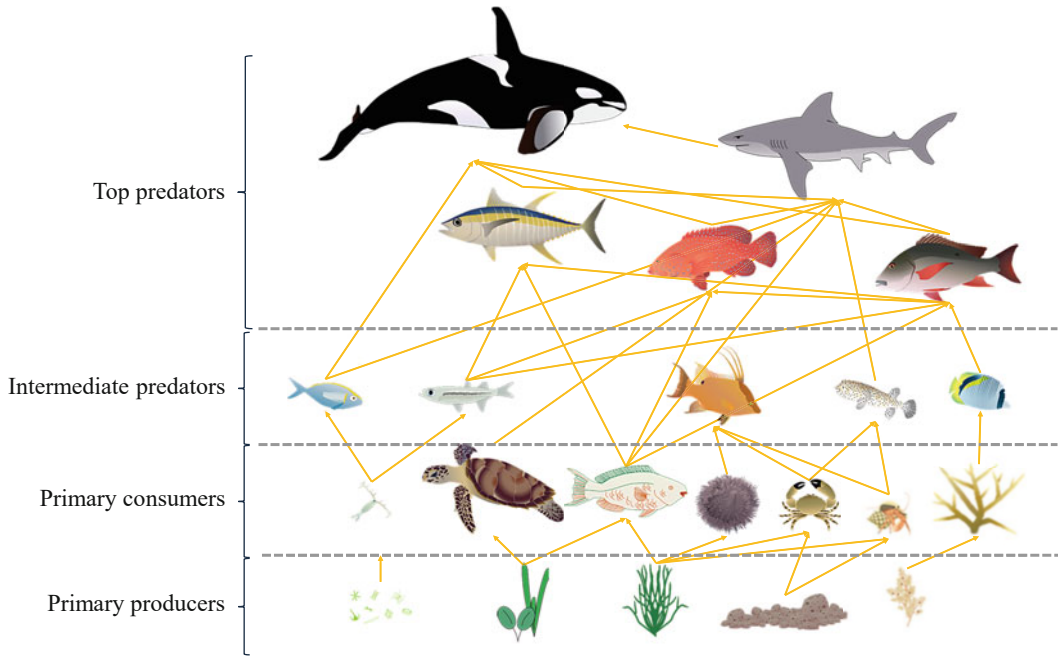
Moving from one link to another in the food chain is accompanied by a certain amount of consumption. From an energy point of view, there is a certain conversion rate. For example, phytoplankton are consumed by zooplankton and converted into zooplankton at a projected rate of 20%, zooplankton are consumed by small fish and form small fish at a rate of 10%, and small fish are consumed by large fish at a rate of 10%. This means that to compose one unit of organism of an animal at a higher level in the food chain hierarchy requires approximately ten units of organismal energy of an animal at a lower level in the food hierarchy. It follows that the closer to the first link in the food chain, i.e., the lower level in the food hierarchy, is to the next link, the more abundant the organisms are. This situation is similar to a pyramid, where the number of organisms decreases higher up in the food chain, and this is called the law of the pyramid (Fig. 6.1).



**Fig. 6.1** Fish food chain pyramid (Chen 2014; Chen and Liu 2017)

As seen from Fig. 6.2, the interrelationship between the trophic levels of a food web, green plants (producers) are at trophic level 1, phytophagous animals (primary consumers) are at trophic level 2, etc., and from the lower level → higher level in a pyramidal pattern, it is generally believed that the energy conversion efficiency of each trophic level is approximately 10%; that is, when energy is transferred from trophic level to trophic level, only approximately 10% of the energy can be transferred to the next level. The largest share of the world's catch composition is accounted for by pelagic fish. At the lowest level of the food hierarchy, phytoplankton and zooplankton, as well as larval classes, are very large in number and sustain highly productive pelagic fish, which are thus able to support other carnivorous and predatory fish. Therefore, to increase the productivity of waters, it is important to keep the number of rings in the food chain as low as possible; i.e., the closer the caught economic fish is to the first ring in the food chain, the greater the yield obtained. However, the fewer the number of rings in a food chain, the more unstable the ecosystem is and the more vulnerable it is to environmental conditions and other factors.

Most of the fish at lower levels of the food chain are small, low-quality fish, such as Clupeidae, Carangidae, and Scombridae, which are not as high quality as those at higher trophic levels, such as Sciaenidae, Sparidae, Bothidae,



**Fig. 6.2** Food webs between catchable marine living resources and primary producers (Chen and Liu 2017)

and Pleuronectidae. Therefore, it is also important to sustainably use fishery resources at different trophic levels.

### 6.1.2.2 Ecoefficiency

Food chains are pathways through which energy flows in an ecosystem. The transition of energy from one trophic level to another does not remain constant, and each subsequent trophic level of the food chain takes up only a small fraction of the food energy provided by the former trophic level. That is, energy flow is gradually reduced. Lindeman (1942) proposed the “one-tenth” law, which indicates the quantitative relationship between the flow of energy between trophic levels in an ecosystem and the efficiency of energy conversion through different trophic levels, and this law is referred to as Lindeman efficiency or ecoefficiency.

Studies have shown that the amount of energy flow in food chains universally decreases sharply as it passes through trophic levels, but the 10% conversion rate is an estimated average that varies considerably between food chains; typically consumers in an ecosystem can only convert up

to 4.5–20% of food energy into their own material (protoplasm). There are usually five trophic levels in a food chain, with green plants as primary producers as the first trophic level, phytophagous animals as the second level, and lower, middle, and higher carnivores as the third, fourth, and fifth levels, respectively. As matter and energy flow from low to high through the trophic levels of the food chain, energy decreases in a stepwise fashion with each trophic level, forming a pyramid shape called the energy cone or ecological pyramid.

## 6.2 Types of Fish Feeding

Because of the wide range of fish diets, the vast majority of aquatic organisms can be consumed by fish, so the prey of fish is very diverse and extensive. In nature, no fish exists that eats all animals and plants, and it is difficult to find a fish that eats exclusively one individual. Usually, a certain fish can eat dozens or even hundreds of species, and these feeding characteristics are closely related to the chewing organs and

foraging methods of fish and are also the result of long-term fish adaptations and evolution. Combined with the feeding characteristics of fish, the feeding types of fish can usually be divided according to the different types of food consumed, food ecological types, predatory nature, and feeding mechanism.

### 6.2.1 Classification According to the Type of the Food Consumed by a Fish

These fish can usually be classified as phytophagous, zoophagous, and omnivorous.

#### 6.2.1.1 Herbivores

Fish that fed on aquatic plant-based food. These fish can also be divided into four categories according to the nature of their staple food.

1. Fish that feed mainly on phytoplankton. For example, *Konosirus punctatus*, *Sardinops sagax*, and *Hypophthalmichthys molitrix*. The gill rakers of this type of fish are very dense and suitable for filtering planktonic single-celled algae, with well-developed intestinal tubes for nutrient absorption. The number of *K. punctatus* is approximately 285 gill rakers. The length of the intestinal canal is three to eight times the body length.
2. Fish that primarily feed on periphyton. This type of fish has a prominent muzzle that facilitates feeding on filamentous algae attached to a reef, such as *Varicorhinus heratensis* and the large-nosed soft-mouthed fish (*Chondrostoma nasus*).
3. Fish that feed mainly on higher aquatic vascular plants. These fish have strong and well-developed pharyngeal teeth and long intestinal tubes suitable for chewing aquatic. For example, *Ctenopharyngodon idellus* pharyngeal teeth are pectinate, and its basal occipital triangular bone pad for grinding can grind plant stems and leaves and cut them to enhance digestion; its intestinal canal is more than three to eight times its body length, and this fish has high amylase activity.

4. Fish that feed on humic substances and detritus. For example, *Mugil cephalus* has a terminal mouth position and a well-developed muscular stomach, similar to the sand sacs of birds, and these are used to grind single-celled algae. The gill rakers in *Mugil soiuy* are in the range of 61–87, and the intestinal canal is more than three times the body length.

#### 6.2.1.2 Carnivores

Fish that primarily prey on animals are characterized by sparse gill rakers and short intestinal tubes. They can usually be subdivided into the following categories:

1. Fish that feed on zooplankton. These fish include *Clupea pallasii*, *Engraulis japonicus*, *Setipinna gilberti*, *Scomber japonicus*, etc. *C. pallasii* has 63–73 gill rakers and long digestive tubes. They feed on krill, copepods, and Amphipods.
2. Fish that feed on benthic organisms. These fish include Bothidae, Pleuronectidae, and Cynoglossidae that are very prey-rich and have diverse dental morphologies, including pavement, cusp, canine, molar, or rostrum morphologies. The number of gill rakers and the length of the gut tube are intermediate between those of zooplankton feeders and swimmer feeders.
3. Fish that feed on swimming organisms. *Trichiurus haumela*, *Scomberomorus niphonius*, and *Pseudosciaena crocea*, which feed on swimming shrimp and small fish, have sharp teeth, short intestinal tubes, and very high digestive protease activity.

#### 6.2.1.3 Omnivores

An omnivorous fish feeds on plants or animals. These fish are characterized by a medium mouth with conical, narrowly flattened or molar-shaped teeth in both jaws. The gill rakers are medium, the length of the digestive tube is less than that of phytophagous fish, and the amylase and protease enzymes for digesting carbohydrates occur at high levels, facilitating digestive growth.

### 6.2.2 Classification Based on the Ecological Type of Food Consumed by a Fish

These types of food can be divided into three categories: plankton, swimming organisms, and benthic animals.

1. Fish that feed on plankton. This type of fish is widely distributed and extremely productive, with a predominantly spindle-shaped body, fast swimming speed, strong digestive capacity, and rapid growth in small and medium fish, such as Clupeidae, Engraulidae, and Carangidae.
2. Fish that feed on swimming organisms. This type of fish is large, swims very well, has a large mouth, is very rich in digestive enzymes, grows rapidly, and feeds exclusively on slightly smaller fish, cephalopods, shrimp, and crabs. They have a high fishery value and include *Trichiurus lepturus*, Sciaenidae, and Sparidae.
3. Fish that feed on benthic animals. These fish are sparsely populated and do not form dense schools. Their dentition is highly variable and specialized to suit the diversity of benthic invertebrate types, such as Bothidae, Pleuronectidae, Dasyatidae, Rajidae, and Soleidae.

### 6.2.3 Classification Based on the Number of Prey Species Consumed

These fish can be divided into generalist and specialist fish.

1. Generalist fish. These fish feed extensively on a variety of prey organisms, and many omnivorous fish belong to this category; for example, *P. crocea* feeds on almost 100 species, and striped bass feeds on 40–60 species of prey.
2. Specialist fish. A small number of fish are distributed in a specific type of water, specializing in hunting certain plants or animal as prey, and their mouthparts and digestive

functions are more specialized and cannot easily adapt to the external environmental conditions that can intensely change. Examples of these fish include *Fistularia* spp., *Tylosurus*, *Syngnathus*, and *Hippocampus* spp.

### 6.2.4 Classification Based on the Nature of Fish Predation

These fish can be divided into two categories: mild fish and aggressive fish.

1. Mild fish. These fish generally feed on small phytoplankton, zooplankton, small benthic invertebrates, organic detritus, or animal carcasses, such as those of *Mugil* spp., *Sphyraenus* spp., *Engraulis* spp., and *Clupea* spp.
2. Aggressive fish. These fish have sharp teeth, are fast swimmers, and live by hunting other fish and smaller invertebrates, and these fish include *Trichiurus lepturus*, *Muraenesox cinereus*, and *Carcharodon carcharias* which can reach a length of nearly 12 m. These fish are extremely aggressive, with sharp triangular teeth and tiny serrated edges, and they can bite large fish or even mammals.

### 6.2.5 Classification Based on the Way Fish Feed

These fish can be divided into five categories: filter-feeding fish, scraper-feeding fish, predatory fish, sucker fish, and parasitic fish.

1. Filter-feeding fish. Specialized filter-feeding fish are characterized by large mouths, fine gill rakers, and weakly developed teeth, and they take in food directly from the oropharynx into the gastrointestinal digestion. Example species include *Engraulis* spp., *Chanos* spp., etc.
2. Scraper-feeding fish. These fish have unique teeth and oral structures that specialize in scraping organisms from rocks. They have

especially well-developed incisors and include fish such as Tetraodontidae.

3. Predatory fish. Characterized by their swift swimming and sharp teeth, they can quickly and accurately pursue their prey and swallow it in one gulp, and these fish include *Trichiurus lepturus* and *M. cinereus*.
4. Suction-feeding fish. These fish form cylinders with specialized mouths that draw in food and water together, causing an attraction current that draws small plants and animals into their stomachs, and these fish include *Syngnathus* spp. and *Hippocampus* spp.
5. Parasitic fish. These fish feed on the nutrients or excreta of their hosts; e.g., *Carassius auratus* specialize in feeding on the excreta or incompletely digested food of larger fish. For example, a male *Ceratias holboelli* feeds on a female by parasitizing her.

Research has shown that the food habits of fish and other organisms are a product of biological adaptations to the environment. For example, the fact that the environment is more stable at lower latitudes than at higher latitudes makes the prey base of fish at lower latitudes more stable, which results in the diets of high-latitude fish species being generally more extensive than those of low-latitude fish. As another example, certain characteristics of fish digestive systems have been developed during the long evolution of fish, thus determining certain food habits. However, the feeding type of fish and other fishery resource species is not fixed; it has a certain stability but also plasticity, especially for omnivorous fish that have a large active sea area, high mobility, very complex food prey, and therefore increased plasticity.

### 6.3 Characteristics of Fish Feeding

When and where a fish consumes its food is related to not only its own biology, such as the developmental stages of its life cycle, but also environmental conditions; thus, it is unlikely that a fish will consume the same food at all times throughout its life cycle, and even

aggressive fish can only take in small algae and zooplankton during their early life history stages. These are important features of feeding habits that need to be understood.

#### 6.3.1 Different Feeding Habits at Different Developmental Stages

Fish tend to feed on different objects at different stages of their development. This is partly due to changes in their nutritional requirements and feeding organs and partly because their living environment tends to change at different developmental stages. For example, *Channa argus* has different characteristics at different developmental stages, feeding on planktonic crustaceans during the fry stage; switching to shrimp, aquatic insects, and small fish during the juvenile stage; and reaching adulthood with a prey composition almost entirely composed of fish, except for a considerable number of shrimp.

#### 6.3.2 Changes in the Composition of Fish Food Across Life Stages

The food consumed by adult fish varies not only in quantity but also in species composition during different life stages. For example, many fish consume very little food during the reproductive and overwintering periods and a substantial amount during the feeding periods. In the case of *Scomber japonicus*, the composition of its diet during different life stages is as follows:

1. Upper north period (transition from reproduction to feeding). In the area where cold and warm currents meet, its main prey types are copepods, krill, amphipods, Salpidae, juvenile *Engraulis japonicus*, and Myctophidae.
2. Southward phase (feeding period). Its main prey types are hornless giant krill.
3. Overwintering period. Its prey species are mainly warm-water copepods, decapods larvae, Salpidae, amphipods, planktonic mollusks, and *Noctiluca*.

4. Spawning period (reproductive period). Its prey are mainly copepods, amphipods, egg and juvenile of *E. japonicus*, *Doliolum*, and Salpidae.

### 6.3.3 Changes in the Composition of Fish Food in Different Waters

Fish have evolved over time to adapt to changes in their environment by changing their biological characteristics, and because the composition of prey organisms varies from one water body to another, fish have had to change their food composition to adapt to their environment. In the case of *Katsuwonus pelamis*, its food composition and changes in different waters have been analyzed and are described below:

1. Eastern waters of Tohoku and Hokkaido. Prey mainly consist of *E. japonicus*, *E. japonicus* larvae, squid, and krill.
2. Waters around the Izu Islands. Prey mainly consist of *E. japonicus*, *E. japonicus* larvae, squid, mackerel, krill, and shrimp.
3. Waters around Ogasawara Islands. Prey mainly consist of Exocoetidae, *Katsuwonus pelamis* larvae, squid, Siganidae, and Holocentridae.
4. Waters off the southern coast of Shikoku. Prey mainly consist of *Trachurus* larvae, *Scomber japonicus*, squid, shrimp, and Gonostomatidae.
5. Balintang waters (southern Taiwan province). Prey mainly consist of squid and Carangidae.
6. Tuko-la-Okinawa waters. Prey mainly consist of *Scomber* spp., *Trachurus japonicus* larvae, Exocoetidae, etc.

As mentioned above, *Trachurus japonicus* typically feed on fish, but the variety of their targets varies considerably in different waters, from migratory to sedentary, feeding extensively on plankton and other invertebrates.

### 6.3.4 Diurnal Variation in Feeding Habits

Due to external environmental conditions such as light, many fish and other prey organisms tend to move vertically, and their feeding habits change diurnally. For example, in one study, the diurnal feeding intensity of black scraper *Thamnaconus modestus* in the East China Sea was greatest from evening to the first half of the night (69.9%), followed by the second half of the night to dawn (27.5%), and the intensity was the lowest in the morning (16.9%); from evening to night, its stomach contents included mainly copepods, isopods, and mesopods of planktonic crustaceans; from the second half of the night to dawn, this fish mainly consumed fish eggs; in addition to mainly consuming fish eggs, the stomach contents of the specimens caught in the morning also included a number of corals.

Numerous observations have revealed that the actions of prey largely determine not only feeding actions but also diurnal variations in food composition.

### 6.3.5 Fish Prey Selectivity

In general, a fish does not have an equal interest in a large number of prey organisms but has a preference, or fish are selective about their food. However, some species show it more obvious selectivity and some less obvious selectivity. Selectivity can be judged by two factors: the ratio of the values of the various prey organisms in a habitat and the ratio of the number of prey organisms consumed by a fish.

Fish are somewhat plastic in their choice of food. When a fish does not have access to its preferred food, it can still feed on other prey types, especially in the juvenile stage, where plasticity is greater. Depending on their preferences and food availability, the food consumed by fish can usually be divided into the following categories:

1. Main food constitutes the main part and is capable of meeting the needs of life entirely.



2. Secondary food is often seen in the intestines of fish but not in large enough quantities to fully satisfy the needs of a fish.
3. Incidental food is prey that is consumed by fish by chance.

In addition, sometimes, due to changes in environmental conditions, fish lack their main food items and ingest some emergency food. For example, sometimes echinoderms and serpentine animals, which are not normally ingested, can be found in the stomach of fish that are apparently forced to swallow them due to a lack of food.

---

## 6.4 Food Security for Fish

### 6.4.1 Fish Food Security

Fish populations and prey biomass, as well as the total biomass of all fish in a water body, depend to a large extent on food security. Food security means that the waters must not only contain prey organisms that fish can feed on but also have environmental conditions that ensure the possibility for fish to feed. The quantity and quality of prey organisms such as plankton, benthic organisms, and fish, which are used as fish food, are also referred to as the prey base. Thus, food security for fish depends on the quantity, quality, and availability of food in their aquatic habitat.

Fish food security is influenced to some extent by the length of the feeding season, but the length of the season does not always affect fish food security; for example, the vast majority of saltwater bream stop feeding after reaching a certain level of abundance and lipid content and begin their overwinter migration to the sea. In fact, when the prey base is at a low level, the length of the prey season has a limiting effect on fish food security. If the prey base is high, then the effect of the length of the season on fish food security is usually felt only at the margins of their range. Fish food security is also affected by the abiotic environment during the feeding season, such as by changes in temperature, light, wind, waves, size of prey distribution, and many

other factors, and to a large extent by the level of defense against predators during the feeding season.

Studies have concluded that fish population size is closely related to biomass and the food security of the species. Fish food security is governed by the following factors: the quantity and quality of prey in the water and its availability, the length of the feeding season, the number of fish seeking prey, biomass, and biomass quality. A fish population influences the prey base, which ensures the growth of that population, the maturity of the sexes, the abundance of the fish, the heterogeneity of individuals within the population, etc. Therefore, when evaluating fish food security, it is best to determine the condition of the fish in terms of growth, abundance, lipid content, heterogeneity of individuals within the population, and other indicators.

### 6.4.2 Adaptation of Fish to Food Security

Individual fish have evolved adaptations that allow them to make the most of the prey base in their complex environments. Fish from the same community are adapted to consume certain types of prey and, through divergence in their diets, to resolve prey conflicts due to feeding on similar food groups with other species. Adults generally have similar diets only in terms of secondary feeding objects, while the main components of their diets are different; in addition, resolution of feeding conflicts by different feeding periods is less common. In contrast, juvenile fish feeding conflicts are mainly resolved by staggering the time of consumption of similar foods. This is generally much less common in the adult stage because of the different composition of food in the juvenile stage. However, different species of juvenile fish consume certain foods at different times. For example, in the same waters, *Esox* spp., *Lateolabrax* spp., and *Parabramis* spp. juveniles mainly consume similar foods such as rotifers and copepods in their anadromous larval stage; however, these *Lateolabrax* spp. and *Parabramis* spp. reproduce earlier, and by the

time the *Lateolabrax* spp. juveniles have switched to feeding on these prey foods, the earlier-born *Esox* juveniles have switched to feeding on other larger size prey. At the same time, the *Lateolabrax* spp. juveniles and *Parabramis* spp. juveniles consume similar foods at longer intervals. Their juveniles are mostly specialists, restricted to feeding on a set species. This scenario may also be the direct cause of the large numbers of mortalities they often suffer, and the prey required for the juveniles is almost always sufficient in water; however, where there is a high concentration of juveniles, there is sometimes not enough prey.

As fish move from one stage to another during development and growth, their food habits also change, an important adaptation for expanding their prey base. During development, in comparison to species with high food security, species with low food security in the early stages of development only switch to external nutrition when they are larger so that they accumulate more yolk in their eggs.

The separation of feeding sites by age is an adaptation that promotes increased food security, and differences in diets between fish of the same size but different sexes are also an adaptive attribute of fish to improve their food security. For example, females of *Sphyrna lewini* often congregate in pelagic waters to feed, while males mainly inhabit nearshore shelf areas; sea cod males consume relatively more crustaceans and worms.

If generations are quite large and food security is low, then a fish population generally shifts to a broad diet, giving it the widest range of recipes. The breadth of a diet varies with food security.

If the parental fish food security is low, then the sizes of its eggs are different, and the duration of hatching small fish from the egg membrane is also different, thus extending the time for juvenile fish to start feeding to the outside world. In the food conversion phase, fish yolk accumulation is different, and the pattern of consuming prey between day and night is also different, thus improving food security. For example, individuals with high yolk accumulation in *Parabramis* spp. will suspend the phenomenon of foraging at night, while individuals with low

yolk accumulation will forage all day. Most of the eggs produced by females with low food security produce different sizes of hatchlings, so the level of prey available to the hatchlings in the same period varies in the environment. Even if the eggs hatch at the same time, the prey base expands slightly when they are transferred to external nutrition, with smaller individuals consuming some types of prey and larger individuals feeding on others. After a certain decrease in food security, fish that were previously the same size began to have different growth rates, with some individuals beginning to grow faster and most lagging behind. Fast-growing individuals move to the next developmental stage earlier, e.g., *Carassius auratus gibelio* in the North Kazakh Lake. Slow-growing individuals have a simpler diet, feeding mainly on detritus, while fast-growing individuals feed mainly on shaker larvae, thus increasing food security.

Migration is an important adaptation for fish to improve their food security, and the sizes of their range change as the density of fish populations change. When fish populations are reduced for one reason or another, they sometimes significantly reduce the size of their feeding grounds. For example, *Clupea harengus* and *Gadus morhua* have reduced feeding ranges and change distances as their numbers decrease.

The clustered lifestyle of many fish species during feeding is an important adaptive attribute for maximum food security. This is evident in pelagic fish, where clustering allows them to locate prey directly and easily and facilitates protection against predators and migration. In comparison to individual fish, groups of fish are more likely to locate and remain in contact with dense groups of prey, and in comparison to a group, an individual fish is more likely to miss a dense, moving group of prey. Certain aggressive fish form schools, making it easier to locate and maintain contact with moving prey and facilitate direct predation. The feeding activity of fish in schools is more intense than that of fish in a dispersed state. Fish in schools generally feed and digest at similar rhythms, which allows the feeding activity of the entire school to begin and end at the same time, making dense groups of prey easily

accessible to the fish. Thus, forming schools during feeding allows fish to expend less energy searching for prey, which means that their food security is improved.

Many fish ingest their own eggs and juveniles when populations experience abundant generations and older fish have unstable food security. This activity is an important adaptive approach for expanding the prey base and regulating fish numbers to match the available prey base in the water, and this activity occurs in *Gadus* spp., *Scomber* spp., *Osmerus* spp., *Esox* spp., *Lateolabrax* spp., and many other fish species.

### 6.4.3 Impact of the Physical and Chemical Environments of a Watershed on Food Security

Changes in the physical and chemical environmental conditions of a watershed greatly affect the food security of fish.

1. Water temperature. For example, the more days in a year that the water temperature is above 14 °C in a lake in England, the faster the growth of *Micropterus salmoides* that year because the appropriate temperature encourages the growth and reproduction of prey organisms, thus increasing the abundance of prey and promoting an increase in fish metabolism. As a result, fish are able to grow faster. Conversely, if the water temperature is lower than normal for the year, it can reduce the metabolic rate of fish, causing them to grow more slowly. For example, there is a clear relationship between the hydrological condition of carp during their feeding season and the fat content and weight of sex products in their liver.
2. Light. The length and intensity of light have an effect on the feeding activity of fish, especially fish that use visual discrimination for food identification, and light is more significant in their foraging process. For example, river cod is likely to feed on *Leucaspius delineatus* when the light is higher than 0.11 x.
3. Waves. Shallow seas are only 8–10 m deep. When storms hit, affecting shallow seas and causing large waves, the waves hit from the bottom to the surface. Some organisms that feed on benthic fish, such as *Abramis brama orientalis*, stop feeding and immediately come up to the surface.
4. Wind. Powerful winds can affect the distribution of insects on land, such as in England in May, August, and September each year in the windy season, and insects can be affected by wind blowing so that the creek, pond, and lake prey increase, making the growth and development of freshwater salmon very favorable. In the Zhoushan area of China, insects on land are pushed into the shallow sea area every autumn due to the influence of wind so that the number of insects in this sea area increases sharply, increasing the prey for fish, especially supplementing those in the juvenile stage.
5. Sea currents. Seawater influences the distribution of prey; for example, the population size of *Engraulis ringens* is closely related to the distribution and abundance of plankton. If tropical warm currents enter Peruvian offshore fishing grounds, thus leading to a decline in the amount of plankton in the fishery, then the anchovy catch is reduced. For example, the annual production of the Peruvian anchovy in 1970 reached 13 million t, and it was mainly distributed off Peru, feeding on abundant plankton. Growth and development was very rapid. In 1972, due to the El Niño phenomenon, plankton was affected by the decline in the productivity of the waters, and the spawning rate of the Peruvian anchovy was also greatly reduced, only one-seventh of the usual, resulting in a significant decline in catches (3.319 million t in 1975, declining to 823,000 t in 1980).
6. Substrate. The distribution and abundance of benthic animals varies with the substrate and affects the amount of energy consumed during fish foraging. There is a close relationship between the energy consumed by foraging

fish and the level of metabolism. For example, sandy, muddy, rocky, and deep-sea areas are different and inhabited by different prey organisms, and the energy consumed by fish foraging activities is naturally different.

Thus, in comparison to other factors, external abiotic environmental conditions are of greater significance for food security. However, these conditions do not affect food security in isolation but work in conjunction with biological conditions.

## 6.5 Methods of Studying Fish Feeding

The standard method for studying fish feeding in modern fish ecology is gastric content analysis. Its purpose is to estimate the trophic structure of the fish community and the trophic level of each fish in the community and further study the cycling of material in the food chain and food web of the ecosystem. Visual, frequency of occurrence, counting, volumetric, and weight methods are the main traditional methods used in fish feeding studies. In recent years, with the development of science and technology and remarkable advances in elemental analysis techniques, new technical tools such as stable isotope tracing, characteristic fatty acid labeling, and DNA barcoding have been continuously applied in the study of fish feeding ecology (Chen 2014; Chen and Liu 2017).

### 6.5.1 Sample Collection and Processing

#### 6.5.1.1 Sample Collection

Due to the degradable nature of animal proteins, fish gut samples must be strictly standardized to ensure reliable analytical results. Thus, the following must be achieved in conducting sample collection:

1. Samples should be fresh or recently have died. When the fish are caught, samples should be

taken immediately to avoid affecting the accuracy of the analysis by continuing enzymatic digestion of the stomach contents over time.

2. Samples should be highly representative. Samples that are truly representative of the target group under study. In the analysis of gastrointestinal contents of fish, samples of all sizes should be taken. In terms of fishing tools, set nets, fish cages, and longlines are generally less representative and are only available for reference, while samples taken from trawls, seines, and drift nets are more representative and can be used for analysis. Gastrointestinal samples caught by tools such as set nets or fish cages have been held for an extended period, and most of the food in the gut has been digested or excreted, which seriously affects the accuracy of the gut analysis; in addition, samples caught by tools such as longlines have a high rate of empty stomachs. Sampling large numbers of fish with mobile gear such as trawls, seines, and drift nets is generally preferred.
3. A certain number of samples should be collected. In fishery resource survey studies, the number of samples taken is usually 1/4–1/8 of the total number of catches, in units of one sample per net. The samples are then numbered, placed in bags, and fixed with 5–8% formalin solution. The length, weight, sex, and gonadal maturity of the fish are recorded during the gastrointestinal analysis for control purposes.

#### 6.5.1.2 Treatment of Stomach Contents

Fish stomach contents must be handled carefully and delicately. Because of the strong digestive capacity of fish, it is important to analyze the stomach contents in a timely manner in a predigested or undigested state so that the analysis of the type and quantity of prey can be carried out accurately.

Before identifying the stomach contents, the number of species, weight, and other parameters of the prey types in the sample area need to be determined. The prey in the stomachs of carnivorous fish can be identified based on the shape and

size of scales, otoliths, hyomandibular bone, cleithrum, operculum, pharyngeal teeth, and fin rays. They prey in the stomach of herbivorous fish and fish feeding on zooplankton can be identified based on the size and number of stems, leaves, fruits, seeds, shape of zooplankton, appendages, mouthparts, bristles, etc. of aquatic plants in the stomach. The identification of prey can occur gradually in the stomach from shallow to deep areas and should not be done roughly.

### 6.5.2 Field Observations of Fish Feeding

Since fishery resource surveys often need to be conducted in relatively difficult environments where highly precise analytical results cannot be obtained, a good approach is to use simple, easy-to-use research methods, such as the visual method. The so-called visual method is to judge the proportional size of each prey item to the volume of the entire stomach by directly estimating the volume of each prey item as a percentage of the volume of the entire stomach contents determined with the naked eye.

When the stomach contents of fish cannot be determined by examining the stomach sac alone, other parts of the digestive organs, such as the intestine, may be used. The visual method is discussed in detail by Soviet scholars in the *Methodological Guide to the Study of Fish Feeding*, which is described as follows:

1. Cylopob E.K. Classifies feeding classes as follows:
  - Level 00: no food either in the stomach or in the intestines.
  - Level 0: no food in the stomach but residual food in the intestines.
  - Level 1: small amount of food in the stomach.
  - Level 2: moderate amount of food in the stomach or 1/2 of the stomach.
  - Level 3: stomach filled with food, but the lining of the stomach does not expand.
  - Level 4: stomach is full of food, and the lining of the stomach is distended.

2. Eotopob T.B. classifies plankton-feeding fish as follows:

Level A: gastric enlargement.

Level B: full stomach.

Level C: moderately full.

Level D: a small amount of food.

Level E: empty stomach.

3. Eotopob T.B. classifies benthic-feeding fish as follows:

Level 0: empty stomach.

Level 1: very few food items.

Level 2: a small amount of food items.

Level 3: multiple quantities of food items.

Level 4: very large quantities of food.

### 6.5.3 Qualitative and Quantitative Versus Analytical Methods

#### 6.5.3.1 Qualitative Analysis Methods

Samples must be thoroughly prepared to ensure a qualitative analysis of prey is easy to conduct. For a qualitative analysis, it is best to take food pieces from the stomach and the front of the intestines, as the prey is more intact and easier to identify there, and if the food has begun to be digested, then it will need to be identified on the basis of residue.

Large prey can be identified by the naked eye, while small prey can be identified with the aid of a dissecting microscope. Depending on the requirements of the biological investigation, identification can be carried out to the phylum, order, family, genus, or even the species level. The degree of digestibility of the prey can be determined by comparing the anterior and posterior segments of the digestive tract.

#### 6.5.3.2 Quantitative Analysis Methods

##### Counting Method

For the counting method, also known as the individual method, the number of individuals of each prey organism consumed by a fish is counted separately in terms of the number of individuals, and then, the percentage of each prey organism of the total number of individuals is calculated. That

is, the number of individuals of a particular type (class) of food component in the stomach contents is calculated as a percentage of the total number of individuals of the food component in the stomach contents. For example, if the stomach of a *Decapterus maruadsi* contains 100 copepods, 75 krill, 50 mysids, 20 *Macrura*, and 5 ostracods, then the percentage of each type of prey type in terms of the total number of individuals is 40%, 30%, 20%, 8%, and 2%, respectively.

The method is rapid, simple, and practical if the food composition can be easily determined. It is particularly convenient in certain situations, such as the analysis of stomach contents of fish-feeding and plankton-feeding fish with similar individual food sizes, where although plankton counts are cumbersome, they can be simplified with the aid of auxiliary sampling, i.e., partial sampling from a known volume of homogeneous water, counting the number of microscopic organisms, and then calculating the total number of organisms, which can be conducted with the aid of Sedgewick Rafter counting baskets (Chen and Liu 2017).

The individual method also does not provide a complete picture of the composition of consumed food alone and is limited by the following factors: (1) the individual method overemphasizes the importance of small organisms that are consumed in large numbers; however, in some cases, small organisms may be overlooked in the food composition because they are digested rapidly; (2) it is difficult to count the number of individuals of all food components because many organisms such as protozoa become paste-like before reaching the stomach sac; (3) the effect of fish size is not taken into account; (4) this method is not applicable to combined foods such as macroalgae and detritus; and (5) the individual prey components derived from this method are often miscalculated because the individual sizes and nutritional values of the various prey organisms are inconsistent, and it is unreasonable to view them in equal amounts. This method is usually used in conjunction with other methods, especially the weight method.

## Weight Method

There are two methods used to determine weight: the first method involves cutting open the stomach of a fish, and removing the contents and immediately weighing them; the second method is the corrected weight method, and it involves carefully selecting intact individual prey organisms during any time of the prey analysis, measuring their lengths or estimating their sizes and weighing them so that after a period of time, the range of sizes and weights of various prey organisms can be known and then multiplied by the number of individual weights. Percent food weight refers to the corrected weight of a particular food as a percentage of the corrected weight of the food mass. There are two types of stomach weights: dry weight and wet weight. The wet weight is generally easier to measure; the dry weight is more time consuming, but it is needed for calculating the energy balance. The corrected weight can be used to calculate the percentage of a prey component to the total weight of the stomach contents according to the following formula:

$$\text{Percent by weight} = \frac{\text{Corrected weight of particular food}}{\text{Corrected weight of food mass}} \times 100$$

In addition to weight percentage, the fullness index can also help analyze the weight of the stomach contents. The total fullness index is defined as the immediate weight of the stomach contents multiplied by 10,000 divided by the net weight of the fish, and the resulting value of the 10,000 parts per million ratio can be expressed by the following formula:

$$\text{Total fullness index} = \frac{\text{immediate weight of the stomach contents}}{\text{net weight of fish}} \times 10000$$

If the weight of the food mass used in the calculation is the corrected weight, then the actual weight of the food mass in the above equation is changed to the corrected weight, and the resulting figure is the corrected total index of fullness. The corrected total index of fullness is more correct

than the total index of fullness derived from the immediately obtained weight. If the components of the stomach contents are separated and the immediate weight of each component is multiplied by 10,000 and divided by the net weight, the value of the 10,000 parts per million ratio is called the fullness index of that component; similarly, the corrected fullness index of that component can be obtained. Again, the corrected fullness index is more correct than the fullness index derived from the immediate weight. The weight stated above is actually the wet weight of the prey.

When determining the wet weight, the water in a food item is usually drained by filter paper, but moisture is still an important cause of error and needs to be further reduced by letting it dry naturally, drying it on a hot plate, or centrifuging it. The determination of dry weight can be conducted by evaporating the water from the food item to a constant weight, and the dry weight temperature varies for different food types (generally between 60 °C and 150 °C). A temperature that is too high may lead to the loss of volatile fat, and the process is time consuming; thus, freeze-drying is more effective.

In food importance studies, the weight method tends to overestimate the importance of individual large food components, and in addition, the weight of food soaked in formalin is different from its wet weight in the field, with resulting measurement errors. This method is somewhat less applicable than the volumetric method but is largely applicable to the analysis of biological components of typical prey items.

### Volume Method

Fish food volume composition refers to the volume of a particular type (class) of food as a percentage of the total volume of the stomach contents. The total volume or fractional volume of the stomach contents is generally determined by the drainage method to determine the percentage of volume of each type of food. A small, graduated test tube or centrifuge tube is commonly used and is filled with 5–10 ml of water. Then, the food mass is placed on filter paper,

blotted dry, and left until moist. The food mass is then placed in a known graduated test tube, and the total volume at this point is used to determine exactly how much water has drained. The composition of the food mass for each major type of prey food is calculated as a percentage of the frequency of occurrence or as a percentage of the number of individuals.

This method is more complicated, and the analysis is cumbersome; however, it allows the volume to be determined more accurately, and then, the weight can be determined. Few people use this method because of its duration.

### Frequency of Occurrence Method

This is one of the simplest and most commonly used methods for determining the composition of prey. The frequency of occurrence is the number of stomachs containing a particular food component as a percentage of the total number of stomachs (nonempty stomachs). Its specific formula is the following:

$$\begin{aligned} & \text{Frequency of occurrence} = \\ & \frac{\text{The number of stomachs containing a particular food}}{\text{The total number of stomachs}} \\ & \times 100 \end{aligned}$$

The frequency of occurrence method has the advantage of being rapid and requiring less instrumentation, but it does not express the relative quantity or volume of each type of prey in the stomach. Nevertheless, this method can provide a qualitative analysis of the types of prey consumed by a fish.

This method is quick and easy if the type of food is easily identified, but it provides only a rough picture of one aspect of fish diets, i.e., the degree of fish preference for a particular food; it does not provide a clear indication of the proportion of a particular (type of) food component to the quantity and volume of the stomach contents.

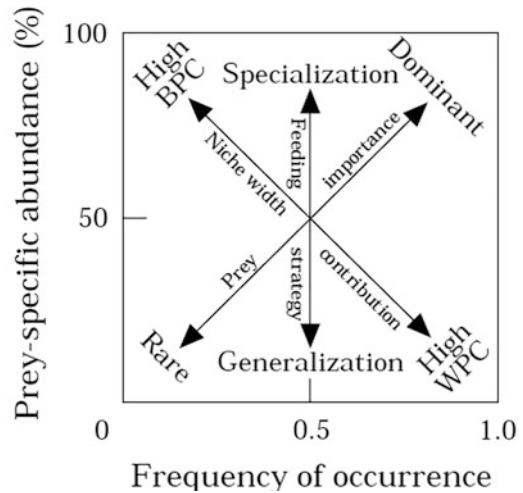
The advantages of the above methods, such as frequency of occurrence, F%; percent quantity, N%; percent volume, V%; and percent weight, W%, are the comparability of the methods in evaluating the importance of each prey category

and their ease in terms of access and handling, where the percent weight indicator can be expressed as wet weight, dry weight, or corrected percent weight. Each method has advantages when evaluating fish feeding habits. The frequency of occurrence method reflects the preference of a fish for a particular prey organism, and the percentage of the quantity provides a good indication of the food composition in the stomachs of fish with similar individual food sizes. Since the weight (volume) of food is related to its caloric value, the weight percentage (volume percentage) reflects the proportion of the total consumption of each prey category by a population. However, these metrics also have some limitations. The frequency of occurrence does not accurately express the actual proportion of each prey organism in a stomach. Percent quantity does not objectively describe the feeding habits of fish whose food varies greatly in terms of individual size, and both frequency of occurrence and percent quantity are strongly influenced by small prey species. Total weight (volume) percentages overemphasize the importance of individual predation on the portion of food that exceeds that utilized by a fish population.

### Integrated Graphical Method

In general, data results are more easily understood as graphical representations. The graphical method uses the frequency and relative abundance of prey as coordinates to directly describe prey composition, the relative importance of prey (primary or incidental food), and the evenness of food selection among predators. This method uses a two-dimensional plot of specific prey abundance and prey frequency to show the importance of the prey, the feeding strategy of the predator, and the composition of the ecological niche width and interindividual composition (Fig. 6.3).

The modified Costello plotting method uses specific prey abundance and frequency of occurrence as indicators to form a two-dimensional plot (Fig. 6.4a). Specific prey abundance,  $P_i$ , and frequency of occurrence are expressed as fractions:



**Fig. 6.3** Costello's improved method for interpretation of feeding strategy, trophic niche width, and prey (Amundsen et al. 1996)

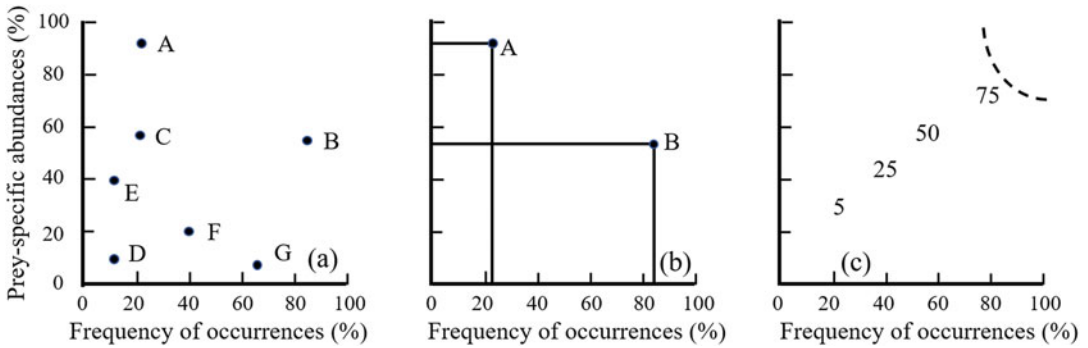
$$P_i = \left( \frac{\sum S_i}{\sum S_{ii}} \right) \times 100$$

where  $S_i$  is the content (volume, weight, and quantity) of prey  $i$  in the stomach contents and  $S_{ii}$  is the stomach content of the ingesting fish with prey  $i$  in the stomach.

The product of a particular prey abundance and frequency of occurrence corresponds to prey abundance and can be represented by a box enclosed together with the coordinate axes (Fig. 6.4b). The sum of the box areas for all prey species equals the total area of the plot (100% abundance), the product of any particular prey abundance and frequency of occurrence represents a particular prey abundance, and different values of prey abundance and frequency of occurrence can be represented by contours in the plot (Fig. 6.4c).

Using Costello's modified graphical method, information about the feeding strategy of a predator and the importance of prey can be inferred by looking at the scatter of points distributed along the diagonal and axes (Fig. 6.3). On the vertical axis, the feeding strategy of a predator is elucidated according to whether it is broadly or narrowly feeding, and the width of the ecological niche of the predator population can be discerned





**Fig. 6.4** Feeding strategy (Yang and Xie 2000)

(a). Hypothetical examples (A, B, C, etc. are different prey types). (b). Prey-specific abundances of prey types A and

B indicated by enclosed areas. (c). Isolines representing different values of prey abundances

by observing the position of the values in the plot. The percentage increase in abundance along the diagonal from the bottom left to the top right is used as a measure of prey importance, with important prey (primary food) at the top and nonimportant prey (secondary food or incidental) at the bottom.

The advantage of the graphical method is that the data can be compared quickly and visually on a graph before further statistical analysis. Rather than simply adding or multiplying weight percentages and frequency of occurrence, the graphical method allows a more detailed fractionation of results by weight percentages and frequency of occurrence to distinguish large prey that are present in only a few fish from small prey that are present in many fish, allowing for a better comparison of results.

### Stable Isotope Tracing

The stable isotope method is a new biogeochemical research method that has emerged in recent years, and it has been widely applied in the study of aquatic ecosystems and has gradually become a powerful tool for fish feeding analysis, trophic level determination, and food web research. Stable isotopes are different stable forms of the same element with different tissue numbers of the same proton number (e.g., carbon has stable isotopes  $^{12}\text{C}$  and  $^{13}\text{C}$ ). The natural abundance of stable isotopes varies in the environment, and because of their complex fractionation mechanisms during

biological metabolism (heavier isotopes linger and become enriched), stable isotope ratios in organisms can be used to trace the flow of material through ecosystems and can provide longer-term information on the feeding of organisms and the transfer of material and energy in food webs. For example, the carbon stable isotope ratio ( $^{13}\text{C}/^{12}\text{C}$ ) is less enriched between trophic levels in the food chain, at approximately 0–1‰, so it is used to indicate food sources and analyze feeding transformation; the nitrogen stable isotope ratio ( $^{15}\text{N}/^{14}\text{N}$ ) is more enriched between trophic levels, at approximately 3‰, and is used for fish trophic level determination; the oxygen stable isotope ratio ( $^{18}\text{O}/^{16}\text{O}$ ) in fish otoliths and the water temperature of habitats have been found to have a linear relationship and are mostly used for life history and habitat reconstruction.

Compared with traditional gastric analysis methods, stable isotope methods provide a more rapid and objective approach for analyzing food web structure and describing the position of food webs in terms of the long-term and short-term changes in organisms' food habits and trophic flows. Stable isotope methods have been widely applied to study energy flow between trophic levels of food chains in lakes, oceans, and estuaries and to map food chain and trophic flow relationships in these ecosystems. For example, carbon and nitrogen stable isotope methods were used to analyze the feeding habits of *Erisphex potti* in the estuary of the Yangtze River and its

adjacent waters and to verify the feeding pattern of *Erisiphex potti* during its growth; carbon and nitrogen stable isotope methods were used to analyze the food web structure of the East Taihu Lake ecosystem, and it was found that despite the large quantity and quality of submerged plants in the lake, the main food source of fish is still the planktonic food chain.

Stable isotope methods have now been developed to the molecular level, namely, compound-specific stable isotope methods. By analyzing stable isotope information (mainly elemental carbon and nitrogen) at the level of specific macromolecules (e.g., amino acids and fatty acids) in organisms, the patterns of material and energy transfer processes of isotopic fractionation in biological tissues can be determined more specifically. In studies of fish feeding ecology, the analysis of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in specific amino acids in fish is the main focus. McClelland and Montoya (2002) studied nitrogen stable isotope ratios ( $\delta^{15}\text{N}$ ) in marine phytoplankton and showed that changes in  $\delta^{15}\text{N}$  values in fish with increasing trophic level are the average result of changes in  $\delta^{15}\text{N}$  values of amino acids in their bodies. Different mechanisms of nitrogen fractionation exist for different amino acids during synthesis and metabolism, where  $^{15}\text{N}$  on glutamate is subject to deamination during metabolism and therefore has a high enrichment between trophic levels, up to 7‰ on average, while phenylalanine has an enrichment close to 0 between trophic levels. Therefore,  $\delta^{15}\text{N}$  values for glutamate can be used for trophic level calculations, while  $\delta^{15}\text{N}$  on phenylalanine can be used to indicate food sources. Dale et al. (2011) applied  $\delta^{15}\text{N}$  to glutamate to estimate trophic levels in the *Lutjanus lutjanus* and found that the  $\delta^{15}\text{N}$  discriminant for glutamate in this fish was approximately 5‰. Notably, both glutamate and phenylalanine are essential amino acids for fish, and essential amino acids cannot be synthesized by the fish themselves and must be obtained from food; therefore, the enrichment of  $\delta^{13}\text{C}$  in essential amino acids is 0, which makes  $\delta^{13}\text{C}$  in essential amino acids a more accurate indicator of food source.

### Characteristic Fatty Acid Labeling Technology

A special class of compounds, such as fatty acids, amino acids, and monosaccharides, which are relatively stable and unlikely to change during the feeding activity of organisms, can be used to identify the source of biological prey and are known as biomarkers. Among them, fatty acids are important components of all organisms and are the most abundant lipids in marine animals. Fatty acids occur mainly in the form of tricarboxylic acid triglycerides and phospholipids. As biomarkers, fatty acids have several advantages: first, the composition and accumulation of fatty acids in organisms are the result of long-term feeding activities, and there is little chance of incorrectly determining organismal food habits on the basis of fatty acids; second, fatty acids are relatively stable during the metabolism of organisms, and their structure remains essentially unchanged after digestion and absorption by organisms; and third, fatty acids in tricarboxylic acid triglycerides in organisms mainly come from the food consumed, and the use of such fatty acids as biomarkers is generally accepted both nationally and abroad. The composition of fatty acids in an organism is directly related to its food intake.

The use of fatty acids as molecular markers in ecosystems has developed rapidly in recent decades. By comparing the composition of fatty acids among organisms, it is possible to trace the process of material transfer in food webs, to indicate the source of organic matter in food webs, and to contribute to the determination of trophic relationships among organisms. Similar to the results reflected by carbon and nitrogen isotopes, the fatty acid composition of organisms is the result of their long-term feeding activities. Phytoplankton-based autotrophs in marine ecosystems can synthesize all the fatty acids they need, and each phylum of microalgae has its own distinctive fatty acid composition characteristics. For example, the major fatty acids of Pyrrophyta are 16:0, 18:4 $\omega$ 3, 18:5 $\omega$ 3, 20:3 $\omega$ 6, and 22:6 $\omega$ 3; the major fatty acids of Bacillariophyta are 14:0, 16:0, 16:1 $\omega$ 7, and 20:5 $\omega$ 3; and the major fatty acids of Chlorophyta are 16:0, 16:4 $\omega$ 3, and 18:3 $\omega$ 3. In addition, 18:4 $\omega$ 3

in Pyrrophyta and 16:1 $\omega$ 7 in Bacillariophyta have been used as characteristic diatom fatty acids to indicate the methanogenic and diatomic components of particulate suspensions in naturally occurring waters. In addition, phytophagous copepods synthesize large amounts of eicosanoid and docosanoid fatty acids and fatty alcohols, both of which are synthesized by copepods from 18:1 $\omega$ 9 through carbon chain extension. In contrast, omnivorous zooplankton and fish synthesize only small amounts of saturated fatty acids, and the proportion of fatty acid synthesis in the diet of predators decreases at higher trophic levels.

Pethybridge et al. (2010) used multivariate analysis of characteristic fatty acids as a complement to gastric content analysis to determine the fatty acid composition of muscle, liver, and digestive glands in 16 cartilaginous fish species collected from the continental slope of Tasmania, Australia. Gastric and fatty acid composition analyses revealed that each species has a specific feeding and survival pattern (e.g., Chimaeridae are low trophic level organisms in this area, feeding mainly on benthic organisms; medium-sized Squalidae mainly feed on fish and cephalopods in the “middle” of the trophic level; and Scyliorhinidae mainly feed on cephalopods). The compositions of the diets of sharks inhabiting different pelagic levels vary considerably; the diets of different species at the same pelagic level are somewhat similar, and there is a divergence in the ecological niches of feeding; in addition, the fatty acid compositions of different tissues vary, but combining the fatty acid composition of muscle, liver, and digestive glands provides continuous and comprehensive information on the diet of an organism over time.

### DNA Molecular Technology

DNA barcoding is a technique that has emerged in the twenty-first century that enables rapid and accurate species identification based on a short, common standard DNA sequence or segments, and this technique has been widely used in the fields of marine fish classification, interspecific kinship identification, molecular genetic diversity, gut microbial diversity, feeding analysis,

etc. DNA barcoding is based on the uniqueness of biological genetic sequences in nature. Aguilar et al. (2017) used DNA barcoding to identify the stomach contents of a native catfish and two invasive catfish species in the Chesapeake Bay, USA, and successfully identified 92% of the species, including *Morone americana* and *Anguilla rostrata*, to the species level. The prey abundance of juvenile *Oncorhynchus keta* was comparatively analyzed using morphological observations and DNA barcoding techniques (Sakaguchi et al. 2017). The results showed that 11 of the 36 food species observed based on morphology failed to be detected by the DNA barcoding technique in an analysis of the stomach contents of juvenile fish, while 61 of 80 food species detected by the DNA barcoding technique could not be discriminated by morphology. Thus, DNA barcoding as a complementary technique can substantially improve the level of resolution in gastric content analysis.

It has been 14 years since the rise of DNA barcoding technology. With the advancement and cost reduction of DNA sequencing technology and the gradual improvement in genetic databases, the emergence of next-generation sequencing (NGS) has further improved the resolution of analysis and given rise to methods for obtaining amplified subsequences of barcoded genes using high-throughput sequencing technology, i.e., DNA metabarcoding. DNA metabarcoding is based on the combination of DNA identification and high-throughput sequencing, where DNA fragments of an entire mixed sample are amplified, and then, high-throughput sequencing is used to automatically identify multiple species in the mixed sample in combination with biological information, which can reduce sampling effort and maximize the identification of semidigested/digested tissue residues. In terms of species identification, Harms-Tuohy et al. (2016) divided the stomach contents of *Pterois volitans* into two samples, digested (digest) and undigested, and sequenced their prey species separately using DNA metabarcoding. The resulting sequences were compared to GenBank and Barcode of Life Database databases, and of the 39 prey organisms identified by the undigested

component, only four species could not be identified from the digest. Thus, DNA metabarcoding is a method that can conduct the “identification of highly digested stomach contents.”

## 6.5.4 Main Factors Affecting Fish Feeding

### 6.5.4.1 Morphological Characteristics of Feeding Organs in Relation to Fish Feeding

The feeding habits of fish and their feeding behaviors are influenced by the morphological characteristics of their feeding organs and environmental and ecological factors such as food security and water temperature. Physiological activities such as spawning and overwintering, as well as the morphological characteristics of the feeding organs of fish, are closely related to their feeding patterns; however, studies on these topics are rare, and systematic theories and research methods have not been developed. For example, Chen Dagang et al. (Chen et al. 1981) used biomathematical methods to study the relationship between the morphological characteristics of the digestive organs of flounder and their feeding habits. The specific method involved selecting the typical quantitative indicators of fish digestive organs, such as the muzzle, head, mouth, intestine, and pyloric pendulum (mean values), and trait indicators, such as teeth, gill rakers, stomach, and anus (the distance between numbers was used to express the differences between various fish species), from which the information matrix  $x$  of fish morphological indicators was obtained, and then, the Euclidean distance (specimen point distance) between each fish species was calculated  $d_{ij}$ :

$$d_{ij} = \left\{ \sum [(x_{ij1} - x_{ij2})/s_i] \right\}^{1/2}$$

where  $s_i$  is the standard deviation of the rows. The ecological types of the feeding fish were classified

by cluster analysis of the distance from specimen sites.

### 6.5.4.2 Food Security in Relation to Fish Feeding

Food security, i.e., the availability of prey organisms in the environment, including the availability of prey biomass and the ability of consumers to capture and use it, is one of the main ecological factors affecting fish feeding. Fish and their prey live in a constantly changing environment. Therefore, natural selection of prey by fish can only be evaluated objectively by simultaneous sampling consumers and prey and comparing the composition of consumer stomach contents based on the composition of prey types in their environment. However, many of the tests needed for such studies are incomplete or immature, and the difficulties in sampling make it difficult to obtain sufficiently precise quantitative information so that the selection of prey by fish is often studied by the food selection index  $I$  in experimental ecology:

$$I_i = (r_i - p_i)/(r_i + p_i)$$

where  $I_i$  is the selection index of fish for prey  $i$ ,  $r_i$  is the proportion of prey organisms in the stomach contents of fish, and  $p_i$  is the proportion of the same prey organism as that in the environment.  $I_i$  values range from  $-1 < I_i < 1$ . For  $I_i > 0$ , fish actively select prey  $i$ ; for  $I_i < 0$ , fish avoid prey  $i$ .

In addition, some scholars use another selectivity index,  $I$ :

$$I = \frac{r_i - p_i}{p_i}$$

where  $I$  is the selection index,  $r_i$  is the percentage of a component in the food, and  $p_i$  is the percentage of the same component in the food base. Size is used to determine how selective a particular fish is for a particular prey. When the selection index is 0, it indicates no selectivity for this component; a positive value of the selection index indicates selectivity; and a negative value of the selection index indicates dislike for this food.

## 6.6 Fullness and Fat Content

### 6.6.1 Fish Fullness

Fish fullness is a measure of weight gain or loss in fish, and it is an indicator of how well the fish feed at different times and in different waters. The formula for calculating fullness is the following:

$$Q = \frac{W \times 100}{L^3}$$

where Q is fullness, W is body weight in grams, and L is body length in centimeters.

This is one of the indicators of fish growth expressed in terms of fish weight in relation to cubed body length. This indicator assumes that fish do not change body size as they grow. A change in the fullness factor indicates a change in the relationship between fish length and weight. An increase in body weight with a constant body length increases fertility; conversely, a decrease in weight indicates a decrease in fertility.

The fertility factor is actually the ratio of two measures, i.e., the volume of the fish (proportional to the weight of the fish) to the cubic product of the length of the fish. Therefore, when comparing the fertility of fish at different times and in different waters, separate calculations should be conducted for each age group and each length group, and the values should be compared for the same age and length groups.

In addition, the maturity of the fish gonads and the fullness of the gut, among other things, affect fatness and cause errors and variations. To eliminate this effect, net body weight is used as a proxy for total fish weight. However, after the gut is removed, some of the body fat will also be removed, which affects the correctness of the fullness determination. To address this problem, it is best to calculate both fullness levels simultaneously for correction.

### 6.6.2 Fat Content

Lipid content is the amount of fat stored in a fish and an indicator of how well a fish is feeding on

nutrients at different times and in different waters, and it is more accurate than fullness.

Fat in fish is a nutrient that gradually accumulates in the body after the assimilation and digestion of food. The accumulation of fat in fish varies with the development of the individual and different life stages. Immature juvenile fish grow rapidly, food taken from the outside world is mainly used for development after assimilation, and very little fat accumulates in the growing body. With the gradual growth of the fish body, body fat gradually accumulates. Before and after sexual maturity of fish, the body fat content is high and often changes with the development of the gonads; generally, when spawning is over and after the resumption of feeding, the gonads and the amount of fat grow at the same time. However, when feeding stops, the amount of fat gradually decreases as the gonads continue to grow, so near the time of spawning, the lipid content decreases due to a shortage of nutrient sources as a result of reduced feeding; the nutrients accumulated in the body are converted to gonad development. The fat content of fish is also related to seasonal changes. Generally, in the late stage of feeding, body fat content increases, and during the overwintering period, because of stopped or reduced feeding, body fat is constantly converted into energy and used for gonadal development; thus, fat content gradually decreases.

The lipid contents of similar species and the same species are related to the characteristics of their habits, in addition to differences in physiological conditions and life stages, with groups that have long migratory routes having higher lipid contents. In the case of overwintering groups, metabolic intensity is reduced because they stop feeding for a certain season, so their body lipid content remains high.

Fish lipid content is usually determined by visual inspection (lipid content classes), chemical determination, and specific gravity estimation. Specific methods of determination can be found in relevant reference books such as the *Aquatic Resources Survey Manual* (1981).

## References

- Aguilar R, Ogburn MB, Driskell AC, Weigt LA, Groves MC, Hines AH (2017) Gutsy genetics: identification of digested piscine prey items in the stomach contents of sympatric native and introduced warmwater catfishes via DNA barcoding. *Environ Biol Fish* 100:325–336
- Amundsen PA, Gabler HM, Staldvik FJ (1996) A new approach to graphical analysis of feeding strategy from stomach contents data: Modification of the Costello (1990) method. *J Fish Biol* 48:607–614
- Chen XJ (2014) Fisheries resources and fisheries oceanography. Ocean Press. (In Chinese)
- Chen DG, Liu CA, Zhang SB (1981) A comparative analysis of the digestive organs and feeding characteristics of the flounders in the Yellow and Bohai Seas. *J Shandong Ocean College* 01:87–106. (In Chinese)
- Chen XJ, Liu BL (2017) Biology of fishery resources. Science Press (In Chinese)
- Dale JJ, Wallsgrove NJ, Popp BN, Holland KN (2011) Feeding ecology and nursery habitat use of a benthic stingray determined from stomach content, bulk and amino acid stable isotope analysis. *Mar Ecol Prog Ser* 433:221–236
- Harms-Tuohy DA, Schizas NV, Appeldoorn RS (2016) Use of DNA metabarcoding for stomach content analysis in the invasive lionfish (*Pterois volitans*) in Puerto Rico. *Mar Ecol Prog Ser* 558:181–191
- Lindeman RL (1942) The trophic-dynamic aspect of ecology. *Ecology* 23:399–418
- McClelland JW, Montoya JP (2002) trophic relationships and the Nitrogen isotopic composition of amino acids in plankton. *Ecology* 83:2173–2180
- Pethybridge H, Daley R, Virtue P, Butler ECV, Cossa D, Nichols PD (2010) Lipid and mercury profiles of 61 mid-trophic species collected off south-eastern Australia. *Mar Freshw Res* 61(10):1092–1108
- Sakaguchi SO, Shimamura S, Shimizu Y, Ogawa G, Yamada Y, Shimizu K, Kasai H, Kitazato H, Fujwara Y, Fujikura K, Takishita K (2017) Comparison of morphological and DNA-based techniques for stomach content analyses in juvenile chum salmon *Oncorhynchus keta*: a case study on diet richness of juvenile fishes. *Fish Sci* 83:47–56
- Yang RB, Xie CX (2000) A review of the contents and methods of fish feeding ecology. *Water Res Fish* 03:1–3. (In Chinese)