Chapter 17

The importance of gut microbes for nutrition and health

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17.1 Introduction

At hatching, the fish gastrointestinal (GI) tract is a simple tube, histologically and functionally undifferentiated (Govoni et al., 1986). During the early development of the GI tract, intestinal cells proliferate, and the GI tract lengthens and twists, as this is crucial for GI-specific functions. The anatomy of the GI tract will eventually stabilize during larval development; during metamorphosis, the GI tract changes significantly, especially when changes in the diet occur.

The GI tract is inhabited by myriads of diverse microorganisms, known as the gut microbiota or microbiome, that play protective, structural, and metabolic functions for the intestinal mucosa. The animal-associated microbiota consists of bacteria, fungi, archaea, viruses and protozoa and is defined as the collective microbial community inhabiting a specific environment, for example, the gut. Relative to mammals, three traditional biomedical models; the fruit fly (*Drosophila melanogaster*), the nematode worm (*Caenorhabditis elegans*) and zebrafish (*Danio rerio*), a freshwater fish of the family *Cyprinidae*, are attracting increasing attention to develop understanding from genes to community. But actually, the first research on the fish gut microbiota dates to the early half of the 20th century, while the first works on this topic were published in the late 1920s and 1930s (Gibbons, 1933; Reed and Spence, 1929; Stewart, 1932). Even though this information and later studies were available, in the 1970s, controversy existed about the role and even the existence of indigenous gut microbiota in fish (e.g., Cahill, 1990; Ringø et al., 1995, 2016). Nowadays, we know that the fish gut microbiota is involved in the barrier functions and maintaining its homeostasis. Gut microbiota provides nutrients, participates in the signaling network, and plays important roles in mediating and stimulating GI development, aiding digestive function, maintaining mucosal immunity, and protecting against diseases (e.g., Li et al., 2019; Meng et al., 2021; Perry et al., 2020; Rawls et al., 2004, 2006; Wang et al., 2018), as well as contributing to fish metabolism (Clément, 2011) and vitamin synthesis (Rowland et al., 2018). There is significant interindividual variability in the number, type, and function of the microorganisms present within the GI tract. As the microbiota responds to homeostatic and physiological changes, some researchers proposed that it could be actually seen as an endocrine organ (Clarke et al., 2014).

The conclusions of early research from the 1930s and until the 1990s on fish gut microbiota were based on culture-dependent methods, but today we know that cultivable microorganisms represent only <0.1% of the total microbial community in the GI tract of some fish species (Zhou et al., 2014). Currently, culture-independent techniques are available for analyzing the fish gut microbiota that has been discussed in detail in several reviews and also addressed briefly in Section 17.7 (Ringø et al., 2016; Tarnecki et al., 2017; Zhou et al., 2014). These methods include quantitative real-time polymerase chain reaction (qPCR), used for quantitative analysis of taxa; fingerprinting methods such as denaturing gradient gel electrophoresis (DGGE), and fluorescent in situ hybridization used to determine the abundance of particular taxa (Wang et al., 2018; Zhou et al., 2014), as well as sequencing technologies. Actually, next- and third-generation sequencing (NGS and TGS) are the latest methods of molecular microbiota analysis. They are beginning to be used more frequently in studies on fish, and Ghanbari

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et al. (2015) discussed the potential of NGS in this field, including the opportunity for rapid and cost-effective acquisition of in-depth and accurate sequence data that provides greater information on even low abundance microbiota as well as the genetic and metabolic potential of the species present.

Lescak and Milligan-Myhre (2017) put forward a controversial hypothesis about using teleost fish as a vertebrate model for understanding the host-microbiota interactions, as traditional models relying on mammals (e.g., mouse) are usually limited by strains and sample sizes, thus compromising statistical power. In addition, mammalian models focus on fecal samples which are composed of mostly transient (allochthonous) strains, and not on the adherent (autochthonous) microbiota. Therefore, it is crucial to increase our knowledge and experience on gut bacteria colonizing the GI tract in the context of improved growth performance and health. For that, different segments of the teleost GI tract must be investigated separately, as differences have been reported betweed the different gut parts (Gajardo et al., 2017; Kokou et al., 2020), while there may be different colonization patterns between beneficial bacteria and pathogens (Ringø et al., 2003). In addition, the gut microbiota differs between those bacteria present in digesta versus those adhering to and colonizing the gut mucus lining (e.g., Nyholm et al., 2022; Ringø et al., 2016; Gajardo et al., 2017). Therefore, depending on the research question, understanding the changes in the autochthonous microbiota might be necessary when evaluating fish health, while allochthonous communities may be more connected to digestion.

Regarding the factors that modulate gut microbiota, both internal and external factors can play a role; these aspects will be further discussed in Sections 17.2 and 17.3. It is well known that genetic effects, rearing conditions (e.g., wild, offshore cage-culture and pond-culture), life stages during growth, environment factors, gender, etc. (Table 17.1) and dietary supplements (e.g., Cahill, 1990; Kokou et al., 2022; Ringø et al., 1995, 2016; Table 17.5) modulate the gut microbiota composition and diversity. Ringø et al. (2016) published a thorough review, where the authors addressed whether dietary manipulation can become a never-ending story. This seems to be true, as aquaculture is expected to rely less on fish meal supply and the industry is growing quite fast, leading to an increasing need of feed resources, such as vegetable proteins (Ringø et al., 2016; Table 17.5), animal proteins (e.g., Gjedrem et al., 2012; Luthada-Raswiswi et al., 2021; Solé-Jiménez et al., 2021), fish-by products (Gasco et al., 2019), insect meals (Li et al., 2022; Rimoldi et al., 2019; Terova et al., 2019; Tran et al., 2022a, 2022b), or single cell proteins; this topic is addressed in Section 17.5.

In an early review devoted to intestinal microbiota by Ringø et al. (1995), the authors stated that "the GI microflora of fish appears to be simpler than endotherms." However, this statement needs revision based on research on the fish gut microbiota in the last three decades. When discussing the gut microbiota of fish, an important question arises, when do the bacteria colonize the gut? Gut microbiota is influenced by the microbiota of the egg, the live feed or the bacteria present in the tank/pond water; Section 17.3 addresses this topic on fish gut microbiota development. The genera in the intestine are generally those from the environment or diet that can survive and multiply in the GI tract. There is evidence for a distinct intestinal microbiota in different fish species (Table 17.2). Earlier studies on fish gut microbiota using conventional culture-based methods revealed only a few taxa within the digestive tract of fry (e.g., Yoshimizu et al., 1976, 1980), but later studies using molecular methods have revealed a diverse microbiota in fish at early stages (e.g., Giatsis et al., 2014, 2016; López Nadal et al., 2018). In a previous study, (Ringø et al., 1996) revealed that the gut of newly hatched turbot (*Scophthalmus maximus*) larvae contained very few bacteria at hatching, but the intestine was rapidly colonized during the first few days in a two-step process. However, it remains to be elucidated what happens with fish species that hatch as fry or what happens in the case of different GI structures, such as stomach or pyloric caeca presence and different relative intestinal length (Olsen and Ringø, 1997; Ray and Ringø, 2014). Moreover, in contrast to the human gut, where microbial density and diversity increase along the gut, in fish some studies using culture-based approaches revealed that the density and diversity of the gut microbiota might be similar in both proximal intestine (PI) and distal intestine (DI) (e.g., Ringø et al., 1995). However, early studies on bacterial genera in the different regions of the fish GI tract showed a progressive increase in aerobic heterotrophic bacteria or bacterial numbers using qPCR (e.g., Kokou et al., 2020; MacDonald et al., 1986; Sakata et al., 1978).

GI microbiota in humans and fish has led to the assumption that some gut microorganisms are autochthonous; they colonize the mucosal surface and the microvilli (Fig. 17.1), while others are allochthonous, which means they are transit passengers. Furthermore, one can generally divide the gut microbiota into beneficial bacteria, and harmless bacteria, while other bacteria are regarded as pathogenic and may lead to disease. Thus, understanding how host and microbiota interact is essential to promote health in aquaculture further; this topic is addressed in Section 17.4. It is crucial to expand the knowledge on fish microbiota, as the microbiota significance is less investigated than in endothermic animals. More studies nowadays show the microbial influence on epithelial

TABLE 17.1 Factors affecting the gut microbiota.

Factors	References
Genetic	Li et al. (2018a)
Between wild, offshore cage-culture and pond-culture	Sun et al. (2021)
Cultured versus wild	Bacanu and Oprea (2013); Kim and Kim (2013)
Migration from fresh water to seawater and migrationfrom seawater back to fresh water	Ringø (2004)
Fish farms	Diler et al. (2000)
Growth	Gadoin et al. (2021)
Fast versus slow growing fish	Sun et al. (2009)
Environment factors	Benson et al. (2010); Lescak and Milligan-Myhre (2017); Li et al. (2018b); Liu et al. (2012)
Gender	Iehata et al. (2015); Li et al. (2018a)
Triploid versus diploid	Cantas et al. (2011)
Different fish species fed similar diet	Li et al. (2014)
Seasonal variations	Ringø et al. (2016)
Stress	Ringø et al. (2016)
Individual variations and different regions of the GI tract	Ringø et al. (2016)
Day-to-day variations	Ringø et al. (2016)
Hierarchy formation	Ringø et al. (2016)
Microbial aspects of live feed	Bakke et al. (2013); Conceição et al. (2010)
Starvation	Xia et al. (2014)
Water quality	Gatesoupe et al. (2013)

Source: Modified after Ringø, E., and Song, S. K. (2016). Application of dietary supplements (synbiotics and probiotics in combination with plant products and β-glucans) in aquaculture. *Aquac. Nutr. 22*, 4–24. https://doi.org/10.1111/anu.12349.

integrity and immune function and how bacterial metabolites such as short-chain fatty acids (SCFAs) control various immune pathways, including the gut and immune homeostasis (Thorburn et al., 2014). Gnotobiotic approaches, which refer to animals cultured in axenic conditions or with defined microbial lineages, have also been used for aquaculture animals, pinpointing the importance of gut microbes (Luna et al., 2022; Zhang et al., 2020d). Gee and Sarles (1942) reported the first gnotobiotic study in aquaculture in evaluating the disinfection of trout eggs contaminated with the bacterium *Aeromonas salmonicida*. Gnotobiotic models have the potential to provide a better understanding of the functions of microbiota in numerous biological processes of their host such as nutrient processing and absorption, development of the mucosal immune system, angiogenesis, and epithelial renewal (e.g., Fiebiger et al., 2016; Pérez-Pascual et al., 2021b; Rawls et al., 2004). In aquaculture, zebrafish is the most frequently used species for gnotobiotic studies due to their brood size, high fecundity, external fertilization, rapid development, optical transparency, and genomic information (Leulier et al., 2017; Pham et al., 2008). However, more studies have evaluated gnotobiotic protocols for aquaculture fish such as rainbow trout, Atlantic cod and Nile tilapia (see review by Luna et al., 2022).

In conclusion, the microbiome present in the GI tract of fish and shrimp, not only contributes to digestion but also has an impact on nutrition, growth, metabolism, reproduction, immune system and affects health and different disease processes. Furthermore, studies of the gut microbial communities of aquatic animals are highly relevant for the development of new and innovative bioproducts, which will be a vital source to build bio- and pharmaceutical industries, including aquaculture. In this chapter, we aim to provide a thorough overview of the development, composition and factors that are affecting

TABLE 17.2 Taxonomic composition of the gut microbiota of important aquaculture fish species.

Fish species	Feeding habit	Habitat	Dominant microbial phyla	Dominant microbial genera	References
Common carp (*Cyprinus carpio*)	Herbivore/ Omnivore	Freshwater	Proteobacteria, Firmicutes and Fusobacteria	*Cetobacterium*, *Aeromonas*, *Pseudomonas*	Eichmiller et al. (2016); Yan et al. (2016)
Nile Tilapia (*Oreochromis niloticus*)	Omnivore	Freshwater	Proteobacteria, Fusobacteria	*Cetobacterium*, *Plesiomonas*, *Bacillus*, *Mycobacterium*, *Clostridium*	Deng et al. (2022); Giatsis et al. (2015); Hallali et al. (2018); Ran et al. (2015)
Atlantic salmon (*Salmon salar*)	Carnivore	Anadromous	Proteobacteria, Firmicutes	*Streptococcus*, *Lactobacillus*, *Leuconostoc*, *Weissella*, *Mycoplasma*	Gajardo et al. (2016, 2017); Schmidt et al. (2015); Zarkasi et al. (2014, 2016)
Rainbow trout (*Onchoryncus mykiss*)	Carnivore	Freshwater	Proteobacteria, Firmicutes	*Streptococcus*, *Lactobacillus*, *Leuconostoc*, *Weissella*	Desai et al. (2012); Ingerslev et al. (2014b); Wong et al. (2013)
European Sea bass (*Dicentrarchus labrax*)	Carnivore	Marine	Proteobacteria, Firmicutes, Acidobacteria, Actinobacteria	*Aeromonas*, *Pseudomonas*, *Acinetobacter*	Kokou et al. (2019); Serra et al. (2021)
Gilthead sea bream (*Sparus aurata*)	Carnivore	Marine	Proteobacteria, Firmicutes, Actinobacteria	*Vibrio*, *Photobacterium*, *Corynebacterium*	Piazzon et al. (2020)
Atlantic cod (*Gadus morhua*)	Carnivore	Marine	Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria	*Vibrio*, *Alivibrio*, *Photobacterium*	Keating et al. (2021); Riiser et al. (2019)
Turbot (*Scophthalmus maximus*)	Carnivore	Marine	Proteobacteria, Firmicutes	*Vibrio*, *Pseudomonas*, *Carnobacterium*, *Lactococcus*	Xing et al. (2013); Zhang et al. (2020a)

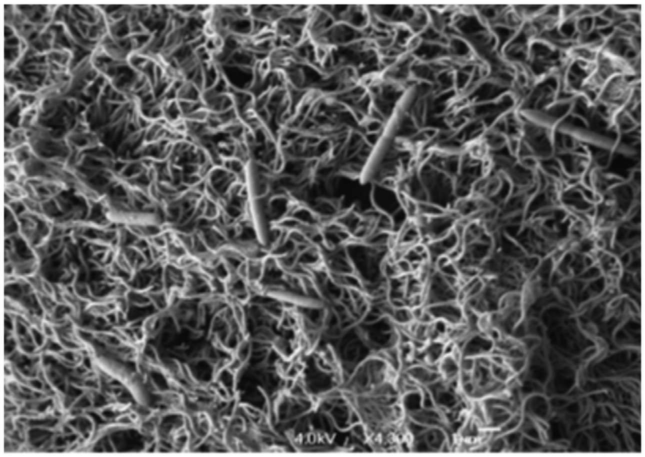

FIGURE 17.1 Bacteria associated with the microvilli in the hindgut chamber of Atlantic cod. *Photo by Lisbeth Løvmo.*

the gut microbiota, including diet and the use of antimicrobials, as well as methods to study the gut microbiota, focusing on the main aquaculture fish and shrimp species.

17.2 Composition of gut microbiota in common farmed fish and shrimp species

Research on the fish gut microbiota started already since the early half of the 20th century (Colwell, 1962; Gibbons, 1933). However, knowledge of gut-associated microbes has grown significantly only in the last two decades, with the advent of nucleic-based techniques (Ghanbari et al., 2015). Teleost microbiota research lags well behind humans, and other mammals (Legrand et al., 2020; Llewellyn et al., 2014), and current knowledge on the fish microbiota is still far from complete as it was described in the previous section. Such outcome also comes from the great diversity of teleost vertebrates, with more than 30,000 described species (Nayak, 2010) having different habitats and dietary habits, making it more challenging to draw general conclusions about the fish and shellfish gut microbiota composition. Nonetheless, compared to studies coming from fish species, there is limited information on the shrimp gut microbiota. This holds true, especially for the cultured fish and shellfish species, as knowledge of healthy gut microbiota can provide essential information on the nutritional and health status of the reared animals. Therefore, given the importance of the gut microbiota for the host's nutrition, health and well-functioning, characterization of their composition and function is highly important.

One of the main reasons for studying fish and shellfish's gut microbiota is that microbial communities can be modulated in order to improve host health and performance (Luna et al., 2022; Nayak, 2010). An important aspect to achieve this is to characterize the gut microbiota of the species of interest. It is, however, important to take into account that the gut microbiota composition can be modulated by several factors, some of which are summarized in Table 17.1, such as host phylogeny, diet, rearing environment, developmental stage, but also biased factors such as sample type, sample storage, DNA extraction, PCR conditions, gene marker and sequencing platform (Tarnecki et al., 2017). Some studies report the environment as the primary driver for gut microbiota composition (Deng et al., 2022; Eichmiller et al., 2016; Giatsis et al., 2015), with salinity having a strong effect—freshwater species have been reported to have different microbiota than marine fishes (Egerton et al., 2018; Sullam et al., 2012; Wong and Rawls, 2012) (Fig. 17.2). However, other studies suggest diet or trophic level as the main factor that shapes microbiome composition and possibly also host phylogeny (Sullam et al., 2012). This section will focus mainly on host phylogeny (fish and shrimp species). Tables 17.2 and 17.3 provide information on the gut microbiota from the most common fish and shrimp species, coming from multiple studies.

17.2.1 Factors affecting the composition of the gut microbiota

Information on the origin of the sample (digesta vs mucosa) is important in order to draw conclusions for gut microbiota composition analysis, since the interaction between microbes and the host enterocytes is very dynamic in the gut, affected by nutrient availability, oxygen levels, host immune system, and thus can alter the composition daily or even hourly. Depending on whether microbes are temporarily present or permanent inhabitants they are referred to as transient or allochthonous, and adherent or autochthonous microbes, which are considered more permanent residents of the gut, attached to the gut mucosa. The transient microbes usually arrive in the gut via feed ingestion, and they are quickly flushed out with defecation. On the other hand, resident microbes are normally attached to the intestinal cell walls and the mucosa of the host, having a symbiotic interaction (Wang et al., 2018). These microbes are usually more resilient to disturbances due to diet, antibiotics or the environment, and can be different in composition compared to the transient microbes (Gajardo et al., 2017). Research in the fish gut microbiota has been often inconsistent even within the same species, due to different sampling approaches, also involving mucosa or digesta microbial community analysis, therefore, this is something to consider before drawing conclusions on different dietary treatments.

Besides the gut microbiota composition, several factors are important to characterize such as the microbial load, which is the number of microbial cells in the gut, and the microbial diversity, which is defined by the number of microbial taxa present in a metagenomic sample (genomic DNA from a gut sample, including both the fish-host and the microbiota DNA). The fish gut hosts approximately 10^6-10^8 microbial cells per g of gut tissue, representing more than 500 microbial taxa, dominated by mainly facultative anaerobes and aerobes, but also strictly anaerobes (Tarnecki et al., 2017). In Section 17.7, more detailed information on the analysis of the gut microbiota load and diversity is provided, including the methodology to be used in order to analyze the gut microbiota composition.

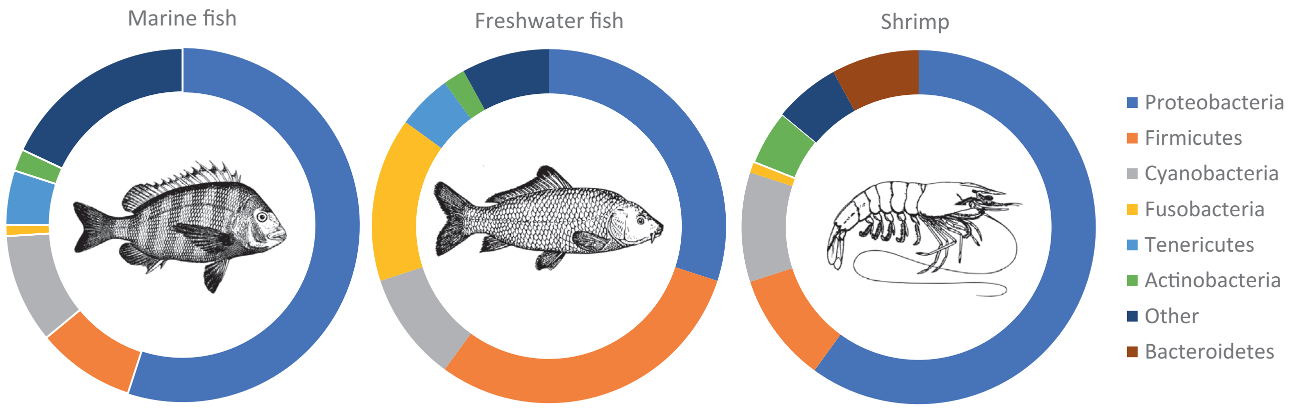

FIGURE 17.2 Gut microbiota composition at the phylum level in marine and freshwater fish, and shrimps.

TABLE 17.3 Microbiota composition of Asian black tiger shrimp *Penaeus monodon* and Pacific whiteleg shrimp *Litopenaus vannamei* from different studies.

Species	Treatment	Dominant microbial phyla	Dominant microbial genera	References
Asian black tiger shrimp (*P. monodon*)	Wild and Cultured	Proteobacteria, Bacteroidetes, Fusobacteria, Firmicutes	*Vibrio* (aquaculture), *Photobacterium* (wild)	Oetama et al. (2016)
	Developmental stage	Bacteroides, Firmicutes, and Proteobacteria	*Photobacterium* (postlarvae), *Vibrio* and *Listonella* (juvenile)	Rungrassamee et al. (2013)
	Functional/ Novel feeds	Proteobacterial, Bacteroides	Genera from Vibrionales, Alteromonadales, Rhodobacterales and Flavobacteriales; *Vibrio* increased with dietary bacterial meals	Simon et al. (2020)
Pacific whiteleg shrimp (*L. vannamei*)	Health and Diseased	Proteobacteria, Cyanobacteria, Actinobacteria, Tenericutes	*Faecalibacterium* and *Aeromonas* (healthy individuals); *Aeromonas* and *Photobacterium* (diseased individuals)	Cornejo-Granados et al. (2017)
	Freshwater and Seawater	Proteobacteria, Chloroflexi, and Actinobacteria (freshwater); Proteobacteria, Actinobacteria and Cyanobacteria (seawater)	*Lactobacillus* (freshwater), *Synechococcus* and *Vibrio* (seawater)	Fan et al. (2019)
	High and low intensity	Proteobacteria, Planctomycetes, Acidobacteria, Bacteroidetes, Chlamydiae (low intensity)	*Paracoccus* (low and medium intensity), *Nocardioides* (high intensity)	Deng et al. (2019)
	Developmental stage	Proteobacteria, Bacteroidetes, Actinobacteria (increase with age)	Genera from *Rhodobacteriaceae*, *Flavobacteriaceae* and *Pirellulaceae*	Xiong et al. (2017)

In order to understand the gut microbiota composition and function, understanding the core microbes of a fish species, that is, members of the gut microbiota present in most or all individuals of a species known as the core microbiota, has been a primary goal for many studies, either in humans (Turnbaugh and Gordon, 2009), but more recently also teleosts (Kokou et al., 2019; Roeselers et al., 2011). However, the definition of the core microbiota is not an easy task since microbial communities are quite complex and several parameters need to be taken into consideration such as composition, phylogeny, and persistence of these microbes in the gut (Kokou et al., 2019; Shade and Handelsman, 2012). The first study looking into a core gut microbiota in fish was conducted by Roeselers et al. (2011), where the authors compared the gut microbiota of wild and domesticated zebrafish to find several microbial taxa that were shared among all individuals no matter the origin of fish, and there were considered as the core community. The core microbiota of invasive carp species was compared between laboratory-reared and wild fish (Eichmiller et al., 2016), and five shared microbial taxa were identified, that comprised up to 40% of the total microbial taxa abundance in the samples, suggesting a key role of those bacteria. Although several studies report nowadays a core microbiome (Mukherjee et al., 2020; Roeselers et al., 2011), it is unclear whether core microbiota exists in all fish species and what is its role. Understanding the forces that affect the core microbiome, dictated by host genetics and/or gut physiology, as well as by microbial interactions is definitely worth exploring (Kokou et al., 2019; Neu et al., 2021). Understanding the role of these communities will bring us a step closer to making informed decisions on how to manipulate the gut microbiota to achieve good health and better performance of the hosts.

17.2.2 Main taxonomic groups in the gut microbiota of fish

So far, research on the fish and shrimp gut microbiome has revealed a dominance of few phylogenetic groups, mainly composed of Proteobacteria, Fusobacteria, and Firmicutes, followed by Bacteroidetes, Actinobacteria, and Verrucomicrobia (Llewellyn et al., 2014). The dominant phyla in the gut of most fish species are mainly Proteobacteria, Fusobacteria and Firmicutes in both marine (Hennersdorf et al., 2016) and freshwater species (Eichmiller et al., 2016; Larsen et al., 2014; Liu et al., 2016), representing up to 90% of the microbiota, observed in both allochthonous (transient) and autochthonous (adherent) microbial communities. The rearing environment can greatly affect the gut microbiome taxa composition, with *Aeromonas*, *Lactococcus*, *Pseudomonas*, *Cetobacterium*, *Enteromonas*, and *Fusobacterium*, being more dominant genera in freshwater species (Llewellyn et al., 2014). Actually, *Cetobacterium somerae* is one of the most interesting species found in the gut, which belongs to the phylum Fusobacteria. This species has been identified in many freshwater fishes representing in some cases, more than 90% of total microbial taxa (Deng et al., 2022; Gaulke et al., 2016; Hallali et al., 2018; Larsen et al., 2014; Maas et al., 2021). It is known to produce high amounts of vitamin B12, degrade proteins, and inhibit the growth of potential pathogens (Sugita et al., 1996), while its decrease in the gut has been associated with impaired health states (He et al., 2017). Isolation and culture of this species are not easy since it is a microaerophilic species, thus the exact role and importance of this species for freshwater fish remains to be investigated. On the contrary, marine fish have been reported to being mainly dominated by members of the Proteobacteria genus like *Vibrio*, *Alteromonas*, *Pseudomonas*, and *Photobacterium* in their gut (Egerton et al., 2018) (Fig. 17.2; Tables 17.2 and 17.3).

With regard to important aquaculture fish species, many studies so far have focused on the gut microbiota composition of cyprinids, cichlids, salmonids, and marine fish (sea bream, sea bass, turbot, Atlantic cod).

Numerous studies have focused on cyprinid gut microbiota, such as common carp (*Cyprinus carpio*), due to the importance of these species for global aquaculture. The dominant phyla were reported to be Proteobacteria, Firmicutes, and Fusobacteria (Eichmiller et al., 2016; Yan et al., 2016; Mukherjee et al., 2020). Depending on the species and the sample type (digesta or mucosa) the phyla abundances like Bacteroidetes or Cyanobacteria, which are typically transient, may differ (Givens et al., 2015; Ye et al., 2014). Like in other freshwater fish species, *Cetobacterium* as well as *Aeromonas* have been reported as core genera in cyprinids (Eichmiller et al., 2016; Yan et al., 2016; Ye et al., 2014), while other genera reported are *Pseudomonas*, *Bacteroides and Clostridium* (Wu et al., 2012, 2013).

Several studies exploring the gut microbiota have been also reported for the Nile tilapia (*Oreochromis niloticus*), as the second most economically important aquaculture species. The dominant phyla are generally Proteobacteria and Fusobacteria although the relative abundances of these phyla have been reported to vary between studies due to differences in rearing environment (Deng et al., 2021, 2022; Giatsis et al., 2015). Like in carps, *Cetobacterium* has been reported as part of the core microbiome members (Deng et al., 2022). Moreover, *Plesiomonas* has been reported as one of the dominant genera, along with *Bacillus*, *Clostridium* and *Mycobacterium* (Deng et al., 2022; Giatsis et al., 2015; Hallali et al., 2018; Ran et al., 2015).

Salmonid species like Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) have also been the subject of numerous sequencing studies. The dominant phyla in those species are Proteobacteria and Firmicutes, in both freshwater or saltwater stage for Atlantic salmon, while factors such as the rearing environment and diet can also affect the composition (Gajardo et al., 2016, 2017; Schmidt et al., 2015; Zarkasi et al., 2014, 2016). Major bacterial families have been reported to be *Ruminococcaceae* and *Mycoplasmataceae* (Cheaib et al., 2021; Dehler et al., 2017), while at the order level several studies reported Aeromonadales and Lactobacillales (Gajardo et al., 2017; Schmidt et al., 2015), with *Streptococcus* and *Lactobacillus* genera to be altered by diet. In two studies during the seawater stage (Gajardo et al., 2016; Zarkasi et al., 2014), *Leuconostoc* and *Weissella* were reported as dominant genera during temperatures approximately 10°C−12°C, whereas the community shifted to Vibrionaceae (*Vibrio*, *Aliivibrio*, and *Photobacterium*) when waters were warmer (14°C−17°C; Zarkasi et al., 2014).

Rainbow trout shares several members of the gut microbiota with Atlantic salmon, with *Weissella* identified as a major genus, along with *Streptococcus*, *Leuconostoc*, and *Lactobacillus* (Desai et al., 2012; Ingerslev et al., 2014a, 2014b; Wong et al., 2013). These similarities suggest that salmonids (rainbow trout, Atlantic salmon) share dominant members of the Firmicutes as a core microbiome, despite differences in the environment that are reared (freshwater vs marine), indicating that host-related microbial selection may be tied to phylogeny (Sullam et al., 2012).

Marine fish of economic interest for aquaculture are mainly carnivorous in feeding habits, such as gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*), Atlantic cod (*Gadus morhua*) and turbots. Like in other groups reported above, Proteobacteria are the main phyla dominating the gut microbiota of such groups, followed by Firmicutes and Actinobacteria (Egerton et al., 2018; Kormas et al., 2014). Among the most dominant genera prevailing in the gut of these species are *Vibrio*, *Aeromonas* and *Pseudomonas*, while *Photobacterium* has also been reported (Keating et al., 2021; Kokou et al., 2019; Piazzon et al., 2020; Riiser et al., 2019; Serra et al., 2021; Xing et al., 2013; Zhang et al., 2020f). *Vibrio spp*., *Enterobacter spp*., *Pseudomonas spp*., and *Aeromonas* spp. isolated from marine fish GI tract have been found to produce proteases while these bacteria along with *Photobacterium spp*. have also been reported to produce chitinases (Ray et al., 2012).

17.2.3 Main taxonomic groups in the fish and shrimp gut microbiota

As mentioned above, shrimp gut microbiota studies are limited compared to those in fish, with only very few reviews reported so far (Holt et al., 2021; Tang et al., 2021b; Xiong, 2018). Although the shrimp microbiota composition (mainly the abundance) can be affected by developmental stage, environment and health status, the main bacterial phyla reported are Proteobacteria, Firmicutes, Bacteroidetes, and Fusobacteria (Holt et al., 2021). The genera *Vibrio* and *Photobacterium*, from the Gammaproteobacteria class, have been reported to be the most abundant taxa in penaeids shrimps, giant tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*Litopenaeus vannamei*), which are the most well-studied species (Holt et al., 2021; Tang et al., 2021b). The presence of *Vibrio* species may be explained by their capacity to produce chitinolytic enzymes (Ray et al., 2012; Sugita and Ito, 2006) in a chitin-rich environment such as the crustacean gut, providing a niche substrate for their utilization. However, *Vibrio* species, as reported also for fish species, may cause health problems, since they are opportunistic bacteria (Jun and Woo, 2003). Other abundant bacterial species reported in both tiger and white shrimps are *Flavobacteriaceae* and *Rhodobacteriaceae* species, which have been reported in healthy individuals, although overabundance of these taxonomic groups was reported to coincide with disease states in shrimps (Xiong et al., 2017).

17.3 Development of the gut microbiota

Our understanding of the development and functional significance of the gut microbiota in fish is lagging behind the terrestrial vertebrates, although fishes represent the greatest taxonomic as well as ecological diversity among the vertebrates (Clements et al., 2014; Nayak, 2010; Ray et al., 2012). Being an aquatic organism with the GI tract that exists as an open channel, the gut microbiome in fish is dynamic in nature rather than static (Yukgehnaish et al., 2020). The fish gut microbiota can be diverse, representing members from bacteria and archaea, along with yeasts, molds, and viruses (Merrifield and Rodiles, 2015). Bacteria are the dominant microbiota within the fish GI tract and the majority of the studies focused on this particular group (Egerton et al., 2018; Rombout et al., 2011). In addition, quite a few studies have demonstrated yeasts as part of the normal microbiota within the fish GI tract (Das and Ghosh, 2014; Naya-Català et al., 2022).

The colonization of fish gut starts early in the larval stage and is continuously driven toward the achievement of a complex assemblage of gut-associated microbes (Nayak, 2010). Development of gut microbiota in fish is determined by the interplay of a variety of factors that depend on both the nature of the available microbiota in the aquatic medium and the host-fish, along with its ambient environment. Talwar et al. (2018) stated that the colonization of gut-microbiota may either be driven by (1) a stochastic (probabilistic) model that is linked with random dispersal of microorganisms, or (2) a deterministic (non-neutral) model that assumes the congregation of the host selective pressures and active dispersal of microorganisms by the interactions between host and microbes. Studies on zebrafish over the developmental period suggested that the gut colonization among larvae was governed by inoculation from the surrounding environments, which was then gradually shifted to the non-neutral processes as the host matured to become adult, suggesting stochastic toward deterministic colonization within the GI tract (Burns et al., 2016). Several biotic (e.g., genetics, sex, age, physiological status, and trophic level) and abiotic (e.g., environmental) factors affect the development as well as the diversity of the fish gut microbiota (Butt and Volkoff, 2019; Ghanbari et al., 2015). As the composition and diversity of the fish gut microbiota have been discussed in the preceding section, this section will provide an overview of the development of the gut microbial community within the fish GI tract with an emphasis on the intrinsic and extrinsic factors that influence the development.

17.3.1 Development of gut microbiota at different stages

Primarily, fish embryos develop within the egg (mostly) in a comparatively stable and bacteria-free environment. Therefore, fish are microbe-free at hatching, at least theoretically, and the gut microbiota is acquired from the surrounding environment posthatch (Li et al., 2017). After hatching, fish are exposed to changing environmentally recognized factors (e.g., pH, nitrate-nitrogen, phosphorous, salinity, temperature, dissolved oxygen, and pollutants) that can extremely influence the gut microbiota throughout their lifespan (Butt and Volkoff, 2019). The composition of fish gut microbiota differs at different stages of development. It is anticipated that microbial colonization of fish larvae starts off from the eggs, the surrounding water and the first feeding. Presently it is established that initial colonization of the bacteria is supposed to be species-specific, and is determined by variation in binding glycoproteins on the egg surface (Larsen et al., 2014). Thus, interspecies variation was noticed in some of the preceding studies. Differences in bacterial colonization with the eggs of Atlantic cod and halibut (*Hippoglossus hippoglossus*) were recorded (Hansen and Olafsen, 1989). Nevertheless, the microbiota of the ambient water could dictate what bacteria might be encountered and would consequently have the chance to colonize the eggs. Upon hatching, the sterile larvae acquire the chorion-associated bacteria that become the first colonizers of the developing GI tract. Subsequently, more bacteria are obtained by the marine teleosts when the fish larvae begin to drink water to maintain ionic balance through osmoregulation (Hansen and Olafsen, 1999). Afterwards, the microbiota becomes further diversified through feeding in both marine as well as freshwater fish species. At the beginning, the GI tracts of newly hatched larvae tend to have only a few bacteria (Ringø et al., 1991). Several studies have indicated that diet is the key factor affecting the shape or composition of the gut microbial community and substantial diversification occurs from the first feeding phase through the developmental stages (Korsnes et al., 2006; Lauzon et al., 2010; Parris et al., 2016; Reid et al., 2009). In contrast, rather than feed, the environment was considered to play a significant part in influencing the composition of gut microbiota at the larval stage of codfish (Bakke et al., 2013).

Gut microbiota compositions in Atlantic salmon varied between developmental stages indicating lower richness and diversity in embryonic communities as compared to the hatchlings (Lokesh et al., 2019). In another study, larval, juvenile, and adult stages of Malaysian Mahseer (*Tor tambroides*) had higher gut microbiota diversity than fingerling and yearling stages (Mohd Nosi et al., 2018). Although the microbial community changes with life stages and habitat, a comparatively stable gut microbiota is set up within the first 50 days of life for many fish species (Larsen et al., 2014; McIntosh et al., 2008). A decisive study on zebrafish, demonstrated that a core microbial community was established through host-specific selective pressures despite the consequences of environmental parameters (Roeselers et al., 2011). Another study on zebrafish also established considerably distinct gut microbiota during the larval and juvenile stages with similar rearing environments and feeding indicating the genotype of the fish as a foundation for the development of the gut microbiota composition (Stephens et al., 2016).

17.3.2 Factors affecting the development of gut microbiota

The factors affecting the development of gut microbiota at different stages of development may broadly be grouped into four classes, which are (1) environmental factors, (2) host-specific intrinsic factors, (3) trophic level and/or feeding behavior, and (4) nature of the microflora. These are discussed in brief in the following section (Fig. 17.3).

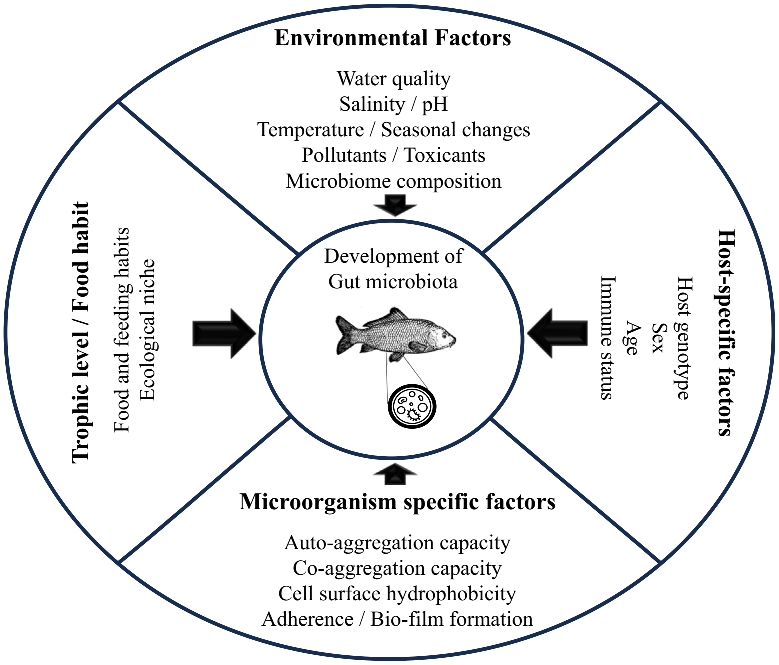

FIGURE 17.3 Factors affecting the development of fish gut microbiota.

17.3.2.1 Environmental factors

Environmental microbial accumulation is significantly correlated with the microbiota composition in the intestine of fish (Jesu et al., 2014). Water quality and its alterations resulting from diverse anthropogenic and natural causes influence the development and maintenance of gut microbiota in fish. Earlier studies revealed that microbes in water could affect the GI tract microbiota in fish. The composition of the GI tract microbiota in grass carp (*Ctenopharyngodon idella*) was correlated with the associated microbiota in the production of water and sediment (Wang et al., 1993). Atlantic salmon reared under indoor recirculating aquarium facilities and open freshwater cage culture showed dissimilar compositions of the gut-associated microorganisms indicating the influence of the rearing conditions on the development of the gut microbiota (Dehler et al., 2017).

Salinity might influence the development of the gut-microbiota. The composition of the gut bacteria was noticed to vary between the fish inhabiting freshwater and marine habitats. In general, *Bacilli*, *Aeromonas*, and *Pseudomonas* predominate freshwater fish (Ghosh et al., 2021; Ray et al., 2012), while *Vibrio* is the most common genus in marine fish (Vatsos, 2017). Zhang et al. (2016b) revealed the gut-microbiota composition of Nile tilapia reared under varying salinity and noticed an increased abundance of *Devosia*, *Pseudomonas*, and *Cetobacterium* in high-salinity conditions. Similarly, an increase in salinity induced changes in dominant bacterial taxa in the gut microbiome of black molly, *Poecilia sphenops* (Schmidt et al., 2015). While evaluating the influence of environmental factors on the development of gut microbial communities in a large number of fish species with varying ecological needs, meta-analysis suggested salinity of water as a decisive factor to determine the microbial composition within fish gut (Sullam et al., 2012) (Figure 17.2).

Seasonal changes along with changes in temperature might induce alteration in food consumption owing to variation in nutrient loads in the aquatic medium, which in turn results in altered composition of the associated microbiota in a particular season. For instance, abundance of the gut bacteria in tilapia declined in winter as compared to the other seasons. Moreover, in the gut of hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*), few genera (e.g., *Pseudomonas*, *Micrococcus*, and *Flavobacterium*) were only detected during winter (Al-Harbi and Naim Uddin, 2004). The authors also indicated changes in the abundance of total bacteria with peaks in the summer and autumn months (Al-Harbi and Naim Uddin, 2004). Seasonal variation in the composition of the gut-associated lactic acid bacteria (LAB) was noticed in three carp species (silver carp, *Hypophthalmichthys molitrix*; common carp, *Cyprinus carpio*; deep-bodied crucian carp, *Carassius cuvieri*) and channel catfish (*Ictalurus punctatus*) (Hagi et al., 2004). Although seasonal changes and temperature variation have been considered crucial parameters for fish gut microbiota, a study on adult Atlantic salmon could not detect significant variation in gut microbial composition when temperature varied between 5.5°C and 18.8°C (Hovda et al., 2012). In contradiction, another study on the same species noticed the disappearance of gut-inhabiting lactic acid bacteria (LAB) and *Acinetobacter* spp. with an increase in *Vibrio* spp. at increased temperatures up to 21°C (Neuman et al., 2016). It was hypothesized that a rise in the temperature of the sea was associated with an increase in potentially virulent *Vibrio* spp., which could be linked to the decreasing stocks of wild salmon. An increase in temperature was associated with increased growth of the microbiota in rainbow trout (Huyben et al., 2018).

The existence of pollutants and toxins in the environment may also interfere with the development of fish gut microbiota. Contamination of crude oils in the environment resulted in abundance of Deltaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria, and Sphingobacteria along with a decline of Alphaproteobacteria and Clostridia within the GI tract of Southern flounder (*Paralichthys lethostigma*) that had a negative impact on the health of the fish (Brown-Peterson et al., 2015). Further, exposures of pesticides (pentachlorophenol) to goldfish (*Carassius auratus*) (Kan et al., 2015), polystyrene microplastics to zebrafish (Jin et al., 2018) and waterborne copper to common carp (Meng et al., 2018) and long-term feeding with antibiotics in zebrafish (Zhou et al., 2018a), were associated with disorders of the GI tract microbiota and induced gut microbiota dysbiosis.

To delineate environmental influence on the host gut microbiota, a comparative account of the microbial composition in the host gut and its ambient environment would be trustworthy. Shared operational taxonomic units (OTUs) between water and gut microbiota in tilapia larvae suggested the potential role of surrounding water on the development of gut microbiota in fish (Giatsis et al., 2015). Studies on wild and laboratory-reared invasive carp species also established that the environment was embraced with a determinative role in shaping the gut microbiome in fish (Eichmiller et al., 2016). In contrast, abundant microbial taxa in the ambient water were not detected in the gut of inhabitant fish species and vice versa, suggesting a much stronger influence of host-associated factors than environment on the development of fish gut microbiota (Schmidt et al., 2015).

17.3.2.2 Host-specific intrinsic factors

Some of the host-specific factors could play an important role in determining the development and composition of gut microbiota in fish (Yukgehnaish et al., 2020). Among these intrinsic factors, host genotype, phylogeny and sex have been indicated as important factors for variations in microbiota composition among fish (Egerton et al., 2018). Fish with higher genetic heterozygosity displayed lesser complexity in variation of the microbiota, suggesting the likely role of MHC-II in controlling the microbial population within fish GI tract (Smith et al., 2015). Thus, the host's immunological genotype might affect the development and composition of the gut microbiota.

Intra- as well as interspecific variations in gut-microbiota due to differences in the genetic background have been anticipated. For instance, some bacterial groups were associated with specific families of rainbow trout from different habitats (Navarrete et al., 2012). Distinct bacterial populations in the gut of cohabitating freshwater fish larvae, for example, silver carp, grass carp, bighead carp (*Hypophthalmichthys nobilis*) and blunt snout bream (*Megalobrama amblycephala*) were detected indicating host species as a strong determinant of the gut microbiota in fish larvae (Li et al., 2012). Species-specific differences in the dominance of the gut associated bacterial phyla have been indicated by several authors (Mukherjee et al., 2020; Sullam et al., 2012). Li et al. (2015) conducted a study on grass carp, crucian carp and bighead carp and suggested that specific endogenous factors could be co-linked with the environmental factors and feed composition to shape the gut microbiota in fish. Similarly, Mukherjee et al. (2020) also noticed variations in the composition of the gut microbiota in the three Indian major carps (IMCs), rohu (*Labeo rohita*), catla (*Catla catla*), and mrigal (*Cirrhinus mrigala*) reared under polyculture system. Higher proportions of Bacteroidetes and Actinobacteria were recorded in both mrigal and rohu, while Proteobacteria was more abundant in catla. The authors opined that gut microbiota might have resulted from niche partitioning and selective pressures that could be species-specific (Mukherjee et al., 2020). On the other hand, channel catfish and blue catfish (*Ictalurus furcatus*) raised under constant environmental and husbandry conditions had similar gut microbiota compositions, suggesting that a shared environment could overcome differences in host genetics (Bledsoe et al., 2018).

Several studies conducted in fish during the last decade demonstrated sex-specific differences in the gut-microbiota, which were attributed to the different sex-specific factors, e.g., hormones, host-microbe interactions, immune responses or diet preferences (Bolnick et al., 2014; Piazzon et al., 2019). Variations in the gut-microbiota between sexes of the three-spine stickleback (*Gasterosteus aculeatus*) and Eurasian perch (*Perca fluviatilis*) were reported (Bolnick et al., 2014). The gut microbial composition of adult gilthead sea bream indicated significant changes with age and sex, although microbiota remained constant in terms of richness estimates and diversity indexes (Piazzon et al., 2019). Composition and actual bacterial pathways being expressed in genetically selected gilthead sea bream demonstrated that the microbiota of these animals is more structurally stable but is able to functionally adapt to diet changes when compared to nonselected animals (Naya-Català et al., 2022; Piazzon et al., 2020). Significant and gradual changes in bacterial composition were noticed in gilthead sea bream beyond the reproductive season (Piazzon et al., 2019; Simó-Mirabet et al., 2018). However, Stephens et al. (2016) and Liu et al. (2016) could not detect significant differences in the gut microbiome composition between the sexes of zebrafish. To conclude, how intestinal microbiota is modulated by sex and the exact mechanisms behind it still remain to be elucidated.

17.3.2.3 Trophic level and/or feeding behavior

It is logical to apprehend that trophic levels as well as food and feeding habits might influence the development and composition of the fish gut microbiota. The trophic position of the host fish species relates to the natural diet developed in the course of evolution and it might have a major impact on the gut microbiota composition. As indicated, gut bacterial diversity is usually lower in carnivores, and progressively increases in omnivores and herbivores (Wang et al., 2018). Liu et al. (2016) conducted studies with fish from distinct trophic levels (viz., herbivores, carnivores and omnivores, including filter feeders) and noticed distinct gut microbiotas with reference to the specified groups. Carnivorous fish appeared to harbor *Halomonas* and *Cetobacterium*, while the omnivorous fish was rich in *Halomonas*, *Clostridium*, and *Cetobacterium*, and herbivorous fish harboring *Leptotrichia*, *Clostridium*, and *Citrobacter* in abundance. Mukherjee et al. (2020) recorded the high abundance of Bacteroidetes and Actinobacteria in both rohu and mrigal that could be related to the presence of complex polysaccharides (PSAs) in their diet owing to feeding on phytoplankton, supplementary feeds or plant detritus arising out from decomposing macrophytes, while Proteobacteria was more abundant in catla (zooplanktophagus).

Carnivorous species were noticed to harbor lipase and protease-producing bacteria, such as *Halomonas* (Liu et al., 2016). On the other hand, cellulose-degrading bacteria, for example, *Anoxybacillus*, *Actinomyces*, *Clostridium*, *Citrobacter*, and *Leuconostoc* populate the gut of herbivorous species such as grass carp (Wu et al., 2012). In a study with rohu, catla and mrigal, the occurrence of plant PSA degrading bacteria in all of these major carp species studied seemed to be specifically important, as these carps were reared under the carp polyculture system and fed on supplementary feeds containing plant materials, for example, mustard oil cake and soybean meal (Mukherjee et al., 2020). Filter-feeding fishes displayed higher diversity as they need to filter large amounts of water covering large areas by swimming rapidly (Li et al., 2017). This trend has been detected in both freshwater as well as marine fish, suggesting that the trophic level is possibly one of the most influential factors affecting the development and composition of gut microbiota (Egerton et al., 2018).

Feeding behavior might also affect the gut microbiome composition in a habitat within closely related, but different species. For instance, four herbivorous Asian carp species (silver carp, bighead carp, grass carp, and common carp) reared in the same environmental conditions exhibited interspecific differences in the gut microbiota composition, particularly with regard to the relative abundance of the cellulose-degrading phylum Firmicutes, most likely due to species-specific diets (Li et al., 2018b). Thus, variation within the species belonging to the same trophic level was established. It was hypothesized that the development and composition of the intestinal microbiota might have coevolved with their host in response to feeding habits, although diverse environmental factors may also interplay (Mukherjee et al., 2020). A later section will discuss diet-related alterations of the gut microbiota in diverse fish species in greater detail.

17.3.2.4 Nature of the microbiota

While considering the factors affecting the development of GI tract microbiota in fish along with other hosts, the majority of the reports addressed environmental as well as host-associated factors as the key determinant of the existing gut microbiota and the nature of the developing microbiota itself has been neglected. The microorganisms that meet the physiological as well as ecological requirements to grow and survive within the GI tract and satisfy the most appropriate niche space would become the permanent dwellers (so-called) of the GI tract and are referred to as 'autochthonous' microbiota (Talwar et al., 2018), whereas frequent visitors of the GI tract derived from the surrounding environment and associated with the digesta are known as 'allochthonous' microbiota (Ray et al., 2012). Therefore, as mentioned also in the previous sections, generally we need to look at the fecal samples to study the allochthonous communities, while mucosal epithelium is examined to consider the adherent residents (autochthonous communities). The adherence of the microbiota onto the gut epithelial cells is considered an essential factor in determining their functions within the host. But what made the autochthonous communities capable of adhering the GI tract mucosa?

Diverse ways by which the microorganisms congregate with the host at first are simply fortuitous, however, that may become obligatory later (Talwar et al., 2018). Aggregation between microorganisms of the same strain (auto-aggregation) or between different strains (co-aggregation) is of considerable importance for several ecological niches (Jena et al., 2013). Bacterial cell surface hydrophobicity and auto-aggregation capacity are directly correlated, and hydrophobicity could be considered as one of the determinants of auto-aggregation (Patel et al., 2009). Auto-aggregation of the bacterial strains appeared to be necessary for adhesion to GI tract epithelial cells, and co-aggregation abilities may form a barrier that prevents colonization by pathogenic microorganisms (Del Re et al., 2000). On the other hand, cell surface hydrophobicity influences the attachment of microbial cells to a surface and thereby formation of bacterial biofilm. Thus, organisms capable of forming biofilms could have successful colonization and exert functions of normal gut microbiota. The aggregating bacteria may achieve adequate colonization to adhere to the gut mucosal surfaces of the host or form biofilms (water additive) as indicated elsewhere (Dutta et al., 2018; Jena et al., 2013). The ability to form biofilms helps bacteria to survive in hostile environments offered by the GI tract which could be seen in both pathogenic and probiotic bacteria (Kalia et al., 2017; Sanchez et al., 2016).

The development of gut microbiota seems to be a complex process involving the interplay of several factors. Still, it is inconclusive whether genetics (host-associated factors) or the environment has a greater influence on the development of gut microbiota. Although the majority of the reports suggested that gut microbiota could result from niche partitioning and selective pressures that might be species-specific, given the small number of published reports and the wide variation in studies, the underlying mechanisms are not yet well-understood and necessitate further comprehensive investigations.

17.4 Homeostatic functions of the gut microbiota

Homeostasis is a self-regulating process by which living organisms maintain their stability with regard to internal, physical and chemical conditions while adjusting to the changing external environment. The micro-ecosystem of the GI tract is constituted by complex and dynamic populations of microorganisms that tightly interact with each other and with the macroscopic host with key effects on host homeostasis. Gnotobiotic approaches using the model species zebrafish showed that, like mammals, fish intestinal microbiota affects epithelial renewal and enterocyte morphology, and stimulates nutrient metabolism and the development of the immune system (Rawls et al., 2004). The absence of microbiota in fish leads to intestinal epithelial cell dysfunction, impaired nutrient absorption and metabolism and faulty immune responses (Bates et al., 2006, 2007; Cheesman et al., 2011; Galindo-Villegas et al., 2012; Kanther et al., 2011; Rawls et al., 2004, 2006). One of the mechanisms by which microbiota affects animal health is the capacity to produce diverse enzymes and certain types of metabolites with direct activity over the host physiology. Among these metabolites, SCFAs, derived from microbial fermentation of dietary fibers, have been thoroughly studied due to their beneficial effects on health (Koh et al., 2016). In addition, the equilibrium within the microbial structures is maintained by competence for cell-surface and mucin-binding sites. Further, the production of antimicrobial substances shape the specific populations within the gut and, when function properly, keep pathogens away from proliferating and invading the host (Liu et al., 2013). In this section, we will review the current knowledge on these different aspects of microbial effects on host homeostasis in teleost fish.

17.4.1 Digestion, nutrient absorption, and metabolism

The gut microbiota is frequently referred to as an "extra organ," as it plays a vital role in the intestinal function along with the overall health and development of the host. It has been hypothesized that the gut microbiota is involved in homeostatic mechanisms by regulating feeding, digestion and metabolic as well as immune function of the host organism (Butt and Volkoff, 2019; Feng et al., 2018). Wide variations in the composition of fish gut microbiota exist between the species, even if they share the same environment (Foysal et al., 2020; Mukherjee et al., 2020). However, several bacterial phyla are dominant in the GI tract of diverse fish species, which include Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacteria, as has been reported in Section 17.2 (Butt and Volkoff, 2019; Eichmiller et al., 2016; Mukherjee et al., 2020). The function of the gut microbiota and the succeeding physiological responses of the host might depend on the composition of the microbial community that is present within the GI tract (Butt and Volkoff, 2019). Recent investigations suggested that data from amplicon sequencing could provide greater information on the abundance of microbiota in the fish GI tract as well as the genetic and metabolic potential of the species (Foysal et al., 2020; Li et al., 2015; Mukherjee et al., 2020).

Studies in mammals indicated that microorganisms within the GI tract are involved in the regulation of appetite, feed intake, digestion and metabolism (Duca et al., 2012; Fetissov, 2017). A pioneering study in mice revealed that colonization of the GI tract with specific microorganisms could modulate the expression of numerous cellular genes that aid the host in vital functions like intestinal maturation, nutrient absorption, metabolism and immune response (Hooper and Gordon, 2001). Similar findings have been recorded in zebrafish (Kanther and Rawls, 2010; Rawls et al., 2004) and in some other fish species where microorganisms are demonstrated to regulate metabolism (Li et al., 2013), while, significant disparity between the mammals and fish may exist, pertaining to host−microbe interactions and its relation to metabolism and homeostasis (Butt and Volkoff, 2019). Thus, this area demands comprehensive studies to get more insight into fish gut microbiota and their connection with the homeostatic mechanisms. The majority of the functions of the gut microbiota ascribed to the host homeostasis are also recognized as providing probiotic attributes for fish as well as other vertebrates. A greater detail of the probiotic functions of the fish gut microbiota has been included in a separate chapter. Therefore, this section will present a brief report on the gut microbiota and their likely involvement in the optimal functioning of the physiological processes relating to digestion, nutrient absorption and metabolism in fish.

17.4.1.1 Gut microbiota in digestion

In contrast to mammals, the likely contribution of the endosymbiotic microbiota to the digestion and nutritional physiology of fish has been recognized only lately. However, a good number of studies conducted during the last two decades indicated positive effects of the gut microbiota in the digestive process of fish (Ghosh et al., 2019; Ray et al., 2012). Despite the endogenous digestive enzymes produced by the host, exogenous enzymes produced and supplemented by the gut microbiota have been considered one of the vital secondary factors affecting digestion and feed utilization in fish (Soltani et al., 2019).

In fact, an extensive array of enzymes including carbohydrases, phosphatases, esterases, lipases, and peptidases produced by the gut microbiota contribute to the digestive processes in fish (Ghosh et al., 2019). The enzymes produced by the gut microbiota that play a significant role in digestion have been categorized into (a) digestive enzymes, for example, protease, amylase, and lipase; and (b) degradation enzymes, for example, cellulase, xylanase, phytase, tannase, and chitinase (Soltani et al., 2019). Studies conducted on IMCs (Banerjee et al., 2016; Das and Ghosh, 2014; Dutta and Ghosh, 2015; Dutta et al., 2015; Mandal and Ghosh, 2013a, 2013b; Mukherjee and Ghosh, 2016; Mukherjee et al., 2016, 2017; Ray et al., 2012), as well as other teleosts (Al-

Hisnawi et al., 2015; Cahill, 1990; Hoseinifar et al., 2016a; Llewellyn et al., 2014; Ringø and Song, 2016; Ringø et al., 2016), have provided conclusive pieces of evidence with regard to the occurrence of symbiotic gut-associated microorganisms in fish and their beneficial role in nutrition and feed utilization.

Endogenous enzymes in the fish digestive system help to hydrolyze organic macromolecules into simpler compounds. Copious reports have provided information that the gut-associated bacteria and yeasts have a positive effect on breakdown of diverse substrates present in natural food/feed ingredients that the fish (including other vertebrates) usually cannot digest utilizing the endogenous digestive enzymes. For example, chitin (e.g., Banerjee et al., 2015; Ringø et al., 2012), starch, cellulose (e.g., Askarian et al., 2012a, 2012b; Bairagi et al., 2002; Kar and Ghosh, 2008; Mondal et al., 2008; Mukherjee et al., 2016; Saha and Ray, 1998; Saha et al., 2006), xylan (Banerjee et al., 2016), collagen (Kar and Ghosh, 2008), phytate (e.g., Das and Ghosh, 2013; Khan and Ghosh, 2013; Li et al., 2008; Roy et al., 2009) and tannin (Mandal and Ghosh, 2013a, 2013b; Talukdar et al., 2016). Thus, it seems that the enzymes produced by the gut microbiota might have a vital role in digestion, especially for the complex feedstuffs, which few animals can digest (Ghosh et al., 2019). Several studies have revealed that enzyme-producing gut bacteria are able to utilize mannose, xylose, raffinose and cellobiose (Banerjee et al., 2016; Kar et al., 2008). Diverse species of the genus *Bacillus*, *Citrobacter*, *Enterobacter*, *Aeromonas*, *Vibrio*, *Photobacterium*, and *Clostridium* among the bacteria along with *Pichia* and *Candida* among the yeasts with efficient exoenzyme-producing ability are detected from the gut of varied fish species (Egerton et al., 2018; Ghosh et al., 2019). Consequently, it has been hypothesized that the gut probiotic bacteria could get the opportunity to secrete by-products which can activate the digestive enzymes for digestion within the gut of host fish (Salam et al., 2021a). Readers who are especially interested on the different exoenzyme producing fish gut bacteria and degradation of plant-derived antinutritional factors by the enzymes produced by gut microbiota are referred to the reviews of Ray et al. (2012) and Ghosh et al. (2019) for comprehensive information.

17.4.1.2 Gut microbiota in nutrient absorption and metabolism

Only a few studies conducted so far addressed the influence of gut microbiota on feeding and metabolism in fish. Studies on six days post-fertilization germ-free gnotobiotic zebrafish revealed that the GI tract microbiota could regulate 212 genes, which were involved in the stimulation of epithelial proliferation, promotion of nutrient metabolism and innate immune responses (Rawls et al., 2004). Further, another study on zebrafish larvae reared in a germ-free environment noticed that the GI tract failed to differentiate properly in the absence of the microbiota as revealed by the reduced levels of intestinal brush border alkaline phosphatase activity, lack of glycan expression and a scarcity of goblet and enteroendocrine cells (Bates et al., 2006). Gut microbial communities in fish as well as other vertebrates have been indicated to play an integral role in the proliferative activity of intestinal epithelial cells, metabolism of xenobiotics and also regulation of energy homeostasis (Rawls et al., 2007), although direct pieces of evidence regarding the influence of gut microbiota on absorption of nutrients are scarce. Pirarat et al. (2011) reported that probiotic gut bacteria *L. rhamnosus* significantly improved growth as well as the length of intestinal microvilli in Nile tilapia. More recently, Salam et al. (2021a) demonstrated that dietary supplementation of gut bacteria (viz., *Enterococcus xiangfangensis*, *Pseudomonas stutzeri*, *Bacillus subtilis*, and a consortium of five gut bacteria) in silver barb, *Barbonymus gonionotus* promoted growth, improved the length of intestinal villi and modulated the intestinal microbiota by reducing the number of pathogenic bacteria. It was likely that the improved length of the villi led to an increase in intestinal nutrient absorption contributing to improvement in feed utilization and growth.

Increased feed intake, enhanced nutrient digestibility and improved feed utilization owing to dietary supplementation of isolated fish gut microbiota as probiotics have been recorded in diverse fish species indicating beneficial effects of the gut microbiota in nutrient utilization and overall metabolic function. For example, Japanese flounder (*Paralichthys olivaceus*) fed a diet supplemented with *Bacillus clausii* isolated from the gut of grouper (*Epinephelus coioides*) displayed higher weight gain, feed efficiency and growth performance compared to fish fed control diets (Ye et al., 2011). In a recent study, the application of *Bacillus tequilensis* (isolated from rohu) through diets or water improved growth, nutrient utilization and nonspecific immunity in rohu fingerlings (Dutta and Ghosh, 2021). Apart from growth and feed utilization, some other studies made an attempt to correlate feeding and/or changes in the microbiota with feeding rate. For instance, carp fed a diet supplemented with fructo-oligosaccharide displayed variation in microbiota composition indicating the increased abundance of heterotrophic aerobic bacteria and lactic acid bacteria without changes in feeding rates as compared to the fish fed a control diet (Hoseinifar et al., 2014). In another report, zebrafish fed *Lactobacillus rhamnosus* supplemented diets had reduced appetite in comparison to the control fish (Falcinelli et al., 2016, 2018). Thus, the results pertaining to dietary supplementation were inconsistent and inconclusive, as different studies used different microbial strains, and diverse additives and were conducted at dissimilar environmental conditions.

The potential effect of the gut microbiome on growth and metabolism has been examined in a few fish species. Studies on transgenic fast-growing common carp suggested that the Firmicutes confer fast growth of fish over the Bacteroidetes indicating an important role of the dominant gut flora on growth of the fish (Li et al., 2013). In grass carp, changes in the composition of gut microbiota was associated with alteration in the biosynthetic as well as metabolic pathways of carbohydrates, amino acids and lipids (Ni et al., 2014). In zebrafish, the colonization of the gut by microorganisms promotes the absorption of fatty acids through the gut epithelium (Semova et al., 2012). Furthermore, fish with intact microbiota were noticed to have increased lipid accumulation in the gut epithelium, along with increased expression of genes linked to lipid metabolism as compared to the germ-free fish that lacked gut microbiota (Sheng et al., 2018). Thus, it appears that the gut microbiota may have a considerable influence on the metabolic processes in fish. However, apart from the influence of gut microbiota on digestive and metabolic processes in fish, the consequences of the metabolites produced by them should also be considered while realizing their contribution to the host. The major metabolites produced by the gut microbial community are the SCFAs, a detailed description of which has been included in the following section.

17.4.2 Production of short-chain fatty acids

SCFAs are organic monocarboxylic acids with a chain length of up to six carbon atoms. Among them, acetate, propionate and butyrate are the major metabolites produced by anaerobic bacteria through the microbial fermentative process in the intestine. SCFAs are mainly generated by the fermentation of undigested dietary carbohydrates and fibers, but also during the catabolism of proteins (Louis and Flint, 2017; Miller and Wolin, 1996). They are important energy and signaling molecules that, among their beneficial effects include strengthening of the epithelial barrier, reduced inflammation, antioxidative effects and increased production of mucins and antimicrobial peptides (Koh et al., 2016; Onrust et al., 2015). Each of the three major SCFAs has specific effects on host physiology, differing in fate and tissue distribution. Butyrate is preferentially used by the gut mucosa as an energy source, propionate is an important contributor to gluconeogenesis in the liver, whereas acetate achieves high concentrations in the blood (Morrison and Preston, 2016).

17.4.2.1 Production of short-chain fatty acids

Production of acetate is widely distributed among intestinal anaerobes, whereas butyrate and propionate are produced by distinct subsets of bacteria (Morrison and Preston, 2016). Gut bacteria mainly produce acetate by carbohydrate fermentation, but a significant amount is also directly synthesized by acetogenic bacteria from hydrogen and carbon dioxide or formic acid through the Wood–Ljungdahl pathway (Ríos-Covián et al., 2016). Intestinal propionate can be produced by three different pathways, the succinate, acrylate and propanodiol pathways. The succinate pathway is present in several members of the Negativicutes class (phylum Firmicutes) and in Bacteroidetes. The acrylate pathway is more limited to some Clostridiales (Firmicutes) members of the families *Veillonellaceae* and *Lachnospiraceae*. The propanodiol pathway is present in phylogenetically distant bacteria such as some Proteobacteria or members of the *Lachnospiraceae* family (Reichardt et al., 2014). Butyrate can be produced by two different pathways. The butyrate kinase pathway is the least common pathway and has been described almost exclusively in some *Coprococcus* species (*Lachnospiraceae* family). The most common pathway for butyrate production is the butyryl-CoA: acetate CoA-transferase pathway, used by the majority of butyrate producers such as *Feacalibacterium*, *Eubacterium* or *Roseburia*, members of the Clostridia order (Louis et al., 2010; Ríos-Covián et al., 2016) (Fig. 17.4).

SCFAs produced in the intestine are not only utilized by the host but also by other intestinal microorganisms through mutualistic cross-feeding interactions, determining the final availability of SCFAs to the host. Bacterial cross-feeding from acetate to butyrate is the most common, but also metabolic fluxes between butyrate and propionate can change the balance of SCFAs in the intestine (den Besten et al., 2013; Duncan et al., 2004). As an example, this metabolic complementarity was demonstrated between an acetate producer *Bacteroides* species and a *Faecalibacterium* species that uses acetate to generate butyrate changing the effect of these microbial metabolites on host mucin production and glycosylation (Wrzosek et al., 2013). Also, the use of lactate by butyrate and propionate-producing bacteria avoids metabolic acidosis in the host (El Aidy et al., 2013).

The interest and importance of SCFAs lies in their ability to inhibit the growth of certain bacterial species changing the microbial composition of the gut, and in their direct effects on host physiology (Fig. 17.4). The beneficial effects of SCFAs have not been ignored and they are considered as attractive molecules to be used as aquafeed additives. In that line, studies in different fish

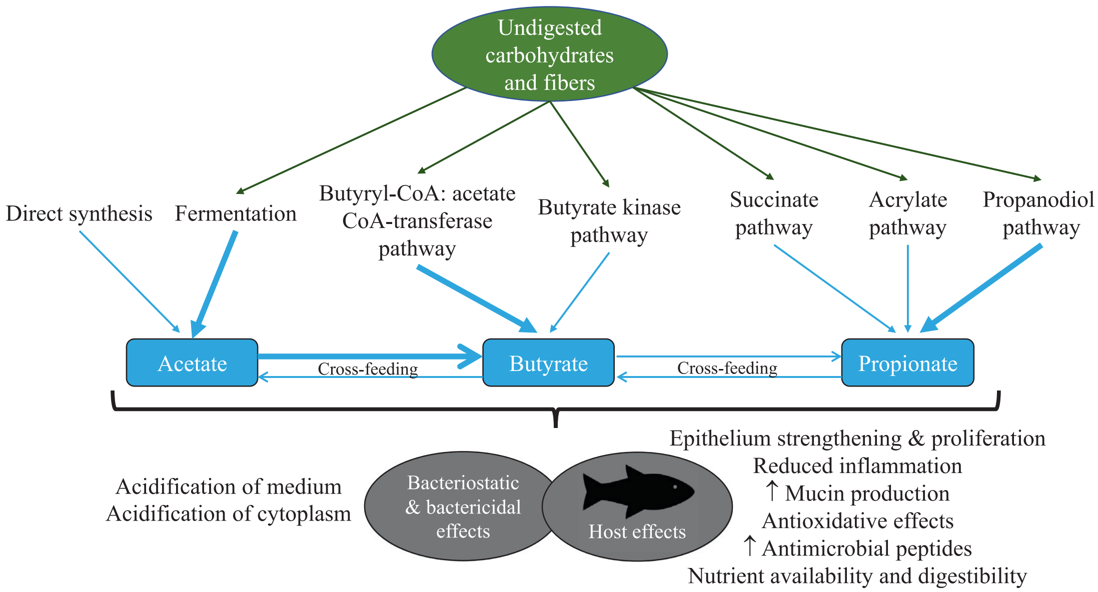

FIGURE 17.4 Short-chain fatty acids synthesis and activity. Synthesis pathways of the main SCFA produced by commensal microbiota: acetate, butyrate and propionate, and their effects on other bacteria and host physiology. Thicker arrows indicate the most common pathways.

species have been conducted and are discussed later on in this section and in Section 17.5.

17.4.2.2 *Antimicrobial effects of short-chain fatty acids*

Most bacterial species have specific requirements and will not grow at low pH conditions. SCFA are lipid-soluble weak acids that in a high pH medium dissociate releasing hydrogen ions producing direct acidification of the surrounding environment and inhibiting the growth and proliferation of acid-sensitive bacteria. However, their bacteriostatic and bactericidal effects are more effective at low pH, when they are in the undissociated lipophilic form. In this form, SCFAs are able to passively diffuse through the semipermeable membrane of gram-negative bacteria dissociating in the pH-neutral cytoplasm. The charged ions released inside the cell cannot diffuse back to the extracellular medium causing acidification of the cytoplasm which will inhibit cell enzymes causing inhibition of bacterial cell metabolism (Cherrington et al., 1991). Changes in intracellular pH can also interfere with the transmembrane pH gradient required for the active transport of nutrients inducing cellular stress, energy depletion and cell death (Salsali et al., 2008).

Due to these properties, these bacterial metabolites have been used as food preservatives to prevent deterioration caused by microbial growth (Ng and Koh, 2017). Their ability to inhibit the growth of undesired bacterial populations also makes them very attractive feed additives to be used as an alternative to antibiotics (Ng and Koh, 2017; Rimoldi et al., 2018a). Dietary supplementation with SCFA reduces the pH of the diet, but the impact on stomach or gut pH is not always significant. In trout, supplementation of acetic acid reduced gastric and cecal pH but did not reduce the pH of intestinal contents, showing that fish are able to regulate gastric acid secretion to maintain normal gut pH (Sugiura et al., 2006). Thus, these weak acids are usually found as anions rather than as undissociated forms in the neutral or slightly alkaline pH of the intestine (Yoon et al., 2018) being easily absorbed in the upper intestinal tract. In any case, several studies showed that the inclusion of SCFAs in fish and shrimp diets was still able to modulate microbial populations decreasing the presence of harmful bacteria in the intestinal tract. Supplementation with sodium butyrate showed a decrease in *Vibrio* sp. counts in Pacific white shrimp intestines (da Silva et al., 2016). In gilthead sea bream, the inclusion of sodium butyrate alleviated the dysbiosis caused by plant-based diets inducing a shift toward a more diverse and beneficial microbial population by decreasing gut abundance of *Photobacterium* sp. and increasing Firmicutes, Bacteroidetes and Fusobacteria (Piazzon et al., 2017). In the same species, supplementation of plant-based diets with a blend of propionate, butyrate and medium-chain fatty acids positively modulated intestinal microbiota by increasing lactic acid bacteria (*Lactobacillus*) and reducing Gammaproteobacteria (Rimoldi et al., 2018a). Similarly, butyrate supplementation increased *Lactobacillus* and decreased *Aeromonas* and *Escherichia coli* in the grass carp intestine (Tian et al., 2017). Further details and examples of intestinal microbial modulation by dietary SCFAs are provided in the next section.

17.4.2.3 Effects of short-chain fatty acids on nutrient availability and digestibility

The increased nutrient utilization related to the presence of SCFAs is mainly associated with their ability to lower the pH increasing enzymatic activities, digestibility and animal performance. This has been demonstrated in vitro and in vivo in different fish and shrimp models. Pacific white shrimp trypsin and chymotrypsin in vitro activities increased in the presence of sodium acetate and propionate but decreased in the presence of lactate or citrate. In zebrafish, sodium acetate was able to promote amylase activity leading to elevated energy intake (Zhang et al., 2020a). In Javanese carp (*Puntius gonionotus*), dietary supplementation with *Enterococcus faecalis* enhanced the production of intestinal butyrate and propionate with a concomitant increase in protease and lipase activities (Allameh et al., 2017).

Besides increased enzymatic activity, SCFAs are also known to increase mineral absorption. Changes in the acidity of the gut can contribute to increased solubility and transport of minerals. In addition, SCFAs act as chelating agents being able to bind various cations increasing mineral absorption. Chelation with calcium ions reduces co-precipitation between calcium and phosphates or trace elements in the brush border increasing absorption of phosphorous and other trace elements (Sugiura et al., 1998). Moreover, organic phosphorous in plant ingredients exists in the form of phytic acid or phytates, undigestible for monogastric animals. However, SCFAs are able to induce microbial phytase activity making phosphorous available for the host (Dibner and Buttin, 2002).

17.4.2.4 Other effects of short-chain fatty acids on host physiology

SCFAs and their salts are readily absorbed to the intestinal epithelia by passive diffusion or through monocarboxylate transporters where they can act as a direct source of energy or be transported to other organs where they are used as substrates to synthesize sugars or lipids or can act as signaling molecules to regulate host metabolism (He et al., 2020; Titus and Ahearn, 1992).

SCFAs, particularly butyrate, are used locally as an energy source by intestinal epithelial cells, stimulating their proliferation and differentiation, and increasing the absorptive capacity of the intestine (Estensoro et al., 2016; Guilloteau et al., 2010). These molecules have high energy value and are used in different metabolic processes for energy generation (Guilloteau et al., 2010) and as substrates in intermediary metabolism. The effects of butyrate on intestinal epithelium are the strongest, followed by acetate and then propionate. Besides being an energy source, butyrate operates as a signal metabolite regulating the balance between proliferation, differentiation and apoptosis in the epithelium and accelerates intestinal mucosa maturation during tissue development or repair (Jacobasch et al., 1999; Kotunia et al., 2004). In that line, supplementation with dietary microencapsulated sodium butyrate was able to repair intestinal mucosal damage and increase microvilli density in common carp prefed oxidized soybean oil, correlating with an improved growth performance (Liu et al., 2014). Similarly, in gilthead sea bream, supplementation with sodium butyrate in diets with extreme substitution of fish meal and fish oil contributed to counteract the intestinal damage and inflammation induced by plant-based diets in this carnivorous marine fish. These effects were detected by histology, gene expression and proteomic studies demonstrating the ability of this SCFA to modulate several aspects of the mucosal health of the host. In addition, ex vivo electrophysiology studies revealed the ability of butyrate to rescue tissue integrity (Estensoro et al., 2016; Piazzon et al., 2017). A metabolomic study in the same species showed that butyrate increases the availability of essential amino acids and nucleotide derivatives and, as it acts as an energy source for enterocytes, reduces glucose and amino acid oxidation in these cells (Robles et al., 2013). SCFAs have also been demonstrated to regulate neural system development (Yang et al., 2020), but this feature is less explored in fish.

The exact mechanisms behind all the effects described for butyrate remain to be elucidated. However, its potent effect on gene expression has been in part attributed to the inhibition of the activity of many histone deacetylases, leading to hyperacetylation of histones, modifying chromatin structure and allowing the binding of transcription factors to start gene transcription (Hamer et al., 2007). The ability of butyrate to cause histone hyperacetylation and regulate the expression of genes involved in epigenetic regulatory mechanisms was confirmed mainly in the liver, but also in the intestine, of European sea bass highlighting systemic and conserved effects in fish when compared to mammals (Terova et al., 2016).

SCFAs also have antiinflammatory and antioxidant properties and the potential to stimulate the immune system. These effects are exerted directly through histone hyperacetylation or by interaction with specific receptors such as the Hcar1/Gpr81 receptor present in mammalian and fish innate immune cells (Cholan et al., 2020; Ranganathan et al., 2018). In zebrafish, butyrate, but not acetate or propionate, reduced neutrophil recruitment and downregulated *tnf*α expression in macrophages via histone acetylation (Cholan et al., 2020). Other examples of the effect of SFCAs in immune response and antioxidant defence are presented in Table 17.4.

The interest in these bacterial metabolites to be used as dietary supplements in aquaculture is leading the research effort toward unraveling the mechanisms behind the

TABLE 17.4 Effects of different dietary short-chain fatty acids on immune and antioxidant responses in fish.

SCFA	Fish species	Effects	References
Sodium acetate	Common carp	↑ Mucus, total Igs and protein ↑ Expression of antioxidant (*gsta*, *gpx*) and immune (*tnfα*, *il1β*, *il8*, *lyz*) genes in intestine	Safari et al. (2020)
Sodium acetate + Sodium propionate	Yellowfin sea bream	↑ Serum lysozyme and ACP activity ↑ Liver catalase and glutathione peroxidase activity ↑ Intestinal *il1β* expression	Sotoudeh et al. (2020)
Sodium propionate	Zebrafish	↑ Intestinal *tnfα*, *il1β* and *lyz* expression ↓ Liver *sod*, *cat* and *hsp70* expression ↑ Lysozyme and Ig levels in skin mucus	Hoseinifar et al. (2017a); Safari et al. (2016)
	Caspian white fish	↑ Lysozyme, total Ig and ACP activity in serum and skin mucus	Hoseinifar et al. (2016b)
	Goldfish	↑ Total leukocyte count in blood ↑ Skin *lys*, *il1β*, *inos* and *tnfα* expression ↓ Parasite intensity in skin	Sheikhzadeh et al. (2021)
	European sea bass	↑ Lymphocyte and monocyte counts in blood ↑ Respiratory burst, phagocytic, MPO and bactericidal activities in blood	Wassef et al. (2020)
	Common carp	↑ Lysozyme and total Ig in skin mucus ↑ Liver antioxidant gene expression (*gsr*, *gsta*, *gpx*) ↑ Intestinal *lys*, *il1β* and *tnfα* expression	Safari et al. (2017)
Calcium or sodium propionate	Silver catfish	↑ Lymphocyte count in blood	Pereira et al. (2018)
Formic and propionic acids + calcium propionate	Nile tilapia	↑ Neutrophil and total leukocyte counts in blood ↑ Serum killing activity, lysozyme and NO ↑ Liver and kidney *tnfα* and *il1β* expression ↓ Mortality upon bacterial infection	Reda et al. (2016)
Sodium butyrate	Grass carp	↑ Intestinal lysozyme activity and amount of IgM and complement proteins ↑ Expression of intestinal antimicrobial genes ↑ Expression of intestinal antiinflammatory cytokines ↓ Expression of intestinal proinflammatory cytokines ↓ Enteritis morbidity upon bacterial infection	Tian et al. (2017)
	Common carp	Improvement of diet induced enteritis via regulation of intestinal *hsp70*, *il1β*, *tnfα* and *tgfβ* expression	Liu et al. (2014)
	Yellow catfish	↑ Resistance to ammonia challenge ↑ Intestinal activity and gene expression of Sod, Cat and Gpx ↓ Intestinal *il1*, *il8* and *tnfα* expression	Zhao et al. (2021)
	Rice field eel	↑ Serum Sod and lysozyme activities ↑ Intestinal nonspecific immune response	Zhang et al. (2020c)
	Atlantic salmon	↓ Head kidney melanomacrophage centers ↑ Immunostimulatory and complement related expression profile in head kidney	Jalili et al. (2020)

(*Continued*)

TABLE 17.4 (Continued)

SCFA	Fish species	Effects	References
	Rainbow trout	↑ Intestinal lysozyme, complement and bactericidal activities ↑ Intestinal *lyz*, *il1β*, *tgfβ*, *il10*, *sod*, *cat* and *gpx* expression ↓ Intestinal *il8* expression ↑ Survival to bacterial infection	Mirghaed et al. (2019)
	European sea bass	↑ Liver *il10* expression ↑ Intestinal *tnfα* expression	Rimoldi et al. (2016); Terova et al. (2016)
	Gilthead sea bream	↑ Survival to bacterial infection and ameliorates disease signs in parasite infections Restores immune and antioxidant responses in diet-induced enteritis model (gene and protein levels)	Benedito-Palos et al. (2016); Estensoro et al. (2016); Piazzon et al. (2017)
	Nile tilapia	↑ Survival to heat stress and bacterial infection ↑ Lysozyme, respiratory burst and phagocytic activities in blood ↑ Liver *hsp70* and *sod* expression	Abd El-Naby et al. (2019); Dawood et al. (2020)

↑ Increase; ↓ Decrease; Ig: Immunoglobulin; ACP: Alternative complement pathway; MPO: myeloperoxidase; NO: Nitric oxide; Sod: Superoxide dismutase.

beneficial activities of SCFAs on immune and antioxidant responses. For instance, an in vitro experiment with turbot head kidney macrophages showed that butyrate, propionate and acetate induced the production of antibacterial effectors and increased bacterial killing *via* Hif1α signaling, being *hif1α* expression upregulated by SCFA-induced histone acetylation (Zhang et al., 2020b). In grass carp, mRNA and microRNA profiling revealed that sodium butyrate is able to affect the intestinal immune system by regulating microRNA-mRNA interactions (Tang et al., 2021a).

17.4.3 Development of the mucosal system

Symbiotic microbial communities are established prior to the complete maturation of the host and their signals play critical roles in host developmental programs. Larval digestive tracts are morphologically and physiologically less complex than those of adult fish. Like in mammals, they start to develop in a sterile environment being colonized by bacteria at birth or hatching before active feeding starts and full development is achieved (Govoni et al., 1986; Ringø and Birkbeck, 1999). First bacteria colonizing the GI tract derive from the environment and are selected for their ability to survive within this niche (Hansen and Olafsen, 1999). Studies in mammalian and teleost model species demonstrated that intestinal microbiota is a key factor in the correct development of the GI system. The current knowledge on how intestinal microbiota affects the development and maturation of the fish GI tract comes from studies with germ-free zebrafish (Bates et al., 2006). However, since most of these features are conserved between zebrafish and mammals (Bäckhed et al., 2005; Bates et al., 2006; Hooper et al., 2001; Rawls et al., 2004), it is plausible to also assume conservation with aquaculture fish species.

In the absence of microbiota, maturation and differentiation of the GI tract in zebrafish larvae is inhibited. A lack of brush border intestinal alkaline phosphatase activity, immature patterns of glycan expression, reduced number of secretory cells (goblet and enteroendocrine cells), and defective protein macromolecule uptake were observed in these germ-free animals. Another feature detected in germ-free animals was increased intestinal motility, which was correlated with the lower numbers of serotonin-expressing enteroendocrine cells. Interestingly, all these effects were efficiently reversed when animals were colonized with microbes from conventionally reared siblings (Bates et al., 2006). Microbiota has a direct influence on intestinal cell fate specification. Epithelial cell proliferation is reduced in germ-free animals and it has been proposed that the host attempts to compensate for this outcome by extending the cell lifespans (Bates et al., 2006; Rawls et al., 2004).

Effects of the microbiota in gut differentiation occur through different pathways. Sometimes, just the presence of bacterial molecules (i.e., lipopolysaccharide (LPS)) are required to activate developmental pathways, whereas other routes depend on the interaction of the host with living and metabolically active bacterial communities. For instance, bacterial LPS is sufficient to induce alkaline phosphatase expression and activity in germ-free zebrafish intestines. This interaction is also key for the microbiota-driven mucosal immune system development as alkaline phosphatase decreases LPS toxicity and regulates homeostatic levels of immune cells in mucosal tissues (Bates et al., 2006, 2007). On the other hand, gut epithelial glycoconjugate expression patterns require specific interactions with bacterial signals (Hooper et al., 1999) (Fig. 17.5). In general, host gene expression is regulated differently by the entire microbiota or isolated bacteria, indicating a dynamic equilibrium involving continuous and active signaling (Rawls et al., 2004). Clearly, microbial regulation of host developmental programs has long-term effects on host mucosal development and health.

17.4.4 Prevention of colonization by pathogens: antagonism and barrier function

A critical function that the gut microbiota exerts for the hosts is the prevention of colonization of the mucosal surfaces by pathogenic microbes. The gut is the primary attachment site for pathogenic bacteria, which can come from either drinking water or through feeding. The gut microbes are important for host protection as they can deprive the invading pathogens by secretion of different antimicrobial substances. An example of such substances is bacteriocins, which are antimicrobial compounds produced naturally by bacteria in order to survive when in a competitive environment. Bacteriocins are small peptides or proteins, which can have different characteristics. According to their characteristics such as molecular mass, chemical properties, producer organism and mode of action, they are grouped into four categories (Nes et al., 2007). In the fish gut, several types of bacteria have been reported to produce bacteriocins (Sahoo et al., 2016; Vijayabaskar and Somasundaram, 2008), such as *Vibrio mediterranei* (Carraturo et al., 2006), that could actively kill several fish pathogens related to septicemia, hemorrhagic septicemia and ulcers caused by *Vibrio* sp. and *Aeromonas hydrophila*. *Lactobacillus* spp., with a common species *L. plantarum*, are the most studied fish gut

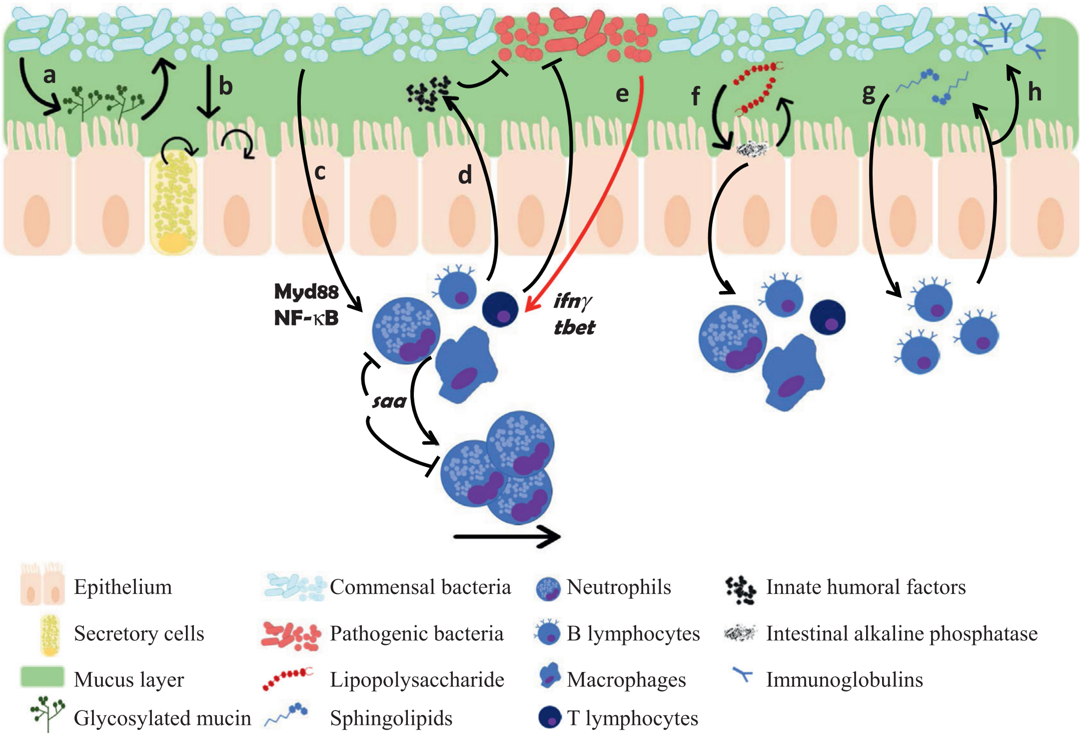

FIGURE 17.5 Host−microbe interactions regulate mucosal tissue and immune system development. Schematic representation of some of the main mechanisms regulating mucosal tissue development and microbial-immune system interactions in the intestine. Mucin expression and glycosylation patterns are regulated by commensal bacteria, while at the same time, these patterns regulate the adherence of certain bacterial taxa (a). Commensal bacteria are key for epithelial and secretory cell proliferation, differentiation and maturation (b). Microbial colonization activates Myd88 and NF-κB pathways in immune cells increasing the expression of serum amyloid A (*saa*). Saa increases systemic neutrophil numbers and migration and suppresses the expression of inflammatory molecules during homeostasis (c). Mucosal immune cells secrete humoral factors to control microbial populations in mucosal surfaces (d). The presence of microbiota is needed to enhance the proinflammatory response to control pathogen invasion (e). LPS induces alkaline phosphatase expression and activity. In turn, alkaline phosphatase decreases LPS toxicity and regulates immune cell homeostasis (f). Sphingolipids induce systemic modulation of IgM^{+} and IgT^{+} B lymphocytes (g). Most bacteria present at mucosal surfaces are coated with antibodies (mainly IgT) as a means of regulating microbial populations and interactions with the host's immune system (h).

bacteria, that produce a nisin-like bacteriocin and inhibit pathogenic bacteria (Vijayabaskar and Somasundaram, 2008). Moreover, the *Bacillus subtilis* and *Lactococcus lactis* have been also reported as beneficial taxa, and have been developed into fish probiotics (Balcázar et al., 2008; Denev et al., 2009; Ghosh et al., 2007). Probiotic bacteria may attach on the mucosal surface of the gut, and although not all may colonize, they can exert beneficial effects in the mucosal microbial communities in several ways such as inhibiting the attachment site for pathogens, secreting antimicrobial proteins or bacteriocins, or enhancing the beneficial commensal microbiota (Ibrahem, 2015; MacKenzie et al., 2010; Ray et al., 2012).

Several mechanisms exist that allow the adhesion of microbes on the mucosal surfaces of the host, some of them are reported in Section 17.3. To know better how commensal pathogens and hosts interact, more studies are required to understand the dynamics of those interactions. A good tool to examine the association of bacteria in the gut is the electron microscope (scanning or transmission). Several studies used this tool to understand the mode of association of bacteria in the GI and how bacteria translocate (Ghosh et al., 2010; Hellberg and Bjerkås, 2000; Lødemel et al., 2001; Ringø and Olsen, 1999). Since the mucosal intestinal layer has a critical role in bacterial attachment, as well as makes the protection barrier, understanding bacterial adhesion or bacterial translocation and the interaction with the immune system is important. Although several studies looked into the mode of association of bacteria on the gut surface, the mechanism is still not fully understood.

17.4.5 Immunologic effects of gut microbiota

The immune system of vertebrates, from fish to mammals, is constituted by the innate and adaptive branches. The first line of defense relies on the innate immune system that rapidly detects and eliminates pathogens in a nonspecific manner. In addition, the adaptive immune system, key for the existence of immunological memory, utilizes somatic recombination of germ-line encoded V-D-J fragments to produce a vast repertoire of antigen-specific receptors expressed by B and T lymphocytes. Although very similar to mammals, teleost fish present some unique characteristics in their immune system (e.g., absence of germinal centers or differences in immunoglobulin isotype repertoire) (Kelly and Salinas, 2017). Moreover, some teleost species show specific characteristics that challenge the current dogma on the functioning of the vertebrate immune system derived from mammals (i.e. lack of MHC-II and CD4 T cell-related molecules in Gadoid fish) (Malmstrøm et al., 2016).

Intestinal microbiota interacts directly with the mucus layer covering mucosal surfaces. This mucus layer has specific physicochemical characteristics and contains immunologically important molecules that shape the composition of commensal microbial communities. Commensal microorganisms have coevolved with the host's mucosal and systemic immune responses, both players having direct consequences on the development and homeostatic states of the other (Kaetzel, 2014; Kelly and Salinas, 2017; Murdoch and Rawls, 2019) (Fig. 17.5).

17.4.5.1 Commensal microbiota influences the development and homeostasis of the host's immune system

Commensal microbiota is key for the correct development of the host's immune system and for maintaining a natural state of activity to keep this defence system functioning correctly. The importance of commensal microbes for the development of the innate immune system has been demonstrated using germ-free zebrafish larvae. Germ-free zebrafish showed impaired neutrophil migration, decreased resistance to viral infection and altered expression of innate immune genes (Galindo-Villegas et al., 2012; Kanther et al., 2014; Rawls et al., 2004). Colonization with natural microbiota was enough to revert these effects. The development of the immune system and mucosal epithelium and their interactions with commensal bacteria are actually correlated. In fact, activation of Myd88 in innate immune cells by microbial proliferation in mucosal surfaces is key for the aforementioned development of intestinal epithelial cells (Cheesman et al., 2011). The crosstalk between intestinal microbiota and the immune system also has systemic implications. Colonization with a normal microbiota in zebrafish results in Myd88- and NF-κB-dependent induction of serum amyloid A (Saa) (Kanther et al., 2011) which is required to increase systemic neutrophil numbers and migration and suppress the expression of inflammatory biomarkers (Kanther et al., 2014). Thus, the presence of a healthy microbiota is needed for systemic neutrophil function and development. Not surprisingly, the occurrence of different types of bacteria has different effects on the state of the host's immune system. Zebrafish mono-associated with *Aeromonas hydrophila* showed expression levels of *saa* and *C3* comparable to conventionally reared fish, indicating that in this case, a single microbial species is enough to induce immunostimulatory effects (Rawls et al., 2004). Another experiment showed that fish associated with *Vibrio* and, in much lower proportion, with *Shewanella* had a significantly reduced number of neutrophils when compared to fish mono-associated with *Vibrio* (Rolig et al., 2015). The latter result highlights that microbial effects on host physiology are not always proportional to taxa abundance.

Beneficial bacteria stimulate the nonspecific immune system of the host helping modulate host immune responses to pathogens. In fact, gut bacteria are necessary to enhance the proinflammatory response during intestinal inflammation. When compared to conventionally reared animals, germ-free zebrafish larvae showed higher resistance to chemically induced enterocolitis due to lower activation of TLR, MyD88 and NF-κB signaling pathways and a consequent lower expression of proinflammatory cytokines (He et al., 2014). Similarly, exposure to *Aeromonas* sp. or *Vibrio* sp. activated TLR and MyD88 pathways and increased expression of innate proinflammatory genes only when zebrafish had normal intestinal microbiota. This response was absent in germ-free animals indicating that host-microbiota interactions are indispensable for activating the inflammatory response when needed (Xin et al., 2020).

The mechanisms behind microbial regulation of the host's innate immune system are being studied using gnotobiotic fish larvae. However, the adaptive immune system is still not fully developed in these stages and it is technically difficult to maintain a germ-free status after a few days posthatching. Although some new models that will allow to study the effect of microbiota on adaptive immune system development in older fish have been produced (Schaeck et al., 2016), there is still an important knowledge gap to be addressed here. From mammals, we know that germ-free animals present fewer, smaller and underdeveloped Peyer's patches with very few T or B lymphocytes and a defective adaptive humoral response (Smith et al., 2007). Colonization with commensal bacteria is often enough to induce a normal development of mucosal adaptive immune responses. Defective development of adaptive immune responses is also observed systemically. In germ-free animals, central lymphoid structures are hypoplastic with reduced numbers of B and T lymphocytes (Smith et al., 2007). Clearly, commensal bacteria are absent in systemic lymphoid tissues. However, microbial molecules that are absorbed into the body are the ones responsible for the systemic effects. This was demonstrated when purified PSA from *Bacteroides fragilis* was given to germ-free mice and corrected T cell systemic deficiencies and imbalances (Mazmanian et al., 2005). In rainbow trout, sphingolipids from the mucosal commensal *Flectobacillus major* induced modulation of systemic IgM^+ and IgT^+ B cells (Sepahi et al., 2016). Oral administration of PHB from *Bateroides thuringensis* in Nile tilapia increased serum antibody titers and systemic innate immune parameters (Suguna et al., 2014).

The exact mechanisms behind microbial regulation of the host's immune system development are still under study in both, fish and mammals. In any case, it is clear that for the optimal development and functioning of this defense system, healthy populations of commensal microbes and their products are essential.

17.4.5.2 The host's immune system shapes commensal microbiota

On its side, the host's immune system is key for reshaping and maintaining a healthy microbiota. While symbionts must suppress or evade the host's defences to get established in mucosal niches, the host's immune system has developed mechanisms to remain tolerant to the natural diversity of symbionts and, at the same time, prevent infection by pathogenic or opportunistic microbes. Mucosal secretions contain several innate humoral components such as antimicrobial peptides, lysozyme, complement components, transferrin or lectins that help maintain microbial homeostasis (Gomez et al., 2013). Antimicrobial peptides are very diverse in teleosts and are able to inhibit several opportunistic pathogens like some *Vibrio*, *Aeromonas* or *Streptococcus* species (Masso-Silva and Diamond, 2014). Feeding grouper with the antimicrobial peptide hepcidin 2−3 from tilapia increased intestinal microbiota diversity and microbial metabolic activity (Ting et al., 2019). Fish upregulate the expression levels of lysozyme, complement, lectin or transferrin genes in mucosal tissues to control infection with opportunistic bacteria (Arasu et al., 2013; Fu et al., 2019; Gao et al., 2016; Poochai et al., 2014). Complex interactions have been found to maintain host-microbe equilibria in mucosal tissues. For instance, *irf8* deficient zebrafish showed a significant loss of intestinal macrophages and a severe reduction in the expression of complement genes, which was correlated with intestinal dysbiosis. Restoration of macrophage *irf8* expression prevented microbial destabilization (Earley et al., 2018). Besides macrophages, other innate immune cells, such as dendritic cells or granulocytes, are also important in shaping intestinal microbiota (reviewed in Yu et al., 2021).

Innate immune responses shape microbial populations according to unspecific signals and broad-spectrum modulatory molecules. On the other hand, the adaptive immune system is able to specifically recognize beneficial or pathogenic bacteria and react accordingly inducing tolerance or responsiveness (Feng and Elson, 2011). All vertebrates have mucosa-specialized immunoglobulin isotypes with important roles in immune exclusion that regulate the composition of commensal bacteria and protect the host from invading pathogens. In fish, this specialized isotype is the teleost-specific IgT (Zhang et al., 2010). Most bacteria present at fish mucosal surfaces are coated with antibodies (mainly IgT) and free secretory components neutralizing adhesion and limiting access to the host (Kelly et al., 2017; Zhang et al., 2010). Indeed, IgT is required for microbiota colonization, anchoring and containment within the mucosal layer. In rainbow trout, IgT depletion caused dysbiosis, loss of IgT-coated beneficial taxa and translocation of bacteria into the gill tissue inducing an increase in circulating LPS (Xu et al., 2020). To a lesser degree, IgM also plays important roles in regulating host-associated microbiota, being able to inhibit growth of gram-positive and -negative bacteria (Tsutsui et al., 2013). The biological role of IgD is still unclear. However, IgD can bind commensal bacteria influencing adhesion to the mucosa and its transcription is affected by microbiota. These correlated findings suggest that IgD can also play an important role maintaining mucosal homeostasis in fish (Perdiguero et al., 2019).

T lymphocytes-mediated inflammatory responses are also important in shaping the microbiota. During the initial phases of development, before the adaptive immune system is fully functional, zebrafish microbiota is characterized by an overgrowth of *Vibrio* species. This high abundance of *Vibrio* is maintained in Rag1-deficient zebrafish (B and T cell deficiency) when compared to wild-type animals. Adoptive transfer of T lymphocytes, but not B lymphocytes, was able to reduce the amount of *Vibrio* to homeostatic levels, indicating that T cells are important in controlling microbiota composition (Brugman et al., 2014). Interestingly, zebrafish T cells significantly increased the expression of *ifnγ* when exposed to microbiota from Rag1-deficient fish. Upregulation of *ifnγ* in the intestine is a common feature of bacterial infection in several fish species (Chen et al., 2015; Evenhuis and Cleveland, 2012; Kumari et al., 2015), and although specific expression by T cells was not demonstrated in the latter experiments, other Th1 cell markers, like the transcription factor *tbet*, were found upregulated in Atlantic salmon intestine in response to pathogenic bacteria (Kumari et al., 2015). Therefore, Th1 cells are very likely involved in the shaping of microbial populations in the fish gut. The interaction of other subsets of T lymphocytes with fish intestinal microbiota remains to be studied, but some research points to a probable role of different T cell subsets in microbial modulation. For instance, the intestinal microbiota of Il17a/f1-deficient medaka (*Oryzias latipes*) is significantly different from that of wild-type fish, hinting that Th17 responses can also be involved in the maintenance of intestinal microbiota (Okamura et al., 2020). Further studies are needed to demonstrate which types of lymphocytes interact with intestinal microbiota and how they are able to modulate different bacterial populations.

17.4.6 Role of microbiota in nervous system development and maintenance

The microbiota-gut-brain axis is a bidirectional cascade in which the central nervous system regulates intestinal microbiota, while signals from the microbiota or the intestine affect the development and functioning of the central nervous system (Lynch and Pedersen, 2016). Microbial metabolites, cytokines produced by intestinal immune cells, or the direct connection between the brainstem and the intestine *via* the vagus nerve mediate the actions of this axis (Sampson and Mazmanian, 2015). Studies performed in mammals demonstrated that intestinal microbiota is required for nervous system homeostasis, and dysbiosis has been associated with neurodevelopmental and neurodegenerative diseases that were also correlated with aberrant systemic inflammatory responses (Erny et al., 2015; Morais et al., 2021).

Our knowledge about the microbiota-gut-brain axis in fish derives from zebrafish studies, as this model has emerged as a very attractive alternative to study these interactions in biomedical research (reviewed in Lee et al., 2021). The zebrafish model revealed that microbial colonization at early life stages is required for normal neurobehavioural development. Germ-free zebrafish show altered locomotor and stress-related responses (Davis et al., 2016a). Locomotor hyperactivity was successfully ameliorated after microbial colonization in the first six days postfertilization, but not at later time points (Phelps et al., 2017). The same study demonstrated that monocolonization with *Aeromonas* or *Vibrio* was enough to achieve normal locomotor activities, but exposure to heat-killed bacteria or microbial-associated molecular patterns did not block hyperactivity, demonstrating that living bacteria are key for normal locomotor functions. In addition, administration of probiotics influenced brain gene expression patterns, changed shoaling behavior, attenuated anxiety-related responses, restored GABAergic and serotonergic signaling in the brain, and protected against stress-induced dysbiosis in conventionally reared zebrafish (Borrelli et al., 2016; Davis et al., 2016a, 2016b).

Microbial compounds are also able to influence feeding behavior by directly reaching the brain *via* blood circulation, by activating vagal terminals or modulating the release of appetite-regulating peptides in the gut (reviewed in Butt and Volkoff, 2019). Moreover, gut microbiota can affect fish reproductive behavior and gonad development (Avella et al., 2012; Carnevali et al., 2013; Ghosh et al., 2007; Mehdinejad et al., 2019). The exact mechanisms regulating the microbiota-gut-brain axis in fish need to be further elucidated in future studies.

17.5 Dietary factors affecting the gut microbiota

The beneficial effects of gut microbiota in host physiology and the tight interplay between intestinal microbes and the macroscopic host, where imbalances in either side can cause important health problems, make microbiota one of the main variables to take into consideration when aiming for animal fitness in aquaculture. Among the different factors affecting gut microbiota as they have been discussed in Sections 17.2 and 17.3, diet is one of the most important. Certainly, the GI tract of marine and freshwater fish, or carnivorous and herbivorous fish present important physiological differences allowing adaptation to different environments and dietary regimes. For instance, carnivorous fish have short GI tracts typically shorter than body length, whereas herbivores can have GI tracts that measure even more than 20 times the body length (Ray and Ringø, 2014). Within the same fish species, the characteristics of the GI tract shift to adapt to changes in functional demands, like different life stages, diet changes, seasons or environments (Ringø et al., 2016). This plasticity is reflected in alterations of digestive enzymes within the gut content of salmonids and sparids upon incorporation of plant material in the diet (Romarheim et al., 2006; Santigosa et al., 2008). Gilthead sea bream can increase the intestinal length and morphology and change liver and adipose tissue metabolism to adapt to plant-based diets avoiding detrimental effects on growth (Menoyo et al., 2004; Monge-Ortiz et al., 2016; Perera et al., 2019).

The "you are what you eat" principle applies not only to the host but also to the resident microbes that utilize the nutrients of the food ingested by the host. Therefore, different dietary ingredients will cause changes directly in the commensal populations inhabiting the fish GI tract or indirectly through modulation of host factors that will eventually modulate associated microbe communities. In this section, we will review how and to which degree different dietary components or feed formulations affect microbial structures in the intestines of aquaculture species. We will focus on the effect of some of the most studied alternative diet formulations. Detailed reviews on the microbial changes driven by different nutritional sources can be found elsewhere (Kononova et al., 2019; Ringø et al., 2016). However, it is important to note that a certain degree of microbial stability has been observed in fish and mammals, mostly reflected on conserved microbial structures mainly at high taxonomic levels. A clear demonstration of this was performed when germ-free zebrafish were transplanted with mice microbiota and *vice versa*. In both cases, these transplanted microbial communities changed in the direction of the typical microbiota observed for each species (Rawls et al., 2006). Interestingly, microbial communities of genetically selected gilthead sea bream, that showed a high degree of intestinal plasticity, were able to metabolically adapt to diet changes without major shifts in taxa composition, whereas the opposite was observed in nonselected animals (Piazzon et al., 2020). Hence, it is clear that when conditions are good, host and resident microbes are able to achieve an equilibrium that can withstand a certain degree of environmental or dietary changes.

Due to the growth of aquaculture production the demand for fish meal (FM) and fish oil (FO) has increased. These ingredients have long been used in different types of animal feeds and are considered the gold standard in aquafeed formulations. However, this high dependence on wild capture resources has become economically and environmentally unsustainable, making the search for more sustainable diet formulations to meet the global demand for fish products a priority in the field. Aquafeeds for herbivorous and omnivorous aquaculture species have easily transitioned to feeds containing little to no FM and FO. Yet, this has been more difficult to implement for carnivorous fish and crustaceans (Turchini et al., 2019). The most obvious alternatives for FM and FO are plant ingredients, and plenty of research has been conducted in that direction (Gatlin et al., 2007; Ghosh and Ray, 2017; Hardy, 2010). But other alternative materials, such as animal by-products, insect proteins, single-cell proteins or algae proteins and oils (Ferrer Llagostera et al., 2019; Glencross et al., 2020b; Nogales-Mérida et al., 2019; Révész and Biró, 2019; Turchini et al., 2019) are also entering the formulator's agendas. The overarching goal of all this research is to achieve well-balanced formulations that, besides being sustainable and economically viable, maintain animal growth and health. In that respect, the possible effects on the host's microbiota is a key factor to consider when evaluating the suitability of alternative formulations to be used in aquaculture, and thus there has been an increasing number of studies focusing on this aspect during the past decade (Kokou et al., 2022; Table 17.5).

17.5.1 Effects of plant-based diets on gut microbiota

The use of plant feedstuffs to replace FM and FO in aquafeeds has been a hot subject of research for more than 20 years (Gatlin et al., 2007). However, just like with any other alternative source, special care has to be taken for all nutritional requirements to be met for each target species. In particular, plant ingredients have a higher carbohydrate content, plant proteins (PPs) have a different amino acid profile being low in methionine, and vegetable oils (VOs) are deficient in essential long-chain polyunsaturated fatty acids (LC-PUFAs). In addition, plant ingredients can contain antinutritional factors. These differences not only affect fish growth, health and intestinal morphology, but they can also modify the composition of the intestinal microbiota (Ringø et al., 2016; Zhou et al., 2018b).

TABLE 17.5 Recent studies that investigated the effect of diet on fish gut microbiota.

Dietary component used	Fish species	References
Two dietary lipid levels	Gilthead sea bream	Pelusio et al. (2021)
Essential oils	Gilthead sea bream	Firmino et al. (2021)
Soybean meal	Northern snakehead	Miao et al. (2018)
Soybean meal	Zebrafish	Patula et al. (2021)
Isonitrogenous and isoenergetic Diets containing faba bean extracts	Grass carp	Ma et al. (2022)
Plant-based diets	Rainbow trout	Pérez-Pascual et al. (2021a)
Fish-based versus plant ingredients	Rainbow trout	Gatesoupe et al. (2018)
Processed animal proteins	Gilthead sea bream	Solé-Jiménez et al. (2021)
Safflower	Common carp	Meng et al. (2022)
Oil from Camelina sativa	Gilthead sea bream	Huyben et al. (2020)
Microencapsulated organic acids	European sea bass	Busti et al. (2020)
Sodium butyrate	Hybrid grouper	Yin et al. (2021)
Chitosan	Silver barb	Salam et al. (2021b)
Hydrolysis lignin	Atlantic salmon	Colombo et al. (2020)
Insect meal from *Hermetia illucens*	Rainbow trout	Gaudioso et al. (2021); Rimoldi et al. (2019); Terova et al. (2019)
	Atlantic salmon	Weththasinghe et al. (2022)
	Dicentrarchus labrax and Gilthead sea bream	Panteli et al. (2021)
Insect meal	Atlantic salmon	Li et al. (2022)
Fucoidan	Zebrafish	Ikeda-Ohtsubo et al. (2020)
Phytonutrient-supplemented diets	Common carp	Feher et al. (2021)
Titanium dioxide nanoparticles	Nile tilapia	Sherif et al. (2021)
Yeast (*Cyberlindnera jadinii*)	Atlantic salmon	Leeper et al. (2022)
Postbiotic	Rainbow trout	Pérez-Sánchez et al. (2020)

Notes: Gilthead sea bream – Sparus aurata; Northern snakehead - Channa argus; Zebrafish – Danio rerio; Grass carp – Ctenopharyngodon idellus; Rainbow trout – Oncorhynchus mykiss; Common carp – Cyprinus carpio; European sea bass - Dicentrarchus labrax; Hybrid grouper – Epinephelus fuscoguttatus ♀ x E. lanceolatus ♂; Silver barb – Barbonymus gonionotus; Atlantic salmon – Salmo salar; Nile tilapia - Oreochromis niloticus.

17.5.1.1 Effects of vegetable oils on fish intestinal microbiota

Dietary fatty acids modify GI microbiota possibly by affecting adherent bacterial attachment sites. In gilthead sea bream, plant-based diets balanced with different dietary ARA/EPA/DHA ratios differentially modulated adherent bacterial communities (Magalhães et al., 2020). It has been demonstrated that the beneficial lactic acid bacteria are correlated with diets containing adequate levels of LC-PUFA, whereas their abundance decreases with VO diets (Ringø et al., 1998). In agreement, complete replacement of FO with soybean oil or linseed oil in golden pompano (*Trachinotus blochii*) diets increased the abundance of pathogenic bacteria and decreased lactic acid bacteria in allochthonous bacterial communities, impacted intestinal enzymatic activity, epithelial integrity and induced inflammation (You et al., 2019). In rainbow trout, 50% replacement of FO with rapeseed oil induced a decrease in fecal bacterial diversity and a drastic decrease in the phylum Bacteroidetes which was also associated with the genetic background of the host (Navarrete et al., 2012). On the contrary, the complete substitution of FO with rapeseed, linseed and palm oils did not significantly affect the composition of metabolically active adherent intestinal microbial communities in first-feeding Atlantic salmon. Among the few differences, it was highlighted that Atlantic salmon-fed VO diets were dominated by the Bacteroidetes *Cloacibacterium normanense*, however, the overall abundance of the phylum Bacteroidetes was more related to the age of the animals than to the diet (Nikouli et al., 2021). In European sea bass, plant-based diets with decreasing levels of FM and FO significantly modulated autochthonous and allochthonous microbial populations with increasing abundance of *Clostridium*, *Corynebacterium*, and *Staphylococcus*, and decreasing presence of *Enterococcus* and *Streptococcus* at higher substitution levels. Interestingly, the abundance of some of these taxa returned to the levels expected in an FM/FO diet when diets were supplemented with LC-PUFA from alternative sources, indicating the importance of dietary fatty acid composition on microbiota architecture (Torrecillas et al., 2017). In Pacific white shrimp fed with soybean oil as a main lipid source, a significant increase of *Aeromonadaceae* and *Enterobacteriaceae* was detected in the microbiota of the intestinal contents (Zhang et al., 2014).

In gilthead sea bream, several studies addressed the effect of FO substitution on intestinal bacteria. Complete FO replacement with palm, linseed and rapeseed oil induced different allochthonous microbial profiles but no differences in diversity or richness were detected (Castro et al., 2019). FO replacement with 60% camelia oil lowered the abundance of lactic acid bacteria and increased, among others, the abundance of *Corynebacterium* in adherent bacterial populations. Functional inference associated the increased *Corynebacterium* with the fatty acid synthesis pathway, which was proposed as an attempt to compensate for the lack of fatty acids in the diet (Huyben et al., 2020). Plant-based diets with increasing replacement of FO with a blend of plant oils induced a dose-dependent decrease of adherent bacterial diversity led by a remarkable increase in *Photobacterium* abundance (Piazzon et al., 2017). Untargeted serum metabolomics using similar diets in the same species revealed significant changes in microbial-derived metabolites such as vitamins (Gil-Solsona et al., 2019). To date, no direct link between specific bacterial taxa and serum levels of microbial metabolites has been established in this species but, in rainbow trout fed plant-based diets, significant correlations between levels of plasma markers and intestinal bacteria have been described (Gatesoupe et al., 2018), demonstrating that microbial changes driven by diet have a systemic effect on the host. High VO replacement with rapeseed oil in low FM diets decreased *Staphylococcaceae* and *Lactobacillaceae* and increased *Streptococcaceae* abundance in allochthonous bacterial communities of gilthead sea bream. These differences were also dependent on rearing density, demonstrating the interaction among different factors in shaping the microbiota (Parma et al., 2020). In fact, low FM diets with high substitution of FO with rapeseed oil significantly affected the adherent microbiota of gilthead sea bream, but the changes were highly dependent on the genetic background of the host. In this sense, gilthead sea bream families selected for high growth showed fewer changes in bacterial composition that were associated with more metabolic differences in these populations. On the other hand, slow-growth families significantly changed the intestinal microbiota when fed the low FM/FO diet but these changes were not reflected in the functional analysis performed by inference and by metatranscriptomic analysis (Naya-Català et al., 2022; Piazzon et al., 2020).

The different effects of FO replacement on intestinal microbiota described in the different studies can be attributed to differences between species, sample type (autochthonous or allochthonous populations), or fatty acid profile and source of the diet, among other variables. However, as a general trend, it seems that the substitution of FO with VO induces a decrease in microbial diversity, can favor the abundance of opportunistic pathogens, and decrease the presence of lactic acid bacteria. The mechanisms by which these microbial changes can affect host health need to be studied. In this line, the use of the zebrafish model demonstrated that intestinal microbiota plays a significant role in the hepatotoxicity of palmitic acid, highly abundant in VO. Palmitic acid-altered microbiota transferred to germ-free zebrafish induced endoplasmic reticulum stress and liver damage (Ding et al., 2018). Although the exact molecular mechanisms remain to be determined, this study highlights that manipulation of gut microbiota can be a suitable strategy for addressing health problems derived from alternative diet ingredients. In agreement, supplementation of low FM and FO diets with sodium butyrate in gilthead sea bream alleviated gut dysbiosis which was correlated with an improvement in disease resistance, inflammatory signs, gut integrity and mucosal proteome (Piazzon et al., 2017).

17.5.1.2 Effects of plant proteins on fish intestinal microbiota

There has been extensive research on the modulation of fish gut microbiota by plant proteins. For instance, the effect of soybean meal on intestinal bacteria has been explored for more than 10 years and has been thoroughly reviewed elsewhere (Kononova et al., 2019; Merrifield et al., 2011; Ringø et al., 2016; Zhou et al., 2018b). Plant proteins as alternative protein sources demonstrated to affect gut bacterial communities in fish (Table 17.6). In this table, we show a compilation of several studies performed on gut microbiota effects of different plant protein sources in different fish species. The variability of the

TABLE 17.6 Effects of plant proteins on fish intestinal bacteria.

Fish species	PP source	Type[1]	Significant results[2]	References
Rainbow trout	Soybean meal	TOT	↓ *Lactobacillus*, *Sphingomonas* ↑ *Bacillus*, *Chryseomonas*	Heikkinen et al. (2006)
Rainbow trout	Soybean meal	AUT & ALLOC	↑ *Psychrobacter* ↓ *Aeromonas*, *Vibrio*	Merrifield et al. (2009)
Rainbow trout	Not specified	ALLOC	↑ Firmicutes (lactic acid bacteria, *Streptococcus*, *Leuconostocaceae*) ↓ Proteobacteria	Pérez-Pascual et al. (2021a)
Rainbow trout	Pea, Soybean, Canola. Meal and concentrates	ALLOC	↑ Firmicutes:Proteobacteria ratio ↑ Effects with meals than concentrates	Desai et al. (2012)
Atlantic salmon (freshwater)	Mixed nut meal, corn protein concentrate	TOT	↑ *Lactobacillus* ↓ *Streptococcus*	Schmidt et al. (2016)
Atlantic salmon (freshwater)	Soy meal concentrate	ALLOC	↑ *Aeromonas*, *Devosia* ↓ Lactic acid bacteria, *Bacteroidaceae* ↓ Diversity	Egerton et al. (2020)
Atlantic salmon (freshwater)	Soybean meal and fermented soybean meal	ALLOC	↑ *Lactobacillus*, *Pediococcus* (fermented) ↑ *Acinetobacter* (soybean meal) ↓ *Cellulomonas*, *Clostridium* (both)	Catalán et al. (2018)
European sea bass	Plant-based diet FM and FO free	ALLOC	↑ *Sphingomonas*, *Staphylococcus*. But not significant trend due to high interindividual variability	Pérez-Pascual et al. (2020)
European sea bass	Soybean, rapeseed, sunflower meals	AUT & ALLOC	↓ Richness and Acidobacteria in ALLOC ↑ *Bacillaceae*, *Clostridiaceae*, *Propionibacterium* in ALLOC ↑ *Pseudomonadaceae* and *Ralstonia* in AUT	Serra et al. (2021)
Gilthead sea bream	Rapeseed or lupin meal	ALLOC	↔ *Vibrio* ↑ *Photobacterium* spp. ↓ *Photobacterium damselae*	Silva et al. (2011)
Gilthead sea bream	Wheat gluten, bean, soybean, pea and sunflower meal	ALLOC	↔ Diversity ↑ Proteobacteria ↓ Firmicutes, Actinobacteria ↑ *Photobacterium* ↓ *Streptococcus*, *Lactobacillus*, *Staphylococcus*	Estruch et al. (2015)
Hybrid striped bass	Modified bioprocessed soybean meals	ALLOC	↓ Diversity ↑ *Lactococcus*, *Plesiomonas*, *Ralstonia*	Fowler et al. (2021)
Common carp	Ultra-micro ground mixed plan proteins	ALLOC	↓ Diversity ↓ Fusobacteria, *Cetobacterium* ↑ *Shewanella*, *Citrobacter*	Xie et al. (2021a)
Amur sturgeon	Soybean and cottonseed protein concentrates	TOT	↓ Diversity ↑ *Clostridium*, *Plesiomonas* ↓ *Turicibacter*	Wei et al. (2020)
Turbot	Soybean meal and fermented soybean meal	ALLOC	Fermented ↔ Control diet ↑ *Lactobacillus* and *Faecalibaculum* (fermented) ↑ *Vibrio* (soybean meal)	Li et al. (2020)
Olive flounder	Defatted soybean meal, corn gluten meal, corn concentrate	ALLOC	↔ Richness ↑ *Lactobacillus*, *Photobacterium* ↓ *Prevotella*, *Paraprevotella*	Niu et al. (2020)

↑ Increase; ↓ Decrease; ↔ No change.
[1]*Type of sample taken for microbiota analysis: AUT (autochthonous), ALLOC (allochthonous) or both (TOT).*
[2]*Main differences found when comparing to commercial diets with high levels of FM.*

results is astonishing and is difficult to extract specific trends. Even within the same fish species, contradictory results are found, for example, increased/decreased amounts of lactic acid bacteria (Egerton et al., 2020; Heikkinen et al., 2006; Pérez-Pascual et al., 2021a; Schmidt et al., 2016). These differences can be due to many variables, but the different sources and processing of the plant proteins could play a major role. Different properties of the plant-based diets can be a source of this variability. Soybean meal, for instance, together with a high protein content, can contain up to 30%−50% of carbohydrates, mainly nonstarch PSAs and free sugars and minor amounts of starch (Choct et al., 2010). Bacteria possessing extracellular enzyme systems to degrade PSAs, such as Bacteroidetes (Cockburn and Koropatkin, 2016), can be favored by these ingredients in the diet. In agreement, an enrichment of carbohydrolytic bacteria was detected in the intestine of European sea bass fed plant-based diets (Serra et al., 2019). In addition, soluble pectins present in plant meals can increase the viscosity of the digesta slowing down its movement and decreasing oxygen availability, thus contributing to the increase of anaerobic microbiota and affecting the growth of aerobics such as *Aeromonas* or *Pseudomonas* (Choct et al., 2010; Kononova et al., 2019). Finally, carnivorous fish seem to be more susceptible than terrestrial animals to antinutritional factors present in plant ingredients (trypsin inhibitors, saponins, lectins, oligosaccharides, and phytates) (Krogdahl et al., 2010).

Protein concentrate forms have been used as an alternative to reduce the impact of plant ingredients on intestinal microbiota. Indeed, in rainbow trout, the use of plant concentrates showed a lower effect on intestinal microbiota than that observed with plant meals (Desai et al., 2012). However, protein concentrates still contain levels of antinutrient factors that impair high levels of substitution (Kononova et al., 2019). A different alternative that has recently become more popular is the use of fermented plant meals or proteins (Dawood and Koshio, 2020). Feeding Atlantic salmon with fermented soybean meal-induced an increase in lactic acid bacteria and a decrease in inflammatory cytokine expression in the gut when compared to animals fed nonfermented plant proteins (Catalán et al., 2018). In turbot, *Enterococcus faecium*-fermented soybean meal enhanced the abundance of probiotic and antiinflammatory bacteria and inhibited the growth of *Vibrio* sp. The opposite was found in turbot-fed nonfermented soybean meal (Li et al., 2020).

Interestingly, some studies revealed that feeding plant-based diets increased the levels of gut commensal yeast, probably as a direct result of the higher presence of fermentable carbohydrates in these ingredients. This was demonstrated by a significantly higher abundance of culturable yeast (*Saccharomyces*) in the autochthonous and allochthonous gut microbiota of rainbow trout-fed soybean meal diets (Merrifield et al., 2009). Metatranscriptomic analysis of gilthead sea bream microbiota fed plant-based diets indeed showed that yeast transcripts in the intestinal autochthonous microbiota significantly increased in abundance reaching even higher levels than bacterial transcripts (Naya-Català et al., 2022). All these results demonstrate the importance of commensal Fungi in gut microbial communities, however, these populations remain largely unexplored as the vast majority of the studies are centered on bacteria.

17.5.2 Effects of diets containing animal by-products on gut microbiota

Since 2013, the ban impeding the use of animal by-products in aquafeeds has been lifted, so these protein sources are now considered as a possibility to reduce FM, mainly by partially or totally substituting plant proteins from alternative formulations to avoid the undesired effects of these ingredients (Glencross et al., 2020a). Unlike plant proteins, animal proteins are rich in most essential amino acids, are balanced in fatty acids, vitamins and minerals, have fewer carbohydrates, are highly digestible and improve feed palatability (Badillo et al., 2014; Cheng and Hardy, 2002). Animal by-products used in aquafeeds include nonruminant processed animal proteins (PAPs) mainly from the poultry and porcine industries.

The studies conducted so far, show that diet formulations containing PAPs can have an impact on fish intestinal microbiota. A study conducted in rainbow trout fed a control FM diet or different blends of PAPs and plant proteins showed that gut microbial populations were shifting according to the ratio between vegetable and animal proteins, regardless of the animal protein source. No differences in richness or diversity were detected when compared to the FM diet but the diet with the highest PAP content was richer in Gammaproteobacteria. Lactobacillales and Clostridiales were also regulated by the protein source. The intestinal abundance of specific taxa belonging to Firmicutes and Proteobacteria was discriminatory for diet type. This study concludes that manipulation of the protein source can help to modulate intestinal microbiota to reach beneficial configurations (Rimoldi et al., 2018b). In the same species, when comparing FM, FM-free plant-based diets, and FM-free plant-based diets containing different concentrations of poultry by-products, a lower diversity of allochthonous microbiota was found in the plant-based diets and PAP inclusion partially restored this diversity to FM levels. However, in this study, no differences in the abundance of Gammaproteobacteria were detected in any comparison (Gaudioso et al., 2021).

In European sea bass, the effect of FM/FO-free diets, composed of 85% of vegetable ingredients and 15% PAPs, was compared to a commercial FM diet and a FM-free vegetable diet. No differences in richness or diversity were found in the allochthonous bacteria and few differences were detected in taxa abundance due to a very high interindividual variability. Among these few differences, *Lactobacillus* were more abundant in the PAP diet when compared to FM or FM-free vegetable diets, and the levels of *Methylobacterium* were similar to the FM diet and significantly higher than those found in FM-free vegetable diets. The few changes observed here drove the hypothesis that the high interindividual variability of European sea bass microbiota could contribute to its adaptability to different protein sources (Pérez-Pascual et al., 2020).

Two studies conducted in postsmolt Atlantic salmon evaluated the effect of poultry by-products in autochthonous and allochthonous microbiota highlighting the differential effect in these two bacterial populations and warning about the possibility of overlooking changes if only allochthonous populations or a mixture of both are studied (Gajardo et al., 2017; Hartviksen et al., 2014). The second study evaluated the effect of diets containing feather meal or poultry meal on intestinal microbiota by qPCR using degenerate primers that allow to detection of differential presence of bacterial families or groups of families (Hartviksen et al., 2014). Dietary feather meal increased the number of total bacteria in allochthonous but not in autochthonous populations. In allochthonous populations, a significant increase of *Corynebacteriaceae*, *Lactobacillaceae*, *Streptococcaceae* and *Vibrionaceae* was detected when compared to the control FM diet. In autochthonous populations, *Pseudomonadaceae*/*Xanthomonadaceae* increased and *Vibrionaceae* decreased in both, distal and PI. Dietary poultry meal had different effects, with an increased number of total bacteria in allochthonous but also in DI autochthonous populations. In allochthonous populations, changes were reflected by an increase in *Corynebacteriaceae* and a decrease in Betaproteobacteria, Bacilli-like, *Streptococcaceae* and *Peptostreptococcaceae*. Whereas, in autochthonous populations, differential changes were detected between distal and PI when compared to FM diets (an increase in *Corynebacteriaceae* and *Streptococcaceae* and a decrease in *Vibrionaceae* in DI, and an increase in *Pseudomonadaceae/Xanthomonadaceae* and *Peptostreptococcaceae* in PI) (Hartviksen et al., 2014). The other study evaluated the effect of a diet containing poultry meal by high-throughput sequencing of the 16S rRNA gene (Gajardo et al., 2017). In this study, poultry meal induced lower relative abundances of *Photobacterium*, *Peptostreptococcus*, and *Clostridiales* in allochthonous populations of the DI. In autochthonous populations, differences were smaller than the ones detected for allochthonous bacteria, only highlighting a significantly higher abundance of *Jeotgalicoccus* (Gajardo et al., 2017). Among the many variables that can induce the differences detected between these two studies, the experimental methodology used seems to be the most obvious. In any case, it is clear that poultry by-products modulate both, allochthonous and autochthonous intestinal bacteria in Atlantic salmon.

In Asian sea bass (*Lates calcarifer*), a diet combining poultry by-products with plant ingredients to significantly decrease FM, induced no detrimental changes in autochthonous and allochthonous bacteria. No differences in diversity were observed and among the few changes detected an increase in lactic acid bacteria pointed toward a potential beneficial effect of this diet (Apper et al., 2016). Similarly, no significant or detrimental differences were detected in the intestinal microbiota of dusky kob (*Argyrosomus japonicus*) fed low FM diets with PAPs and plant ingredients (Jones et al., 2021).

In addition to terrestrial animal by-products, aquaculture by-products can be also considered as alternative ingredients to fisheries' products and represent an even more attractive source of protein and lipids in fish diets (Stevens et al., 2018). For instance, fish protein hydrolysates can be made from whole fish or fish by-products, and can contain higher protein than the original fish protein source (Chalamaiah et al., 2012). Several positive impacts have been described for these products in aquaculture, like improved growth performance, digestibility, nutrient utilization, immune response and disease resistance. In addition, they can also modulate intestinal microbiota (Ringø et al., 2016). Supplementation with tuna hydrolysate induced an increase in *Bacillus*, *Lactococcus*, and *Cetobacterium*, improving antioxidant capacity and inflammatory responses in Asian sea bass (Siddik et al., 2020). However, it is difficult to find many studies determining the specific effect of these ingredients on fish microbiota as they are usually applied in formulations containing other alternative sources. For instance, supplementation with tuna hydrolysate and insect meal in diets with a high content of poultry by-products modulated the abundance of Proteobacteria and Firmicutes, decreasing the amount of *Vibrio* in Asian sea bass intestine (Chaklader et al., 2021b). An alternative formulation containing turbot and salmon by-products (protein hydrolysates and oils) together with insect and single-cell proteins, to completely substitute FM and terrestrial PAPs and significantly decrease the levels of FO and plant proteins, was studied in gilthead sea bream. The results showed that microbial changes in the autochthonous populations were significantly correlated with an improved lipid metabolism of the host, and these differences were attributed to the high content of salmon oil in the alternative formulation (Piazzon et al., 2022).

17.5.3 Effects of diets containing insect proteins on gut microbiota

Insect meal is increasingly used in aquafeeds as a protein source to substitute FM. Insects are natural food sources for fish, they have a high nutritional value and can also act as functional ingredients due to their ability to boost the immune system. The impact of dietary insects on gut microbiota is not only driven by the difference in nutrient source. Insects have antimicrobial peptides that can protect against pathogenic microflora (Mousavi et al., 2020; Nogales-Mérida et al., 2019). Also, being rich in chitin, they can favor the growth of beneficial bacteria like lactic acid bacteria, thus insect meals can also be considered prebiotics in some fish species (Perry et al., 2020; Ringø et al., 2012). The most common insect meals used in aquaculture are from *Tenebrio molitor*, *Hermetia illucens*, and *Musca domestica* and their effects on fish health, including gut microbial modulation, will be dependent on different variables such as insect or host species, insect life stage, rearing substrate or processing method. According to a recent review of insects-based diets by (Gasco et al., 2019), it was reported that larval meals are most frequently used with less effects on digestibility, growth performance and product quality of aquatic species.

Several studies have assessed the effect of insect meals on fish intestinal microbiota. Of course, species-specific or experiment-specific differences, including different insect species, have been found (Antonopoulou et al., 2019; Panteli et al., 2021) but there are several common trends. For instance, the presence of chitin, a form of insoluble fiber, can display antibacterial activities against some pathogenic bacteria (Zhou et al., 2013) and stimulate colonization and growth of bacteria with chitinolytic activity. Indeed, chitin was described as a preferential substrate for lactic acid bacteria in the intestine of salmonids (Bruni et al., 2018). Other bacteria with chitinolytic capacity include *Actinomyces*, *Pseudomonas*, *Clostridia*, and many other enteric and marine bacteria (Beier and Bertilsson, 2013; Ringø et al., 2012). In this line, several fish species, including rainbow trout, Siberian sturgeon (*Acipenser baerii*), Asian sea bass and gilthead sea bream, showed a significant increase in chitinolytic bacteria with probiotic potential such as *Lactobacillus*, *Bacillus*, *Enterococcus*, *Carnobacterium*, or *Actinomyces* (Chaklader et al., 2021a; Gaudioso et al., 2021; Huyben et al., 2019; Józefiak et al., 2019; Piazzon et al., 2022; Terova et al., 2019). On the contrary, other studies reported a decrease in *Enterococcus*, *Carnobacterium* and *Lactobacillus* when insect proteins were included in sea trout (*Salmo trutta*) diets (Mikołajczak et al., 2020). These differences were attributed to the different microbiological value of the specific insect meals used, probably due to different rearing methods and substrates, as was previously described in zebrafish (Osimani et al., 2019).

A reduction in Bacteroidetes and Vibrionales has also been reported in several studies with different fish species fed insect proteins (Huyben et al., 2019; Pérez-Pascual et al., 2020; Terova et al., 2019, 2021). Nonetheless, in pikeperch, insect meal induced a dose-dependent increase of Bacteroidetes with a concomitant decrease of Proteobacteria (Tran et al., 2021). The latter study described that FM replacement with up to 18% of insect proteins induced an increase in beneficial probiotic bacteria including *Clostridium*, *Lactobacillus*, *Bacillus*, and *Bacteroides*. However, higher levels of replacement did not show these differences and were correlated with worse growth parameters (Tran et al., 2021). Hence, the complete replacement of FM exclusively with insect meals does not yield good results in terms of growth and health, including microbiota composition. However, these ingredients still arise as a promising alternative source of nutrients to formulate FM-free diets when combined with other protein sources. Combinations of insect meal (up to 25%) with PAPs allowed complete replacement of FM with no negative effects on growth enhancing bacterial richness and diversity and increasing *Lactobacillus*, *Clostridium*, and *Ruminococcus* abundance in Asian sea bass (Chaklader et al., 2021a). Plant-based diets formulated with insect meals (5.5%) and PAPs or yeast protein reduced the adverse effects of the plant-based diet on growth and increased microbiota richness and the abundance of Lactobacillales and Bacillales without impact on disease resistance in rainbow trout (Pérez-Pascual et al., 2021a).

17.5.4 Effects of diets containing single cell ingredients on gut microbiota

The short production times, small land requirements and independence of weather conditions make the use of single-cell products very attractive over other animal or vegetal protein or oil sources (García-Garibay et al., 2003). Single-cell proteins (SCP) and oils (SCO) used in aquafeeds are mainly produced from yeast, microalgae and bacteria (Naylor et al., 2009; Tlusty et al., 2017). The most studied single-cell ingredients (SCI) are from microalgae, interesting for their content of LC-PUFA, and some species can be a source of prebiotics as they can contain SCFA, nondigestible oligosaccharides and dietary fiber such as inulin (De Jesus Raposo et al., 2016). SCI from yeast have high amino acid and micronutrient content and possess high levels of fibers and immunostimulants such as mannan-oligosaccharides, beta-glucans, chitin and nucleosides. These substances are expected to have an impact on intestinal microbial populations. Bacteria as a source of proteins for aquafeeds is the least developed SCP but is currently arising as an interesting alternative ingredient (Glencross et al., 2020b).

The use of microalgae in fish diets does not seem to profoundly impact intestinal microbiota. In any case, some effects have been described. The inclusion of 5% of microalgae from the genus *Schizochytrium* to substitute fish ingredients, mainly FO, induced an increase in *Streptococcus*, *Leuconostoc*, *Lactobacillus*, *Lactococcus*, and *Weissella* in rainbow trout total gut microbiota (Lyons et al., 2017). Supplementation with the same microalgae genus (1.2% on a commercial diet) in Nile tilapia diets decreased Fusobacteria and increased Firmicutes, but in this case, the increase in Firmicutes was due to a higher presence of Clostridiales and not lactic acid bacteria as observed in the previous study (Souza et al., 2020). In gilthead sea bream, diets supplemented with *Nannochloropsis gaditana*, *Tetraselmis chuii*, or *Phaeodactylum tricornutum* induced significant changes in the gut microbiota reflected by different DGGE profiles. Regretfully, specific microbial taxa were not characterized in these studies (Cerezuela et al., 2012; Jorge et al., 2019). The cyanobacteria *Arthrospira platensis* and *A. maxima*, known collectively as spirulina, do not seem to significantly impact the intestinal microbiota of fish. When tilapia was fed a commercial diet supplemented with 3% of *A. platensis* no significant differences were observed in the total intestinal microbiota (Plaza et al., 2019). In African catfish, complete substitution of FM with spirulina did not affect the overall allochthonous gut microbial communities and only a slight increase in the genera *Plesiomonas* and *Bacteroides* was observed (Rosenau et al., 2021). Substitution of plant proteins with 15% spirulina in FM-free diets did not significantly change European sea bass intestinal microbiota (Pérez-Pascual et al., 2020). As a general trend, whole-cell microalgae supplementation tends to increase the richness and diversity of gut microbial populations (Carballo et al., 2019; Lyons et al., 2017) with some exceptions reported (Cerezuela et al., 2012). In European sea bass, the complete substitution of FO with *Schizochytrium* biomass in plant-based diets with different alternative ingredients showed no major changes in the allochthonous microbial composition when compared to a commercial diet (Pérez-Pascual et al., 2020).

Yeast as a source of protein has been applied in aquafeeds to decrease FM levels. Besides, depending on the level of processing of the yeast, they can also act as probiotics (live yeast) or prebiotics (when yeast cell walls are still present in the ingredient). Arctic charr fed extracted or intact *Saccharomyces cerevisiae* or *Rhizopus oryzae* to substitute 40% of FM in the diet showed a higher microbial diversity with higher levels of *Photobacterium* and *Lactobacillus* and lower levels of *Porphyromonas* in total intestinal microbiota (Nyman et al., 2017). The same group, when investigating the effect of diets with different levels of dried yeast meal from *S. cerevisiae* alone or in combination with *Wickerhamomyces anomalus* to substitute FM in rainbow trout, found lower abundance of *Photobacterium* and *Lactobacillus* when compared to the FM group which possibly indicates a fish species-specific response to yeast components (Huyben et al., 2017). Interestingly, this study also evaluated the presence of yeast in the intestinal microbiota and found no significant differences between diets. A follow-up study showed that rainbow trout fed live yeast substituting 40% of FM, also induced a lower abundance of *Photobacterium* in intestinal microbiota, but, in this case, a significant increase in *S. cerevisiae* was found in the gut (Huyben et al., 2018). These differences are probably due to the processing of the feeds, as the former study used extrusion, which destroyed most yeast cells in the diet, while the second used cold-pelleting which maintained yeast viability. Thus, live yeast in the diet can reach fish intestines and become part of the microbiota composition. Different dietary formulations will of course have different effects. This way, rainbow trout fed plant-based diets containing 5.5% yeast mixed with insect proteins or PAPs to lower the levels of plant proteins, decreased allochthonous Proteobacteria and increased Firmicutes when compared to an FM control diet, and increased Actinobacteria when compared to the full plant-based diet (Pérez-Pascual et al., 2021a). In a different fish species, European sea bass, 15% of yeast proteins substituting plant proteins in an FM-free plant-based diet did not induce significant changes in intestinal microbiota (Pérez-Pascual et al., 2020). However, 5% of FM replacement with autolyzed yeast in gilthead sea bream diets led to an enrichment in Bacillales and Clostridiales (Rimoldi et al., 2020a).

Few studies have addressed the effect of feeding bacterial biomass on fish intestinal microbiota, but we know from mammalian studies that feeding bacterial lysates can change host microbiota inducing systemic metabolic changes (Jensen et al., 2021). Feeding the ornamental fish smallmouth grunt with different concentrations of SCP from *Methylobacterium extorquens* up to complete FM substitution did not induce changes in intestinal bacteria (Tlusty et al., 2017). However, dietary fermentation products from *Cetobacterium somerae* did have an impact on common carp. It alleviated the proinflammatory profile, circulating LPS concentrations and liver fat accumulation induced by plant-protein-based diets by regulating host gene expression and gut microbiota. One of the proposed triggers for such changes was butyrate, one of the metabolites present in *C. somerae* fermentation products (Xie et al., 2021b). In gilthead sea bream, an FM-free diet based on plant ingredients, aquaculture by-products, algae oil, insect meal and *C. somerae* fermentation products induced a shift in intestinal microbiota with some species statistically correlating with host fat metabolism gene expression (Piazzon et al., 2022). However, it is not possible to assess the effect of the microbial SCP specifically as the entire formulation was being tested. Similarly, in the same fish species, partial and total replacement of FM with a mixture of PAPs and bacterial SCP reshaped host intestinal microbiota decreasing proinflammatory Gammaproteobacteria and increasing Actinomycetales, *Arthobacter*, *Paracoccus*, and *Propioniciclava*. These changes were hypothesized as an attempt to compensate for proinflammatory effects induced by the diet, but the underlying mechanisms remain to be identified (Solé-Jiménez et al., 2021).

17.5.5 Formulating the perfect diet is not a matter of a single ingredient: additives, prebiotics and whole formulations

So far, we have focused on the effect of alternative protein or lipid sources on the microbiota of aquaculture fish. But diets can also contain supplements or additives to improve the formulation and enhance the effect of the diet on growth and health through the modulation of GI microbiota. These additives can also help to balance or counteract the negative effects of some alternative ingredients. For instance, intestinal inflammation, dysbiosis and increased disease susceptibility can be caused by diets with high replacement levels of plant ingredients (Dai et al., 2020; Estensoro et al., 2016; Piazzon et al., 2017; Romarheim et al., 2013; Urán et al., 2008), but supplementation with fish protein hydrolysates (Egerton et al., 2020), antimicrobial peptides (Dai et al., 2020), or sodium butyrate (Liu et al., 2019; Piazzon et al., 2017) remediated, at least partially, these drawback effects by modulating intestinal microbial populations.

The beneficial effects of SCFA (described in Section 17.4) drove researchers and formulators to consider direct supplementation in aquafeeds to improve microbiota and fish health. They are also commonly used as preservatives in terrestrial livestock feeds and aquafeeds (Ng and Koh, 2017). Dietary supplementation of SCFA modulates fish intestinal microbiota but their effect depends on different variables such as organic acid type and form, concentration, fish species and age, diet composition, microbial community studied, etc. For instance, when butyrate and propionate were tested alone or in combination with supplementing plant-based diets, the changes detected in the allochthonous communities of red drum (*Sciaenops ocellatus*) gut, both in composition and associated metabolic functions, were different in the different diets (Yamamoto et al., 2021). Still, some common trends can be identified from different studies. This way, supplementation with dietary SCFAs increased the abundance of intestinal lactic acid bacteria in grass carp (Tian et al., 2017), common carp (Hoseinifar et al., 2017b), tilapia (Abu Elala and Ragaa, 2015; Zhou et al., 2009) or gilthead sea bream (Rimoldi et al., 2018a). Different studies in gilthead sea bream also reported a decreased abundance of *Photobacterium* species upon SCFA supplementation (Piazzon et al., 2017; Rimoldi et al., 2018a). In general, SCFAs are considered very attractive additives to modulate host and microbial health in aquaculture.

Seaweeds, rich in PSAs, can also be used as feed supplements. They can have prebiotic and immunostimulatory effects interesting for animal feed formulations (O'Sullivan et al., 2010). Dietary supplementation with *Ulva* or *Gracilaria* species modulated the intestinal microbiota of gilthead sea bream or sole (*Solea senegalensis*) increasing beneficial Firmicutes species such as *Lactobacillus* or *Clostridium* and decreasing *Vibrio* species (Rico et al., 2016; Silva-Brito et al., 2021; Tapia-Paniagua et al., 2019). Increased Firmicutes and decreased *Vibrio* were also observed in banana shrimp (*Fenneropenaeus merguiensis*) intestinal microbiota when fed *Enteromorpha* PSAs. Interestingly, an abundance of Firmicutes was positively correlated with intestinal immune-related gene expression in shrimp (Liu et al., 2020a). In Atlantic salmon parr, dietary inclusion of *Asparagopsis taxiformis* supported growth, boosted innate immunity and increased *Shewanella* and *Aeromonas* species while decreasing *Streptococcus* and *Pseudomonas* (Thépot et al., 2022). Modulation of digestive tract microbiota by *Ulva* single cell detritus was also demonstrated in Pacific oyster (*Crassostrea gigas*), where an increase in Vibrionales, Campylobacterales, and Clostridiales, and a decrease in Pseudomonodales, Chromatiales, Rhizobiales, Sphingomonodales, and Burkholderiales orders was detected (Peña-Rodríguez et al., 2020). Dietary supplementation with mannan-oligosaccharides, important components of seaweeds and yeast cell walls, induced an increase in intestinal lactic acid bacteria in Nile tilapia and rainbow trout (Gonçalves and Gallardo-Escárate, 2017; Levy-Pereira et al., 2018).

Several plant-derived components have attracted increased interest to be used as aquafeed additives. Plant PSAs or phytochemicals demonstrate potent immunomodulatory and antioxidant activities and can promote intestinal health by regulating gut microbiota. For instance, supplementation with plant PSAs, alone or in combination with essential oils, in plant-based diets ameliorated the effects caused by FM substitution in European sea bass or in totoaba (*Totoaba macdonaldi*) (Fuentes-Quesada et al., 2020; Rimoldi et al., 2020b). In both studies, the beneficial effects were attributed, at least in part, to an increase in butyrate-producing bacteria. Similarly, plant-based diets supplemented with the alkaloid sanguinarine improved growth, intestinal morphology and microbiota structure and function in grass carp (Liu et al., 2020b). Essential oils, in particular, are active substances that constitute promising replacers for antibiotic growth promoters due to their positive effect on digestion, growth performance, gut microbial community and welfare (Sutili et al., 2018). Several studies investigated the effect of dietary essential oils on fish intestinal microbiota. As expected, diverse results were obtained depending on essential oil and fish species. In rainbow trout, different essential oils failed to induce significant differences in allochthonous gut microbiota (Ceppa et al., 2018; Navarrete et al., 2010). Similar to the findings in rainbow trout, thyme essential oils did not alter the population of beneficial *Bacillus* in the gut of Nile tilapia (Valladão et al., 2019). However, also in Nile tilapia, a combination of essential oils with butyrate had positive effects on intestinal structure and microbiota, which were related to increased numbers of lactic acid bacteria (Alves Jesus et al., 2021). Modulation of koi carp microbiota by oregano oil was connected to enhanced antioxidant capacity, disease resistance and immunomodulatory effects (Zhang et al., 2020e). An integrative study performed in gilthead sea bream showed that supplementation with garlic, carvacol and thymol oils modulated host and microbe metabolism promoting intestinal immunity (Firmino et al., 2021).

Fucoidan has been suggested as a functional supplement due to its health benefits (Abdel-Latif et al., 2022). Fucoidans are PSAs consisting of α-(1 → 3) or α-(1 → 4) -linked L-fucose residues with sulfate substitutions, and occasionally contain monosaccharides such as mannose and galactose, acetate, and glucuronic acid from different algal origins (Ale and Meyer, 2013). Dietary fucoidan showed beneficial properties in aquaculture species such as improved growth (Tuller et al., 2014), boosted immunity (Isnansetyo et al., 2016; Kitikiew et al., 2013; Setyawan et al., 2018), improved resistance against bacterial diseases (Sivagnanavelmurugan et al., 2014; Traifalgar et al., 2012, 2013) and antiviral effects (Immanuel et al., 2012; Sivagnanavelmurugan et al., 2012). However, the effects of fucoidan on the intestinal microbiota are less investigated (Ikeda-Ohtsubo et al., 2020). A promising approach of fucoidan is that it has potential as prebiotic, as no enzymes digesting fucoidan have been reported in the animal GI tracts, thus these ingredients can reach the distal part of the GI tract and potentially confer beneficial effects on the gut microbiota (Brugman et al., 2018; De Jesus Raposo et al., 2016; Michel et al., 1996).

In this section, we tried to individualize the effects of the different components in an attempt to extract specific trends and changes. However, different dietary formulations will have varying amounts of diverse ingredients that can have individual or synergistic effects. Also, the formulations and effects will be different for different fish species. Therefore, each formulation as a whole needs to be tested for each target species to really evaluate the final effect on animal growth and intestinal health. The latest research consequently aims to elucidate how dietary formulations impact both, host and associated microbes, and their interactions in trying to find certain biomarkers for health or disease. For example, recent studies in gilthead sea bream have been evaluating several FM-free formulations on host and microbial health. Different combinations of PAPs, bacterial and yeast SCPs, insect proteins, aquaculture by-products, macroalgae and microalgae oils to substitute FM and decrease the use of plant ingredients, supported optimal growth and nutrient utilization in this species (Naya-Català et al., 2021; Piazzon et al., 2022; Solé-Jiménez et al., 2021). Fish adapted to the different formulations by modulating intestinal microbiota and host gene expression, demonstrating tight cross-talk between certain microbial taxa and the macroscopic host at local and systemic levels (Naya-Català et al., 2021; Piazzon et al., 2022). Future studies should focus on unveiling the exact mechanisms behind these interactions in order to uncover specific pathways, genes or microbial markers that will allow accurate evaluation of new formulations for their use in aquaculture.

17.6 Antibiotics and antimicrobials: impacts on gut health and disease susceptibility

Antibiotics are a heterogeneous group of natural or synthetic compounds produced by microorganisms that possess the ability to inhibit, eradicate or kill the bacteria. While commonly used for the treatment of infectious diseases, antibiotics are also used as additives or feed supplements to promote growth of the livestock (Carvalho and Santos, 2016). Most of the antibiotics used for human medicine are often used in fish feed either as additives or to treat bacterial diseases. For example, erythromycin, tetracycline, sulfamethoxazole, norfloxacin and trimethoprim are frequently used in the aquaculture industry along with other farming systems including poultry, pig and cow industries (Ha Pham Thi et al., 2022). Antimicrobial compounds may reach the aquatic environment through (1) exogenous feed applied for prophylactic or preventive measures; (2) metabolites discharged through feces and urine of treated humans/animals; or (3) may be produced naturally by the producer organisms (Zhou et al., 2018a). Fish do not actively metabolize antibiotics. Therefore, most of the antibiotics are poorly absorbed and approximately 25%−75% of the antibiotics applied with feed are released back into the aquatic environment (Burridge et al., 2010; Karthikeyan and Meyer, 2006). Owing to high water solubility, low bioavailability and widespread misuse, residues of diverse antibiotics have been detected in many aquatic environments (Chang et al., 2010; Zhou et al., 2018a). Uncontrolled use of antibiotics in the aquatic environment has been presumed to induce selective pressure for the emergence of drug-resistant bacteria (Romero et al., 2012; Sørum, 2005), which has become a serious concern worldwide (Kolndadacha et al., 2011). A study concerned with the effects of oxytetracycline on Nile Tilapia (Payne et al., 2021) demonstrated that antibiotic treatment can exert selective pressures on the fish gut microbiome in favor of resistant populations that may have long-term impacts on fish health. Thus, the indiscriminate use of antibiotics was strictly criticized and several countries imposed restrictions on antibiotic usage for aquaculture production (Tang et al., 2017). The emergence of multidrug-resistant pathogens might lead to sudden infectious disease outbreaks resulting in serious economic loss (Thankappan et al., 2015). Apart from the likely appearance of antimicrobial resistance among pathogenic bacteria, alteration in the gut microbial community, accumulation of antibiotics residues in fish tissues and degraded environmental conditions are the related consequences of antibiotic misuse (Cabello, 2006; Ghosh et al., 2021; Ringø et al., 2016).

Like their terrestrial counterparts, the importance of the gut microbiota and gut health for the normal well-being of the fish has now been established (Ray et al., 2012; Soltani et al., 2019). Moreover, the gut is the main target area of antibiotic absorption and it is likely to be the primary organ affected by dietary/environmental antibiotic residues. As compared to the other organs like muscle or liver, the gut appears to be more sensitive to the antibiotic residues in water (Zhou et al., 2018a). It has been indicated that antibiotics may induce huge and extremely variable changes in the intestinal microbiome even at sublethal concentrations (Schlomann et al., 2019). Since intestinal health is very important to fish, the influence of the antibiotics on intestinal health of fish needs to be properly addressed. Although reports on this particular aspect are scanty, this section will provide an impression of the impacts of antibiotics on gut health and disease susceptibility in fish. Table 17.7 summarizes the works carried out on diverse antibiotics and their effects on fish gut microbiota and/or gut health.

17.6.1 Gut microbiota

Antibiotics or antimicrobial compounds can be considered as anthropogenic as well as environmental factors affecting the gut microbiota. Antibiotics can deplete commensal microbiota or directly affect host tissues, causing adverse effects on animal physiology (Morgun et al., 2015). In general, antibiotics alter the gut microbiota, as they kill or inhibit the growth of specific bacteria (Butt and Volkoff, 2019). Although the administration of antibiotics may not totally eliminate gut microbiota communities, it can induce significant changes in the microbial composition. When intestinal health is evaluated, the intestinal microbiota should be considered an essential component for the preservation of the integrity of the mucosal barrier function, as is addressed in Section 17.5 (Sommer and Bäckhed, 2013).

Reports on the effects of antibiotics on the gut microbiota are somewhat contradictory and inconclusive as variations exist with regard to the aquaculture species examined, antibiotics used and concentration/dose applied. An earlier study by Austin and Al-Zahrani (1988) noticed a rapid reduction in bacterial quantity within the GI tract of rainbow trout exposed to erythromycin and penicillin-G. However, the administration of oxolonic acid (20 mg/kg) did not affect the gut bacteria of goldfish (Sugita et al., 1989). The population of *Aeromonas hydrophila* increased following chronic dietary exposure to virginiamycin and terramycin for a period of 70 days (Ahmad and Matty, 1989), which might be indicative of gaining resistance due to prolonged treatment. Depaola (1995) recorded the development of tetracycline-resistant bacteria in channel catfish exposed to oxytetracycline-contaminated feed. Further, administration of oxytetracycline to Atlantic salmon also developed resistance among the gut microbiota (Kerry et al., 1996). On the other hand, oral application of oxytetracycline (75 mg/kg/day) did not influence the bacterial quantity in Atlantic salmon, while alteration of the bacterial composition was noticed (Navarrete et al., 2008). In another study conducted by Roy and Ghosh (2008), rohu fingerlings were fed tetracycline-incorporated diets (250 mg and 500 mg/kg feed) for 3 weeks. The fish-fed diets with tetracycline were noticed with a reduction in the gut bacterial population and a significant decline in the activities of digestive protease and amylase (Roy and Ghosh, 2008). Exposure to oxytetracycline (> 250 mg/L) caused significant mortality of the intestinal bacteria in grass shrimp, *Palaemonetes pugio*. However, lower oxytetracycline concentrations (1, 16, and 32 mg/L) did not exert significant bacterial mortality (Uyaguari et al., 2009).

Navarrete et al. (2010) assessed the effects of oxytetracycline treatment on bacterial populations present in the GI tract of healthy juvenile salmon. Analyses of gut microbiota by restriction fragment length polymorphism (RFLP) and 16S rDNA amplicon sequencing revealed that microbiota from the GI tract of untreated fish was more diverse, representing *Acinetobacter*, *Bacillus*, *Flavobacterium*, *Pseudomonas*, *Psycrobacter*, and

TABLE 17.7 Summary of the works carried out on diverse antibiotics and their effects on fish gut microbiota and/or gut health.

Antibiotics used	Fish species	Mode of application/ exposure	Effects on gut	Reference
Erythromycin and Pencillin-G	Rainbow Trout, *Salmo gairdneri*	Feed	Quantity of GI-tract bacteria reduced rapidly	Austin and Al-Zahrani (1988)
Oxolonic acid (20 mg/kg)	Gold Fish, *Carassius auratus*	Oral/feed	Gut-bacteria were not affected	Sugita et al. (1989)
Virginiamycin and terramycin	Common carp, *Cyprinus carpio*	Feed	Population of *Aeromonas hydrophila* increased within the gut	Ahmad and Matty (1989)
Oxytetracycline/ tetracycline	Channel catfish, *Ictalurus punctatus*	Contaminated feed	Tetracycline resistant bacteria evolved	Depaola (1995)
Oxytetracycline	Atlantic salmon, *Salmo salar*	Feed	Gut-microbiota developed resistance against the antibiotic	Kerry et al. (1997)
Oxytetracycline (75 mg/kg/d)	Atlantic salmon, *Salmo salar*	Oral/feed	It did not influence the bacterial quantity while alteration of the bacterial composition was noticed	Navarrete et al. (2008)
Tetracycline (250 mg and 500 mg/kg feed)	Rohu, *Labeo rohita* fingerlings	Feed	Gut-bacterial population reduced; digestive protease and amylase activities declined	Roy and Ghosh (2008)
Oxytetracycline (> 250 mg/L)	Grass shrimp, *Palaemonetes pugio*	Solution prepared in de ionized water	Induced mortality of the intestinal bacteria; lower oxytetracycline concentrations (1, 16, and 32 mg/L) didn't exert significant bacterial mortality	Uyaguari et al. (2009)
Oxytetracycline	Atlantic salmon, *Salmo salar*	Feed	Gut microbiota of the oxytetracycline-treated group was less diverse consisting of only *Aeromonas* species	Navarrete et al. (2010)
Sulfamethoxazole and trimethoprim	Gilthead sea bream, *Sparus aurata* and European sea bass, *Dicentrarchus labrax*	Fishes were exposed to 40 ppm concentration for 1 h	Effects on gut was not addressed; plasma cortisol and glucose level increased, C-reactive protein decreased	Yildiz and Altunay (2011)
Rifampicin	Mosquitofish, *Gambusia affinis*	Aquatic medium for 1 week (25 μg/mL)	Lower community diversity and taxonomic composition of the gut microbiome; abundance of *Cetobacterium* increased within the gut; resistance to the pathogen *Edwardsiella ictalurid reduced*; increased susceptibility to osmotic stress	Carlson et al. (2017)
Oxytetracycline (0.3125–5 mg/L)	Rainbow trout, *Oncorhynchus mykiss*	Aquatic medium	Catalase activity in the gills declined	Rodrigues et al. (2017)
Oxytetracycline	Zebrafish, *Danio rerio*	Aquatic medium (420 ng/L)	Disruption of the intestinal microbiota, abundance of Proteobacteria decreased, *Cetobacterium* increased	Zhou et al. (2018a)
Sulfamethoxazole (260 ng/L) and oxytetracycline (420 ng/L)	Zebrafish, *Danio rerio*	Aquatic medium	Intestinal goblet cell numbers, alkaline phosphatase and acid phosphatase activities decreased, expression levels of TNF-α and IL-1 increased	Zhou et al. (2018a)

(*Continued*)

TABLE 17.7 (Continued)

Antibiotics used	Fish species	Mode of application/ exposure	Effects on gut	Reference
Oxytetracycline and amoxicillin	Olive flounder, *Paralichthys olivaceus*	Oral administration (80 mg/kg/d for 10 d), or intramuscular injection (12.5 mg/kg and 50 mg/kg)	Oral treatment group dominated by *Virbio scophthalmi*, *V. harveyi* and *Photobacterium damselae*; injection treatment group dominated by *V. scophthalmi* and *Streptococcus parauberis*; number of OTUs decreased (from 178 in control to 42 and 78) following antibiotics treatments	Kim et al. (2019)
Oxytetracycline	Adult zebrafish, *Danio rerio*	Administrated via water (0, 10 and 10000 μg/L for two months)	In water samples, Deltaproteobacteria (Myxococcales and Bdellovibrionales orders) increased, even at the lowest (10 μg/L) OTC concentration; decrease in Gamaproteobacteria and increase in Actinobacteria were recorded at the highest concentration	Almeida et al. (2019)
Florfenical	Pacu, *Piaractus mesopotamicus*	Medicated feed	Relative abundance of Antibiotic-Resistant Genes (ARGs) increased; shifts in the gut microbiome toward well-known putative pathogens such as *Salmonella*, *Plesiomonas*, and *Citrobacter* were noticed	Sáenz et al. (2019)
Ciprofloxacin (sublethal doses)	Zebra fishlarvae, *Danio rerio*	Media	Caused severe drops in bacterial abundance; antibiotic treatment promoted bacterial aggregation and increased susceptibility to intestinal expulsion	Schlomann et al. (2019)
Antibiotic mixtures	European sea bass, *Dicentrarchus labrax* juveniles	Diets coated with mixtures of antibiotics	Showed a negative effect on the gut microbial diversity and composition; abundant microbiome with antibiotic persistence also detected	Kokou et al. (2020)
Tetracycline (0.285 and 2.85 μg/L)	Goldfish, *Carassius auratus*	Feed	The predominant species were changed, both at phylum and genus levels; tetracycline resistance bacteria increased	Jia et al. (2020)
Oxytetracycline	Nile Tilapia, *Oreochromis niloticus*	Feed 100 mg/kg/d for 8 d	Enrichment in *Plesiomonas*, accompanied by a decline in other bacteria taxa; increased the proportion of tetA in the distal gut of fish and tank biofilms of the treated group	Payne et al. (2021)
Erythromycin	Vietnam koi, *Anabas testudineus*	Feed	After infection with antibiotic-resistant *Aeromonas dhakensis* the nonmedicated group has significantly higher mortality than the medicated group	Ha Pham Thi et al. (2022)

Brevundimonas/Caulobacter. In contrast, the microbiota of the oxytetracycline-treated group was less diverse consisting of only *Aeromonas* species. Thus, a reduction in the diversity of the gut microbiota noticed after oxytetracycline exposure might have facilitated the growth of opportunistic microorganisms. Similarly, Mosquitofish (*Gambusia affinis*) exposed to the antibiotic rifampicin displayed lower community diversity and taxonomic composition from both skin and gut microbiomes, compared to the untreated control group. Further, the abundance of

Cetobacterium increased in the gut of *G. affinis* exposed to the antibiotic (Carlson et al., 2017). In zebrafish, exposure to oxytetracycline resulted in a disruption of the intestinal microbiota (Zhou et al., 2018a). In their study, the abundance of Proteobacteria decreased in the oxytetracycline-exposed group, while OTUs related to *Cetobacterium* increased significantly in the antibiotic-treated groups, which was in agreement with Carlson et al. (2017). Further, a decrease in the abundance of Gamaproteobacteria and an increase in Actinobacteria was noticed in adult zebrafish following administration of oxytetracycline through water (Almeida et al., 2019).

The relative abundance of antibiotic-resistant genes was noticed to be increased in *Piaractus mesopotamicus* exposed to florfenical (antibiotic) in the diets (Sáenz et al., 2019). In addition, shifts in the gut microbiome toward well-known putative pathogens, viz., *Salmonella*, *Plesiomonas*, and *Citrobacter* were noticed. Generally, *Plesiomonas* and *Citrobacter* are known to harbor genes that code for multidrug and phenicol efflux pumps (Sáenz et al., 2019). Increased abundance of *Plesiomonas* OTUs accompanied by a decline in other bacteria taxa was recorded in Nile tilapia gut microbiome following dietary exposure to oxytetracycline. Thus, it was likely that antibiotic treatment was associated with a decrease in the number of antibiotic-sensitive bacteria and an increase in antibiotic-resistant bacteria. The reduction in diversity and number of the gut microbial community following antibiotic treatment was further documented by Kim et al. (2019). As compared to the 178 OTUs retrieved from the control group, only 42 and 78 OTUs were detected in olive flounder (*Paralichthys olivaceus*) treated with amoxicillin and oxytetracycline (80 mg/kg/day for 10 days), respectively. Variation of gut-bacterial diversity was noticed between oral administration and intramuscular injection treatment groups. *Vibrio scophthalmi*, *V. harveyi*, and *Photobacterium damselae*, were dominant in the oral treatment group, whereas *V. scophthalmi* and *Streptococcus parauberis* were dominantly isolated from the injection treatment group (Kim et al., 2019). In another study, the proportion of tetracycline resistance bacteria in goldfish increased owing to tetracycline administered through feed (Jia et al., 2020). A large number of gut-associated bacteria isolated and characterized from the IMCs were noticed to be susceptible to the common antibiotics (Ghosh et al., 2021; Mukherjee et al., 2016, 2017). Antibiotic treatment can eradicate susceptible microorganisms within the fish gut and promote the growth of opportunists that may occupy ecological niches previously unavailable to them. In contradiction to the above reports, a differential effect of antibiotics on the composition along the gut of European sea bass was recorded by Kokou et al. (2020). Despite of administration of two broad-range antibiotic mixtures, a high portion of the European sea bass gut microbiome persisted indicating high stability acquired by the microbiota against interference caused by specific antibiotics.

17.6.2 Gut health and disease susceptibility

Intact intestinal structures and normal intestinal function help animal hosts to prevent many diseases. The crucial role of gut microbiota in toxicity and xenobiotics metabolism has also been indicated (Choi et al., 2013). It appears that an undamaged intestine might afford a barrier function to the exogenous toxins through oxidative as well as reductive processes that are the key elements of drugs and xenobiotics metabolic pathways (Rodrigues et al., 2017). Disruption of the normal gut microbiota may be a critical determinant of disease expression. The normal commensal microbiota generates resistance to the colonization of pathogenic bacteria through the process of competitive exclusion, thereby suppressing the disease progression. Moreover, gut microbiota forms an integral component of the mucosal immunity that promotes antiinflammatory cytokines and downregulates proinflammatory cytokines (Pérez et al., 2010). Therefore, antibiotics-induced disruption of the microbial communities might increase susceptibility to the diseases. Involvement of the gut microbiota in the progression of a wide range of disorders, viz., metabolic dysfunctions, dysbiosis, and inflammatory disease has been suggested (Xiao et al., 2014; Zhou et al., 2018a).

Zhou et al. (2018a) conducted a study where zebrafish were exposed to sulfamethoxazole (260 ng/L) and oxytetracycline (420 ng/L) for a period of six weeks. The study revealed that exposure to antibiotics resulted in a significant decrease in intestinal goblet cell numbers, alkaline phosphatase and acid phosphatase activities, and the antioxidant response that are known to play important roles in host defense mechanisms and nonspecific immunity. Antibiotic exposure increased the expression levels of inflammatory factors (*tnfα* and *il1*) in zebrafish, indicating that antibiotics disturbed the intestinal inflammation process (Zhou et al., 2018a). Further, antibiotic treatment caused higher mortality when treated fish were challenged with *Aeromonas hydrophila* (Zhou et al., 2018a). Similarly, *G. affinis* exposed (3-day) to rifampicin (25 μg/mL) resulted in reduced resistance to the pathogen *Edwardsiella ictaluri*, reduced weight gain at the initial stage, and increased susceptibility to osmotic stress as compared to the controls (Carlson et al., 2017). Impairment of intestinal structure and function may be a possible reason for the increase in mortality, suggesting that although the environmental concentrations of antibiotics were very low, there was still an augmented risk to fish health through impaired immune function and increased inflammation in the gut (Zhou et al., 2018a).

Apart from this limited information on antibiotic-induced changes in gut health and disease susceptibility, quite a few studies have recorded other physiological effects following antibiotic exposure. However, whether the physiological effects were anyhow linked to gut health and gut microbiota, has not been clearly indicated. For example, a combinatorial application of sulfamethoxazole and trimethoprim at a concentration of 40 ppm resulted in increased plasma cortisol and glucose, and decreased C-reactive protein in gilthead sea bream and European sea bass following one hour of treatment in each case (Yildiz and Altunay, 2011). Nevertheless, chronic and acute antibiotic exposures revealed different patterns with regard to tissue functions. For instance, chronic exposure (28 days) to oxytetracycline (0.3125−5 mg/L) was associated with a decline in catalase activity in the gills of rainbow trout. Whereas, acute exposure (96 hours) to oxytetracycline (5−50 mg/L) did not alter catalase activity in the gill tissues of rainbow trout (Rodrigues et al., 2017). As a metabolic function of the gut bacteria was well established, likely consequences of the antibiotics-induced changes in the gut health may not be ruled out in all such instances.

Besides, fish provide an indispensable link between antibiotics released into aqueous environments and human health (Zhao et al., 2015). Antibiotics can bio-accumulate in animal tissues (Carnevali et al., 2017) and lead to the development of drug-resistant bacteria, which can be passed along the food chain (Fu et al., 2017). The occurrence of antibiotic-resistant bacteria in diverse fish species reared under farm conditions as well as collected from wild habitats has been demonstrated on several occasions (Carlson et al., 2017; Jacobs and Chenia, 2007; Zhao et al., 2015). It has been widely accepted that the presence of bacteria harboring resistance determinants could be related to the widespread use of antibiotics in aquaculture (Cabello, 2006). However, limited information on the issue might suggest that the specific role of the resident gut microbiota in the dispersal of resistant genes needs to be worked out.

17.7 Methods to analyze microbiota in the fish gut

The study of the fish gut microbiota and the understanding of the interactions between host and microbes or between microbes within these microbial communities require a detailed view of the taxonomic composition and the metabolic potential of the different members of the gut microbiota. Culture-dependent techniques were the main methods to isolate and characterize the fish gut microbiota until the late 2000s. However, numerous reports have indicated the limitations of such techniques, since we can isolate and culture only a limited fraction of the microbial communities (Austin, 2006). Several methods using culture-independent techniques helped us gain more information about the gut microbial communities, using molecular approaches, such as DGGE and automated rRNA intergenic spacer analysis (ARISA), as well as Sanger sequencing (Austin, 2006; Ghanbari et al., 2015; Ringø et al., 2016; Sun et al., 2013). In the past two decades, rapid and low-cost approaches, with the capacity to analyze a larger number of samples than Sanger sequencing, have been developed, known as Next-Generation sequencing (NGS) and Third-generation sequencing (TGS) technologies (Foster et al., 2012). These technologies were also introduced in the fish gut microbiota research to study the taxonomic composition and genetic potential of microbiota. There are various platforms developed, with different capacities, but with a common feature: the high number of reads in a single run, enabling the rapid and cost-effective acquisition of sequence data, with a higher depth and accuracy compared to Sanger sequencing. This allows the detection of low-abundance taxa within the gut microbiota and the study of more complex communities, which would be otherwise impossible to characterize (Ghanbari et al., 2015). In the section below, we will present several commonly used and novel NGS and TGS platforms for the study of the fish gut microbiota, as well as the targeted analysis.

17.7.1 Sequencing technologies

One of the most widely used NGS platforms in fish gut microbiota studies is the Illumina technology, which was introduced in 2006. This technology is based on sequencing-by-synthesis and the incorporation of dye terminator nucleotides into the sequence by a DNA polymerase (similar to Sanger; Siqueira et al., 2012). Single DNA fragments go through a flow cell surface, coated with adapters and complementary adapters, and each of these fragments makes a bridge with its complementary adapters. The sequencing starts when a DNA polymerase reaction mixture is added, together with the sequencing primers and four reversible terminator nucleotides, that are labeled with different fluorescent dyes. After incorporation, the terminator nucleotide and its position on the support surface are detected and recorded by a four-channel fluorescence scanner (Metzker, 2010). Illumina offers different platforms that give an output of different read lengths (number of reads per run): HiSeq platform produces the largest amount of data per run (up to 1500 Gb) with the shortest reads; MiSeq platform generates 44−50 million paired-end reads 2×300 bp in length and is more common for amplicon sequencing projects since it produces longer read length and lower read number compared to the HiSeq instrument. The Illumina NextSeq platform is another product relatively new compared to the other two platforms, it performs at an intermediate scale in terms of output, read length and costs per base, which is so far less used in fish microbiota studies (Perry et al., 2020). The Illumina NovaSeq offers a deep and broad coverage of the genome with an output range from 80 to 6000 Gb and a read length of 250bp, which is ideal for shotgut metagenomic analysis.

The 454 Roche technology, which was mostly used in the initial studies of the fish gut microbiota (Ghanbari et al., 2015; Perry et al., 2020), is based on sequencing-by-synthesis (Mardis, 2008; Metzker, 2010), also known as "pyrosequencing" chemistry, because it involves the incorporation of deoxynucleotide triphosphate (dNTP) bases into a synthesized DNA chain followed by the release of a pyrophosphate. The pyrophosphate serves as a substrate for the enzymatic production of ATP, which in the presence of luciferase, produces light that can be detected by a camera. This reaction is performed on beads containing millions of copies of a single DNA molecule, which is clonally amplified in an oil–water emulsion containing PCR reagents in micelles (Dressman et al., 2003). The beads carrying single-stranded DNA are placed into single wells of a picotiter plate with millions of wells. Sequencing occurs through the repeated cyclic flow of thin films of dNTPs across the wells, where base incorporation leads to the production of photons that are detected by a camera. The amount of light discloses whether a specific dNTP and how many were incorporated in that flow and the information is then translated into a DNA sequence for each bead (Siqueira et al., 2012). The 454 platform family provides approximately an average read length of 700 bp and a total output of 1 million reads, which is less coverage than the Illumina platform (Ghanbari et al., 2015). It is important to mention that this technology is obsolete and reagents are no longer produced.

The Ion Torrent PGM (Personal Genome Machine) technology is another widely used platform in fish microbiota studies in the past, which has been available on the market since the end of 2010. This platform is based on semiconductor chips of different scales that allow sequencing between 0.4 and 5.5 million reads. This system is similar to 454, as it uses a plate of microwells containing beads to which DNA fragments are attached, however, the base incorporation is different than 454. The attachment of a specific base to a growing DNA strand leads to a pH alteration, which is detected through the semiconductor chip, and the changes are recorded. The read length ranges from approximately 100 bp to 400 bp, and sequencing is very fast, lasting only a couple of hours, while the sequencing costs per base range between the 454 and Illumina technologies costs (Ghanbari et al., 2015).

A novel platform that is gaining attention for gut microbiota studies is the Oxford Nanopore Technology (third generation sequencing technologies, TGS). Nanopore technologies are capable of producing long sequences with no theoretical read length limit, which can target the entire 16S rRNA gene, allowing the identification of bacteria with more accuracy and sensitivity (Leggett et al., 2020). Several sequencing platforms depend on the desired coverage and speed, but MinION seems to be an attractive one in terms of time and cost-effectiveness (Nakagawa et al., 2019). MinION produces sequencing data in real-time, which reduces turnaround time for data processing. Nanopore sequencing uses small protein pores (nanopores) embedded in a synthetic membrane, and by applying electrical potential across the pore, different bases in the nucleic acid are identified (charged differently). This sequencing technology has also some drawbacks, such as a relatively lower throughput and higher error rates compared to other sequencing platforms (Kono and Arakawa, 2019). Despite the lower per-read accuracy, the nearly unrestricted read length provided by the MinION sequencer offers promise in sequence-based bacterial analyses (Matsuo et al., 2021). This technology is beginning to be used in fish microbiota research, although the standardized commercial protocols need to be adapted and optimized for the particularities of fish mucosal samples (Toxqui-Rodríguez et al., 2023).

17.7.2 Sequencing analysis

Amplicon sequencing is the most widely used method for gut microbiota analysis, which targets different variable regions of the bacterial 16S ribosomal RNA (rRNA) genes. The backbone of the 16S rRNA gene is highly conserved across prokaryotes, however, it contains nine hypervariable regions (V1–V9) with relatively low levels of sequence conservation. Thus, sequences from those hypervariable regions allow us to identify the different prokaryote taxa and reveal the bacterial diversity in a sample. Most fish microbiota studies so far have focused on either V3, V4, or V3–V4 region to study the taxonomic composition of the sample (Perry et al., 2020). However, recent studies show that in order to have the correct taxonomic composition, ideally the full length of the 16S gene, which is 1500 bp, should be sequenced (Johnson et al., 2019), which can be achieved with TGS technologies like MinION sequencing. Moreover, a methodological problem that arises from sequencing the different variable regions is the comparability between studies, since these can produce different results. The protocols for the DNA extraction methods can also alter the results as well as the sequencing platforms selected. Thus, a solution to this problem would be to create a "gold standard," which at the moment does not exist in fish gut microbiota studies, that depending on the sample type, researchers can select between specific markers, sequencing platforms and DNA extractions protocols so that microbiota research can be standardized.

Besides amplicon sequencing, which is the most widely used method for the taxonomic profiling of the gut microbiota, shotgun metagenomic analysis is a powerful tool that provides comprehensive insights into the repertoire of gene functions within the microbiota by extracting and directly sequencing genomic DNA, without PCR amplification of a specific gene marker (Handelsman, 2004; Quince et al., 2017). This approach also provides an opportunity to explore the genetic and physiological traits of uncultivated microbes through the reconstruction of their genomes (Tyson et al., 2004). The method is widely implemented in different environments and from different host-associated microbiota, however, within fish, relatively few studies exist, which give important insights into the functional involvement of the microbiota.

Alternative to the metagenomic approach, metatranscriptomic analysis can provide information on the expressed functions of the microbiota. This method is widely implemented in human and other animal models, but less in fish. The method is based on RNA sequencing, providing a global transcriptomic profiling of the gut microbiota, not only focused on bacteria, by simultaneous sequencing and analysis of enriched microbial transcripts or mRNA. In this way, it allows the identification of genes that are differentially expressed and which may be of particular importance and can be later manipulated for industrial or clinical applications (Bashiardes et al., 2016).

To understand the functional interactions between host and specific pathogens, a very recent application is the Dual RNA-Seq technology. This approach allows to detection of pathophysiological changes through the capture of the coding and noncoding transcripts from both the host and an invading pathogen, simultaneously (Westermann et al., 2017). For fish, its utility was originally demonstrated in zebrafish models of human disease, revealing the transcriptional networks underlying cancer−host interactions (Zhang et al., 2016a). In aquaculture, this approach can offer a great tool to understand microbiome dysbioses in the host and the involvement of specific pathogens in disease. A recent study was published that explored the kinetics of skin microbiome and fish host responsiveness to infection by *Tenacibaculum maritimum* using batfish (*Platax orbicularis*) as a model (Le Luyer et al., 2021).

17.7.3 Diversity analysis

The next step after sequencing is to proceed to the downstream analysis, where the sequencing data will be binned into the different taxonomic groups, based on the DNA sequences. For that, there are several different platforms with the most common ones the qiime2 (Hall and Beiko, 2018), Mothur (Schloss et al., 2009) and DADA2 (Callahan et al., 2016). The rationale of all of these platforms is similar, as also outlined in Fig. 17.6: first, similar DNA fragments are grouped either into OTUs (those that have a level of similarity more than 97%) or into Amplicon Sequence Variants - ASVs (those that have a level of similarity at the single nucleotide level, so 100% similar). The next step is to compare these OTUs or ASVs to sequence databases like those provided by the National Center for Biotechnology Information or the SILVA high-quality ribosomal databases to assign the taxonomy of these sequences. Once these taxonomic groups are characterized, then the diversity analysis can be performed with the most common indicators being

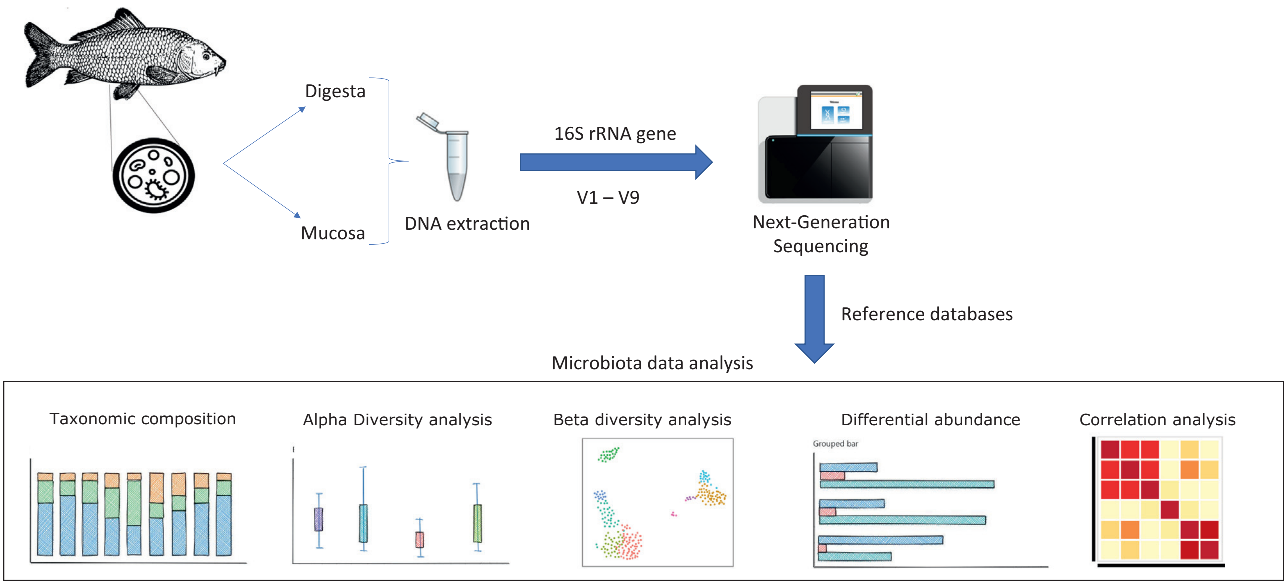

FIGURE 17.6 Outline of the gut microbiota analysis using amplicon sequencing.

the alpha and beta diversity analysis. Alpha diversity looks at the microbial diversity within a sample, while beta diversity is evaluating the diversity among different samples and expressing it as the similarity between the different samples. Further analysis that can provide information on the type of taxa that are within the samples is the taxonomic composition, and the differential abundance analysis, which reveal the type of microbes that are present in a sample and which of those are enriched in one treatment versus another. Correlation analysis can also provide important information with regard to the type of correlations between different microbes and environmental variables.

Bioinformatics tools that have been recently developed can be used to extend 16S rRNA gene amplicon studies to include the functional assessment of the fish microbiota (Ortiz-Estrada et al., 2019). Such tools like PICRUSt (Langille et al., 2013), Tax4Fun (Aßhauer et al., 2015), and Piphillin (Iwai et al., 2016) have been already implemented for profiling the functional potential of fish microbiota. These tools provide the gene abundances belonging to different functional groups such as KEGG Orthologs, which are derived from metagenomes reconstructed from the reference genomes of closely related taxa sourced from different repositories. Although inherent biases like the variable 16S rRNA gene copy number are taken into account by these tools to improve their quantitative capacity, the reliability to predict function may vary and depends on the availability of the reference genomes (Aßhauer et al., 2015). Given that the fish microbiota is underrepresented in the genome databases, it would be important for the future to increase the coverage of microbial genomes from aquaculture and fish gut ecosystems to improve the reliability of these tools. In any case, a recent study comparing results from inferred metagenomes and actual mRNA expression (metatranscriptome) in gilthead sea bream demonstrated that even though inference programs do not fully predict the metabolic potential of the microbial populations, they are useful tools to indicate the direction and magnitude of the changes in comparative analyses (Naya-Català et al., 2022).

17.8 Conclusions

The fish microbiota research lags well behind that in humans and mouse models, while it is still far from being complete. Yet, there is considerably growing interest in understanding this fascinating topic, with most studies coming from aquaculture, thus indicating the increasing importance of this industry as a source of animal protein in the global food supply. Several studies have accumulated over the past decade, highlighting the importance of gut microbes for host health through their involvement in biological processes such as nutrient processing, detoxification, immune modulation, fish development, and mucosal tolerance. In this chapter, we summarized the current knowledge and advances in the composition and functionality of fish microbiota and provided the current methodology to understand these diverse communities. In Sections 17.2 and 17.3, we provided an overview of the main microbiota compositions in important aquaculture fish and shrimp species, highlighting not only the importance of the surrounding environment, the trophic level, the age, genetics and sex on the outcomes of the different studies, but also the differences between autochthonous and allochthonous microbial communities. Considering this information is essential when planning microbiota sampling or interpretation of results, as these can significantly affect the conclusions of the different studies. In Sections 17.4 and 17.6, we describe the role of the gut microbiota on digestion, nutrient absorption and metabolism, as well as the immune response and disease resistance in fish, and the impacts of antimicrobials. We also focused on the importance of evaluating the composition of microbial metabolites such as SCFA. In Section 17.5, we aimed to present the dietary effects of different feed ingredients to understand the general impacts on gut microbiota compositional changes. It is to be noted that dietary formulations can vary significantly between the studies and thus have variable effects on the microbiota compositions. So far, very few studies have linked the gut microbiota and host parameters like growth or immune responses. Thus, future studies should connect these different parameters, as it will enable us to understand how microbiota changes relate to specific phenotypes and provide several valuable biomarkers for fish health and performance. Moreover, exploring the functional properties of the gut microbiota and connecting them to metabolic and physiological changes in the fish hosts is the next step to improve our understanding of fish–microbe interactions.

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