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Review

# The Fish Microbiota: Research Progress and Potential Applications

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## ARTICLE INFO

## ABSTRACT

The gut microbiota plays an important role in host health and disease. Our understanding of the fish microbiota lags far behind our knowledge of that of humans and other mammals. Nevertheless, research has highlighted the importance of the microbiota in the health, performance, and various physiological functions of fish. The microbiota has been studied in various fish species, including model animals, economic fish, and wild fish species. The composition of the fish microbiota depends on host selection, diet, and environmental factors. The intestinal microbiota affects the nutritional metabolism, immunity, and disease resistance of the fish host, while the host regulates the intestinal microbiota in a reciprocal way through both immune and non-immune factors. Improved and novel gnotobiotic fish models have been developed, which are important for the mechanistic study of host-microbiota interactions in fish. In this review, we discuss recent progress in fish microbiota research. We describe various aspects of this research, including both studies on fish microbiota variations and fundamental research extending our knowledge of host–microbiota interaction in fish. Perspectives on how fish microbiota research may benefit fish health and industrial sustainability are also discussed.

## 1. Introduction

As in humans and other mammals, a large and complex community of microbes reside in the gut of fish. It has been generally considered that the gut bacterial community of fish differs significantly from those of other vertebrates (i.e., reptiles, birds, and mammals) [1–3]. In the human gut microbiota, Firmicutes and Bacteroidetes are predominant. Similar results have been observed in other mammals [4]. In contrast, although variation exists among different fish species, Proteobacteria is the dominant phylum in fish microbiota, with Fusobacteria constituting another abundant component in many cases [1,5]. Our understanding of the fish microbiota lags behind our knowledge of that of humans and other mammals, but it has been improved with the application of gnoto-

biotic fish models. As a milestone of fish microbiota research, Rawls et al. [6] revealed evolutionarily conserved responses to the gut microbiota by using gnotobiotic zebrafish (*Danio rerio*) models. Notably, some responses specific to zebrafish–microbiota interaction were also observed in this study, suggesting the specificity of host–microbiota interaction in fish. Overall, the specificity of microbiota composition and host–microbial interaction in fish highlights the importance of fish microbiota research.

Fish microbiota research has been conducted on various fish species, including model animals, economic fish, and some important wild fish species. Involvement of the microbiota in health, performance, and other physiological functions has been reported in many studies, but a causal relationship is lacking in most cases. By using zebrafish and some other important fish models such as rainbow trout (*Oncorhynchus mykiss*), the mechanisms of host-microbiota interaction in fish have been investigated, including the microbiota's regulation of fish nutrition, immunity, and other physiological functions, and the reciprocal regulation of the micro-

biota by host immune components and non-immune factors. In addition to theoretical value, fish microbiota research may benefit the aquaculture industry, which is currently facing problems such as high intensity, metabolic diseases, water quality, and antibiotics abuse. The industry is eager for novel, environmentally friendly, and green solutions for these problems, and some advances in fish microbiota research are giving rise to products that can be used in the industry, such as probiotics derived from the microbiota and corresponding prebiotics [7]. Several reviews on fish microbiota research have been carried out [8–11]. However, many updated studies have been published in the past few years. In this review, we focus on recent progress in fish microbiota research, including pioneering studies that provide novel ideas about the mechanism of host–microbiota interaction in fish. It is notable that there have been many studies on the microbiota of the skin, gill, and other mucosal surfaces of fish [12–14]. However, we focus on the intestinal microbiota in this review.

## 2. Factors influencing the gut microbiota

The structure of the fish gut microbiota can be affected by host genetics, developmental stage, feeding habits, diet, and the environment. The relative contributions of different factors to the formation of the fish microbiota are still unclear, but host selection has been suggested as the major factor. In this section, we summarize recent studies involving factors that influence the gut microbiota of fish.

### 2.1. Host selection

The gut microbial community is not a simple reflection of the environment but is generated by species-specific selection. As a milestone study on this subject, Roeselers et al. [15] revealed a striking similarity between the gut microbial composition of zebrafish reared in lab facilities and those caught in nature, with a shared core gut microbiota, which indicated that the host intestinal habitat selected for specific bacterial taxa. A core microbiota was also identified in European sea bass (*Dicentrarchus labrax*) across different dietary conditions and time series [16]. In the same study, one aspect of the mechanisms associated with microbial interactions and strain variability was revealed to be responsible for the maintenance of the core microbiota [16]. Nevertheless, the presence of a core microbiota suggests the importance of host selection in microbiota assembly. Vestrum et al. [17] studied the influence of different source bacterial communities on the gut microbiota assembly of Atlantic cod larvae (*Gadus morhua*). The results showed that host selection was the major determinant of bacterial community, as the fish microbiota was highly dissimilar from the environmental bacterial communities. Similarly, Li et al. [18] investigated the intestinal microbiota of different species of carp (grass carp, crucian carp, and bighead carp) reared in the same pond. The gut microbial communities of the different carp species were dominated by Fusobacterium, Firmicutes, Proteobacteria, and Bacteroidetes, but the abundance varied significantly among species, suggesting that the gut microbiota resulted from species-specific selective pressures. Daly et al. [19] investigated the intestinal microbiota of common carp (*Cyprinus carpio*) and rainbow trout from different facilities, and found intra-species uniformity of the bacterial community for both fish species, despite distinct rearing environments, thereby demonstrating the importance of host selectivity. As another reflection of host selection, the intestinal microbiota of fish can be affected by life stages. A study of intestinal microbiota at key developmental time points of zebrafish revealed stage-specific signatures in the microbiota. Moreover, the difference between intestinal microbiota and the microbial community in the rearing water increased with age [20]. Li et al.

[21] conducted a long-term study for 1 year of the gut microbiota of newly hatched heterotrophic silver carp (*Carassius auratus gibelio*). The gut microbial community composition showed significant changes corresponding to host age, suggesting that some features associated with the development stages of the host contribute to the determination of the gut microbial community.

### 2.2. Diet

Studies on the human diet and gut microbiota have found that the human gut microbiota is associated with different dietary patterns, foods, and nutrients [22]. Similar conclusions have been made in fish. Walburn et al. [23] found that the largest change in the microbiome of larval Yellowtail Kingfish (*Seriola lalandi*) occurred during a transition of the diet from live feeds to formulated pellets, suggesting that diet plays a key role in microbiome development and assembly. Liu et al. [11] analyzed the gut contents of eight species of wild fish with different feeding habits in the same lake and found distinct gut bacterial communities in carnivorous versus herbivorous fishes. Herbivorous fish were rich in cellulose-degrading bacteria such as *Clostridium*, *Citrobacter*, and *Leptotrichia*, while *Cetobacterium* and *Halomonas* were dominant in carnivores. Although this difference is likely to involve host-specific selection, it also supports the influence of diet on the gut microbiota [11].

Fishmeal replacement is a key topic in aquaculture. A series of studies have shown the impact of fishmeal replacement by other protein sources on the intestinal microbiota of fish. Rimoldi et al. [24] investigated the effect of partial replacement of dietary fishmeal with a mix of poultry byproduct (PSB) meal and vegetable proteins on the intestinal microbiota of rainbow trout. Interestingly, the results showed that the ratio between vegetable and animal proteins plays an essential part in determining the microbiome profiles of rainbow trout; moreover, Firmicutes and Proteobacteria were particularly discriminatory for diet type, with plant ingredients favoring a higher Firmicutes:Proteobacteria ratio than animal proteins. Feeding with plant-based protein results in a significant reduction in the diversity of gut microbiota [25]. Li et al. [26] observed that feeding an insect diet significantly changed the gut microbiota composition of Atlantic salmon (*Salmo salar* L.), with a significant increase in the relative abundance of *Bacillus* in the gut. In a study investigating the effect of insect meal and PSB meal as partial replacements for plant proteins on the microbiota of rainbow trout fed fishmeal-free formulations, Gaudioso et al. [27] found that insect meal inclusion induced a higher abundance of chitin-degrading genera, while PSB increased the relative abundance of protein degraders, suggesting a specific enrichment of bacterial taxa with functions targeting the nutrient ingredients in the diet.

The effect of dietary lipids on the intestinal microbiota of fish has also been investigated. Guo et al. [28] found that vegetable oils with different fatty acid compositions had a significant effect on the abundance and diversity of gut microbes in juvenile golden pompano (*Trachinotus ovatus*), and that fish fed with saturated fatty acids had greater gut microbial diversity. Dietary fatty acids can alter fish microbial community composition. The abundance of *Pseudomonas* was found to be elevated in fish fed a high saturated fatty acids diet, and fish oil supplementation limited the growth of *Pseudomonas* [29].

Similar to the impact of diet, starvation influences the intestinal microbiota of fish. Starvation of hybrid grouper (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂) caused a significant reduction in gut microbial abundance and diversity. After refeeding, the abundance of *Vibrio* increased, and the abundance of *Brevibacillus*, *Bifidobacterium*, and *Alloprevotella* decreased [30]. Tran et al. [31] studied changes in the gut microbiota of grass carp during starvation. Starved fish had higher abundances of *Vibrio* and lower

abundances of *Bacteroides*, *Fusobacterium*, *Coprococcus*, and *Citrobacter* compared with fed fish. Mekuchi et al. [32] investigated the microbiota and host metabolism of leopard coral grouper (*Plectropomus leopardus*). Proteobacteria was found to be the dominant phylum during fasting and Firmicutes during feeding. Furthermore, the microbial diversity under feeding was greater than that under fasting. These results suggest that starvation generally leads to a decrease in gut microbial diversity and impacts the microbiota structure.

## 2.3. Environmental factors

Host selection and diet play major roles in the formation of gut microbiota, but environmental factors also influence the gut microbial community [21,33]. The environmental factors influencing fish microbial communities are mainly water and sediments [17,34–36]. It is notable that the relative contribution of water and sediments to fish intestinal microbiota can differ among fish species. Sheng et al. [37] studied the bacterial communities in water, sediment, and the intestines of three omnivorous fish. The results showed that the intestine microbial communities of *T. houdemeri* and *H. leucisculus* were more similar to the water microbiota, while the intestine microbiota of Oreochromis mossambicus (*O. mossambicus*) was more similar to the bacterial communities of the sediments, which was consistent with the benthic habit of *O. mossambicus*. In terms of the influence of water, the gut microbiota of fish is closely related to the microbial species, temperature, and salinity of the surrounding water [38–41].

The influence of water temperature on the fish intestinal microbiota is an important question, as fish can encounter high water temperature due to global warming or low temperature during overwintering. Zhou et al. [42] treated rainbow trout with higher temperature and found that the diversity of the intestinal microbiota decreased with an increase in temperature, while the abundance of Mycoplasma, Firmicutes, and Tenericutes increased significantly. Guerreiro et al. [43] investigated turbot (*Scophthalmus maximus* L.) juveniles at two rearing temperatures—namely, 15 and 20 °C—and found that the number of operational taxonomic units (OTUs), the richness, and the diversity were higher at 20 °C. Lv et al. [44] evaluated the effect of cold stress on the intestinal microbiota of the large yellow croaker (*Larimichthys crocea*) and found that the diversity and abundance of intestinal microbiota were altered, although the change was not obvious. Kokou et al. [45] found that cold stress reduced the diversity and richness of the intestinal microbiota of blue tilapia, as well as the microbial composition, with an enrichment of some orders of Proteobacteria phylum. Interestingly, the microbial response of cold-resistant and cold-sensitive fish were compared in this study, and the results showed that the microbiome of cold-resistant fish was more resilient to temperature changes, supporting the supposition that host selection is the major factor shaping the microbiota, while water temperature acts as an influencing environmental factor.

Lai et al. [41] evaluated the effects of three different salinity conditions on the gut microbiota of *Oryzias melastigma*: seawater (SW), seawater to 50% seawater transfer (SFW), and seawater–semi-seawater–freshwater transfer (FW). The results showed that the microbial diversity of the SFW and FW groups was higher. The dominant genus in the SW and SFW groups was *Vibrio*, whereas this population was replaced by *Pseudomonas* in FW group. Changes in bacterial communities in environments with different osmotic pressures suggest that gut bacteria may play a role in facilitating host acclimation. Rudi et al. [46] studied the effect of a transition from fresh to salt water on the gut microbiota of farmed Atlantic salmon and found that the bacterial quantity increased by 100-fold in salt water, with enrichment of Firmicutes and decreased abundance of Actinobacteria and Proteobacteria.

## 2.4. Antibiotics and chemicals

In aquaculture, antibiotics are often used to prevent and treat bacterial diseases in fish [47,48]. Antibiotics have changed the way fish interact with exogenous or endogenous microorganisms, as well as with their gut microbial community [49–52]. However, the abuse of antibiotics can cause short-term or long-term damage to the microbial structure of the gut, such as a significant decrease in the abundance and diversity of the microbiota [53–55]. At the same time, the use of antibiotics may lead to an increase in the relative abundance of opportunistic pathogens, including *Osthomonas* and *Aeromonas*, creating a potential threat to fish health [56,57]. Sun et al. [58] established a reliable grass carp model of gut microbiota disruption through treatment with an antibiotic cocktail. The disruption featured a significant expansion of Proteobacteria and suppression of Fusobacteria.

In terms of chemicals, Bozzi et al. [59] found that the intestinal microbiota of Atlantic salmon treated with formalin showed more similarity to that of sick fish than healthy ones, indicating a negative effect of formalin treatment on fish microbiota. de La Torre Canny et al. [60] investigated the effect of the organotin compound tributyltin (TBT)—a chemical previously used in antifouling paints that accumulates in marine sediments—on the intestinal microbiota of zebrafish and observed that TBT altered the intestinal microbiota composition, with a reduced relative abundance of *Plesiomonas* bacteria.

## 3. Effects of the intestinal microbiota on the fish host

Commensal bacteria can regulate many key aspects of host functions involving feeding behavior, energy balance, nutrient metabolism, immune response, and more [61]. In this section, we focus on the roles of the fish commensal microbiota in host metabolism, immune responses, and other aspects of functions.

## 3.1. Nutritional metabolism

The gut microbiota has been regarded as an important factor in regulating nutrition and metabolism in fish. By predicting metagenome function, researchers found that the gut microbiota showed differential metabolic capacity in fish with different microbial compositions, which may contribute to the nutritional metabolism of the host [11,62]. Furthermore, gut microbiota composition had a close relationship with the activity of the intestinal digestive enzymes of fish, such as cellulase, amylase, trypsin, and lipase [62]. Similarly, when studying the gut microbiome of freshwater fish with different feeding habits, Liu et al. [11] found that the gut microbial composition of carnivorous fish species was more related to trypsin activity, while the gut microbiota of herbivorous fish was correlated with cellulase and amylase activities.

The gut microbiota plays a key role in regulating the energy metabolism of mammalian hosts. In fish, studies have shown similar results. Gut microbes promote host energy absorption and regulate the expression of genes related to energy and lipid metabolism in zebrafish [63,64]. By using a gnotobiotic zebrafish model, Semova et al. [64] revealed that the microbiota can stimulate fatty acids uptake and lipid droplets formation in the intestinal epithelium and liver. Guo et al. [65] found that the gut microbiota of zebrafish fed nucleotides can reduce the standard metabolic rate of fish, leading to higher energy gain and growth performance of these fish versus the control group. Zhang et al. [66] isolated a *Citrobacter* strain from the intestine of Nile tilapia (*Oreochromis niloticus*) and found that the bacterial strain could increase the energy harvest of fish on a high-fat diet, which is a conserved fea-

ture of host-microbe interaction. de La Torre Canny [60] observed reduced abundance of *Plesiomonas* bacteria in the gut microbiota of zebrafish exposed to the chemical obesogen tributyltin (TBT), and found that the application of a *Plesiomonas* strain reduced host adiposity in both conventional and germ-free zebrafish. This finding underscores the influence of the microbiota and commensal bacterial strain(s) on the energy balance and metabolic health of fish.

Compared with mammalian studies, the mechanisms underlying the microbiota's regulation of energy metabolism are less understood in fish. Nevertheless, it is likely that some conserved mechanisms exist between fish and mammals, such as the mechanisms involving SCFAs and their receptors, which await further investigation in fish hosts.

Bile acids, as the main component of bile, play an essential role in glucose and lipid metabolism, intestinal homeostasis, and liver and intestinal health. The gut microbiota has been shown to regulate the metabolism of bile acids, which in turn affects the digestion and absorption of fat. Wen et al. [67] revealed that zebrafish bile salts mainly consist of a C27 bile alcohol (5α-cyprinol sulfate, 5αCS) and a C24 bile acid (taurocholic acid, TCA). Both 5αCS and TCA are further modified by the intestinal microbiota of zebrafish, which can augment Fxr activity. Notably, an *Acinetobacter* strain from the zebrafish microbiota was identified as being capable of deconjugating TCA.

The ability of carbohydrate utilization in fish is low compared with that in mammals. It has been suggested that the gut microbiota of herbivorous fish can convert dietary carbohydrates into SCFAs, which play an important role in the nutrition and health of the host [68–70]. Recently, studies have provided more evidence of the gut microbiota's regulation of carbohydrate utilization in fish. *Bacillus amyloliquefaciens* SS1 isolated from the intestine of Nile tilapia was found to improve the metabolic phenotypes of fish fed a high-carbohydrate diet, leading to reduced fasting glucose and lipid deposition. Interestingly, SS1 supplementation increased the abundance of acetate-producing bacteria, which was implicated as the reason for the beneficial effects of SS1 [71]. Similarly, Wang et al. [7] found that *Cetobacterium somerae* (*C. somerae*), which is a common commensal bacterial taxon in fish gut, promoted glucose homeostasis in zebrafish. Further mechanistic investigation revealed that the beneficial effect of *Cetobacterium* was mediated by acetate via parasympathetic activation.

The feed conversion rate of farmed fish determines the growth and yield of fish to some extent and is directly linked to the economic effect. The intestinal microbiota plays an important role in the feed conversion of fish. Studies on the effect of gut microbes on feed conversion have mainly focused on monogastric animals, and related studies are still lacking in fish [72–73]. Probiotics, such as *Lactobacillus acidophilus* [74], *Bacillus coagulans* [75], and *Acinetobacter* [76], can improve fish feed conversion, but the mechanisms involved are not yet known. A study by Dvergedal et al. [77] identified significant associations between the abundance of three microbial OTUs and the metabolic phenotypes of Atlantic salmon involving feed efficiency and carbon metabolism. Bozzi et al. [59] also found that *Mycoplasma* showed a positive correlation with the health and body weight of Atlantic salmon; however, the underlying mechanism requires further investigation.

### 3.2. Immune regulation

Commensal microbes are essential in maintaining the development and maturation of the immune system. The colonization of microbiota in germ-free zebrafish was found to induce the intestinal expression of genes related to innate immune responses, including serum amyloid A1 (*Saa1*), myeloperoxidase (*MPO*), and complement component 3 (*C3*), indicating the function of the microbiota in priming the innate immunity of fish [6]. The NF-κB pathway plays an important role in innate and adaptive immune responses. Microbiota colonization was found to activate NF-κB in the intestinal and extra-intestinal tissues of germ-free zebrafish, supporting the role of the microbiota in immune regulation [78]. More recently, by using the same germ-free zebrafish and microbiota colonization model, Koch et al. [79] found that the microbiota affected intestinal leukocyte populations, with a decrease in macrophages but a rise in neutrophils. Furthermore, the researchers demonstrated that the microbiota-induced intestinal immune regulation was dependent on TLR2 and Myd88 [79]. Notably, zebrafish do not have adaptive immune responses until 4 weeks postfertilization, and the microbiota's regulation of adaptive immunity cannot be studied by using gnotobiotic larval zebrafish. Further studies exploring long-term gnotobiotic zebrafish or other fish models are warranted.

Immunity regulation can be mediated by bacterial components or microbial metabolites. Although related knowledge in fish is far behind that in mammals, recent studies have yielded interesting results in this area. Rolig et al. [80] studied the interaction of a commensal bacterium *Aeromonas veronii* and a zebrafish host, and found that the bacterium can secrete an immunomodulatory protein, AimA, which can ameliorate inflammation in the gut of zebrafish. In terms of microbial metabolites, Shan et al. [81] revealed that microbiota-derived butyrate enhanced the expression of IL-1β, which increased the percentage of intestinal neutrophils and enhanced resistance against pathogenic infection in zebrafish. Interestingly, the initial purpose of this study was to investigate the mechanism of the anti-bacterial effect of a commensal probiotic bacterium, *Pediococcus pentosaceus*, isolated from fish gut. The probiotic strain modulated the microbiota with an enrichment of SCFA-producing bacteria. Thus, this study provides an example of microbiota modulation leading to enhanced immune stimulation. Effects of the intestinal microbiota on fish nutrition and immunity are shown in Table 1 [6,7,59,60,62–67,71 ,77–81].

### 3.3. Endocrinology, the nervous system, and fish development

The microbiota also affects fish development, the nervous system, and endocrinology. Phelps et al. [82] found that early colonization of the intestinal microbiota or individual bacterial species *Aeromonas veronii* or *Vibrio cholerae* in germ-free zebrafish was required for normal neurobehavioral development, while exposure to heat-inactivated bacteria or the microbial-associated molecular patterns pam3CSK4 or Poly(I:C) was not adequate to block hyperactivity in germ-free larvae, emphasizing that the gut microbiota is required for normal neurobehavioral development. A study by Casadei et al. [83] reported that the commensal microbial colonization of germ-free zebrafish resulted in a widespread transcriptional response in the olfactory organ, and that this effect may be associated with the increased expression of RE1 silencing transcription factor (REST), which is a transcriptional factor related to neuronal development and differentiation.

The gut microbiota can also regulate the expression of proliferation-related genes in fish intestinal epithelial cells and can govern tissue maturation and organ function during fish development [60]. In addition, control of the host endocrine system by commensal microbes has been reported. Giorgia et al. [84] found that the use of probiotics (*Lactobacillus* preparations) in tilapia provoked gastrointestinal microbial changes that coincided with the activation of the endocrine system, including increased expression of the genes of the insulin-like growth factors (*igfs*) system.

**Table 1**
Effects of the intestinal microbiota on fish nutrition and immunity.

| Microbial taxa | Phenotype/function | Species | Reference |
|---|---|---|---|
| **Nutritional metabolism** | | | |
| Microbiota | Relates to the activity of intestinal digestive enzymes | Black Amur bream (*Megalobrama terminalis*) | [62] |
| Microbiota | Regulates genes related to lipid metabolism, such as *Apoa4*, *Hsl*, *Cox15*, *Slc2a1a*, and *Lss* | Zebrafish | [63] |
| Microbiota | Stimulates fatty acids uptake and lipid droplet formation in the intestinal epithelium and liver | Zebrafish | [64] |
| Microbiota in fish fed a nucleotide-supplemented diet | Reduces the standard metabolic rate and increases energy gain | Zebrafish | [65] |
| *Citrobacter* sp. S1 | Increases energy harvest | Nile tilapia | [66] |
| *Plesiomonas* sp. ZOR0011 | Reduces host adiposity | Zebrafish | [60] |
| Microbiota | Modifies primary bile salts including 5αCS and TCA | Zebrafish | [67] |
| *Acinetobacter* sp. ZOR0008 | Deconjugates TCA | Zebrafish | [67] |
| *Bacillus amyloliquefaciens* SS1 | Improves metabolic phenotypes of fish fed a high-carbohydrate diet | Nile tilapia | [71] |
| *Cetobacterium somerae* | Promotes glucose homeostasis | Zebrafish | [7] |
| *Pseudoalteromonas* sp., *Bradyrhizobium* sp. | Associated with feed efficiency and carbon metabolism in adipose tissue | Atlantic salmon | [77] |
| *Mycoplasma* sp. | Positively correlates with fish weight | Atlantic salmon | [59] |
| **Immune regulation** | | | |
| Microbiota | Stimulates the expression of serum amyloid A1 (*Saa1*), myeloperoxidase (*Mpo*), complement component 3(*C3*), etc. | Zebrafish | [6] |
| Microbiota | Activates NF-κB signaling | Zebrafish | [78] |
| Microbiota | Regulates intestinal leukocyte status through TLR2 and *Myd88* | Zebrafish | [79] |
| *Aeromonas veronii* Hm21 | Secretes an immunomodulatory protein (AimA) | Zebrafish | [80] |
| Microbiota associated with probiotic *Pediococcus pentosaceus* treatment | Microbiota-derived bytyrate enhances the intestinal expression of IL-1β and subsequently increases the percentage of neutrophils and pathogen resistance | Zebrafish | [81] |

## 4. Host regulation of the intestinal microbiota

Many studies have demonstrated the impact of the microbiota on the host. In contrast, the reciprocal question regarding host–microbiota interaction—that is, how the host regulates the intestinal microbiota—has been much less investigated. Host factors, including immune components and non-immune factors, underpin the homeostasis of the intestinal microbiota and contribute a great deal to microbiome assembly. Knowledge in this area can provide not only insights into the mechanism of host–microbiota interaction but also hints on novel strategies for gut microbiota regulation via targeting host factors.

The innate immune system is the primary defense of the host against pathogens, and immune cells (i.e., macrophages, neutrophils, phagocytes, DC cells, natural killer (NK) cells, etc.) and immune factors play essential roles in the maintenance of innate immune homeostasis. Earley et al. [85] studied the effect of intestinal macrophages on the microbiota by using a macrophage-deficient interferon regulatory factor *irf8* zebrafish mutant. Their results showed that the intestinal microbiota was significantly altered in the mutant compared with wild-type zebrafish, and that this destabilization of the intestinal commensal microbiota was associated with a reduction in gut C1q genes expression, while macrophage rescue of *irf8* mutants recovered commensal microbes.

Adaptive immunity, which involves immune responses mediated by T and B lymphocytes, also plays a role in maintaining homeostasis of the gut microbiota [86]. Although related knowledge in fish have been insufficient, some studies have demonstrated regulation of the fish gut microbiota via adaptive immunity. Okamura et al. [87] knocked out IL-17A/F1, a hallmark inflammatory cytokine produced by Th17 cells, in Japanese medaka (*Oryzias latipes*). The results showed that the intestinal microbiota of IL-17A/F1-KO fish differed from that of the wild type, with significantly higher abundance of *Verrucomicrobia* and *Planctomycetes*; moreover, *Plesiomonas shigelloides* was the dominant species in the mutant. Studies have also demonstrated the interac-

tion of mucosal secretory immunoglobulins (sIgs) with commensal bacteria in the gut of rainbow trout, indicating an evolutionarily conserved role of sIgs in microbial homeostasis. Xu et al. [88] depleted the mucosal IgT of rainbow trout and found that IgT deficiency leads to gut dysbiosis, as evidenced by the loss of sIgT-encapsulated beneficial bacteria and the expansion of pathobionts. Perdiguero et al. [89] revealed that secretory IgD was capable of interacting with the intestinal commensal microbiota of rainbow trout, which in turn stimulated the transcription of IgD genes in intestinal B cells, suggesting that secretory IgD may play an evolutionarily conserved role in mucosal homeostasis.

Apart from the immune system, other host-derived factors may also regulate the colonization and homeostasis of commensal bacteria. A pioneering study in zebrafish showed that the commensal bacterium *Aeromonas veronii* modulated motility by sensing host-released amino acid signals through proline diguanylate cyclase enzyme (SpdE). The sensing of free proline by SpdE resulted in lower production of intracellular c-di-GMP, a second messenger controlling bacterial motility, leading to increased bacterial motility and colonization in zebrafish. This study demonstrated for the first time how bacteria sense host-emitted cues, which trigger immigration into hosts [90]. Table 2 [85,87–90] lists the host regulation of the intestinal microbiota in fish.

## 5. Interactions between the intestinal microbiota and pathogens

Studies have shown that the intestinal microbiota is the third player in the interaction between the host and pathogens. This finding brings novel insights into a triangular relationship among the host, microbiota, and pathogens. The intestinal microbiota hinders pathogens through direct inhibition or nutrient competition, colonization resistance, or interaction with host factors. Pathogen infection can also impact the intestinal microbiota. Studies on microbiota-mediated pathogen inhibition via the stimulation of host factors in fish are rare. In this review, we discuss two aspects

**Table 2**
Host regulation of the intestinal microbiota in fish.

| Host factors | Phenotype | Species | Reference |
|---|---|---|---|
| Intestinal macrophages | *irf8* mutants with deficient intestinal macrophages have disrupted gut microbiota and the outgrowth of a rare bacterial species | Zebrafish | [85] |
| IL-17A/F1 | IL-17A/F1-knockout fish show different intestinal microbiota, with significantly higher abundance of *Verrucomicrobia* and *Planctomycetes*, and a drastic enrichment of *Plesiomonas shigelloides* | Japanese medaka | [87] |
| Mucosal IgT (sIgT) | Depletion of sIgT induces gut dysbiosis, with the loss of sIgT-encapsulated beneficial bacteria and the expansion of pathobionts | Rainbow trout | [88] |
| Secreted IgD | Secreted IgD binds to gut commensal bacteria and establishes a mutualistic relationship with commensals | Rainbow trout | [89] |
| Host-released amino acid signals | Increases the motility and host colonization of commensal *Aeromonas veronii* | Zebrafish | [90] |

of the triangular interactions among the host, microbiota, and pathogens: direct inhibition of pathogens by the microbiota and the impact of pathogen infection on the intestinal microbiota in fish.

### 5.1. Direct inhibition and nutrient competition

Many studies have reported the isolation of probiotic bacteria exhibiting inhibitory activity against aquatic pathogens [91,92]. Similarly, commensal bacteria can produce antagonistic substances to inhibit pathogens [93]. From marine fish, Sequeiros et al. [94] isolated *Lactococcus lactis* TW34, a nisin Z producer (bacteriocin nisin Z) that can inhibit the growth of *Lactococcus garvieae*. *Enterobacter* sp. C6-6 has been isolated from trout intestines and was shown to inhibit *Flavobacterium psychrophilum* both *in vitro* and in a challenging study of rainbow trout. Further investigation showed that the inhibitory effect was mediated by a small lipoprotein entericidin [95]. Notably, in many studies, the antagonistic effect of commensal bacteria against fish pathogens has been investigated *in vitro*, while the contribution of inhibitory compounds *in vivo* has rarely been confirmed. By constructing an isogenic mutant deficient in the production of the entericidin, Schubiger et al.'s [95] study was the first to demonstrate the contribution of inhibitory substances to the protection of fish against pathogens. Microbiota components can also inhibit pathogens by means of nutrient competition. For example, Smith and Davey [96] isolated a strain of *Pseudomonas fluorescens strain* F19/3 from salmon, which inhibited *Aeromonas salmonicida* by competing for free iron.

### 5.2. Colonization resistance

Colonization resistance is the ability of the microbiota to resist the invasion of pathogens. Commensal bacteria can compete with pathogens directly through their ecological occupation to protect the host from infection [97]. Germ-free zebrafish were found to be more susceptible to *F. columnare* infection than their conventional counterparts, indicating that the microbiota conferred resistance against infection. Further investigation showed that 10 culturable bacterial species were sufficient to protect zebrafish from infection. Among them, the bacterium *Chryseobacterium massiliae* can provide protection individually, while an assembly of the other nine species—each of which had no individual protection activity—conferred a community-level resistance [98]. Similarly, conventional rainbow trout were found to be more resistant to *F. columnare* infection than their germ-free counterparts. Recolonization of 11 culturable bacterial strains recapitulated the protection of the microbiota, and this protection was attributable to one *Flavobacterium* strain [99].

### 5.3. Effect of pathogen infection on the fish gut microbiota

Disease not only marks the overall loss of gut microbiota diversity but also correlates with changes in fish immune status and the microbiota barrier system [14]. The intestinal hindgut microbiota was found to differ between healthy Atlantic salmon and unhealthy ones with *Aeromonas salmonicida* infection, especially at the taxonomic levels of family and genus [100]. dos Santos Silva et al. [101] infected Nile tilapia with *Streptococcus agalactiae* and found that bacterial infection reduced gut microbiota variability. Similarly, Zhang et al. [102] infected zebrafish with *Streptococcus agalactiae* and found that the composition of the intestinal microbiota was partially affected by bacterial infection, with enrichment of potentially harmful bacteria such as *Aeromonas veronii* in the intestine. Zhou et al. [103] analyzed the intestinal microbiota of grass carp after *Aeromonas hydrophila* infection. The results showed fluctuations of the intestinal microbiota at different time points after infection, with the greatest fluctuation occurring in Proteobacteria. In a study of a spring viremia of carp virus (SVCV) infection model of common carp, after viral infection, the abundance of Proteobacteria in the gut increased, whereas the abundance of Fusobacteria decreased. Compared with normal fish, the abundance of dominant commensal microbiota was found to be decreased in SVCV-infected fish, while the abundance of the opportunistic pathogen increased, which increased the possibility of secondary infection of carp [3].

The interaction among parasites, host, and microbiota is increasingly being understood as an important determinant of disease progression [104]. Perturbation of the skin mucosal microbiome by salmon lice infection has been observed in Atlantic salmon [104]. Moreover, Vasemägi et al. [105] studied the effect of myxozoan *Tetracapsuloides bryosalmonae* infection on the gut microbiota of wild brown trout (*Salmo trutta*). The results showed that the parasite load was positively related to the richness of the gut microbiome, suggesting that brown trout with large parasite burdens were prone to lose microbial homeostasis [105].

## 6. Gnotobiotic fish models

Germ-free animals are important tools for the study of the intestinal microbiota. Gnotobiotic zebrafish are a classic model used to study host-microbe interaction in fish and vertebrates [24,106–110]. As an improvement of this model, Canny et al. [60] established a long-term sterile zebrafish-rearing method (raised to 35 days post fertilization using sterile Artemia and Tetrahymena as feed. This long-term sterile zebrafish model can make up for the drawbacks of the previous model and will be very useful for many aspects of host-microbe interaction research with a zebrafish model.

Gnotobiotic models of economical fish species have also been reported, which have more relevance for fish farming. Pérez-Pascual et al. [99] established a germ-free and gnotobiotic rainbow trout model, which can be raised in sterile conditions with no growth difference with control fish 35 days post-hatching. Situmorang et al. [111] developed a gnotobiotic Nile tilapia larvae model via axenic Artemia feeding, providing a research tool to investigate the effects and modes of action of probiotics under con-

trolled conditions. Dierckens et al. [112] used gnotobiotic sea bass larvae to study the three opportunistic pathogens *Aeromonas hydrophila* and *Listonella anguillarum* serovar O1 and O2a, and showed that only *Listonella anguillarum* serovar O2a caused a significant increase in juvenile mortality.

## 7. Development and application of green inputs in aquaculture based on fish microbiota research

Our understanding of the key role played by the microbiota in fish health and diseases opens up new possibilities for developing solutions to regulate intestinal microbiota composition, with the aim of improving the growth, metabolism, immunity, and disease resistance of fish. The ban on antibiotics in animal feed creates an urgent need to develop novel green inputs as alternatives. Research on the fish microbiota may give rise to green inputs as antibiotic alternatives that will be critical for the sustainable development of the aquaculture industry.

### 7.1. Probiotics and prebiotics

The health of the host can be improved effectively by improving the abundance of beneficial bacteria in the gut, which may include the direct addition of probiotic bacteria and the addition of prebiotics that can improve the abundance of beneficial bacteria in the gut. Traditional probiotics such as lactic acid bacteria and yeasts isolated from terrestrial animals or other sources are not the dominant indigenous microbes in the digestive tract of fish, and their use in fish may risk causing intestinal injury or microbial dysbiosis in some cases [113,114]. Identifying commensal beneficial bacteria in fish is of great value for the development of novel probiotics for aquaculture. Our work has demonstrated that *C. somerae*, which is an abundant bacterium in many fish species, can be used as a novel probiotic product with multiple beneficial effects [7,115–117]. The isolation of commensal bacteria belonging to traditional probiotic species has also been reported in some studies [71,82]. *Lactococcus lactis* L19 and *Enterococcus faecalis* W24 isolated from the *Channa argus* intestine increase the activity of digestive enzymes in the digestive tract, and *Lactococcus lactis* (*L. lactis*) L19 promotes an increase in intestinal beneficial bacteria and decreases the abundance of pathogens. *Bacillus cereus* KAF124 and *Bacillus thuringiensis* KAF135 isolated from the marine fish *Moolgarda seheli* can effectively improve the survival of *Moolgarda seheli* after *Aeromonas hydrophila* infection [118].

Prebiotics are feed additives that can stimulate the growth of probiotics in the host [119–121]. Common aquatic prebiotics include inulin, fructo-oligosaccharide, and mannan oligosaccharides [120–122]. Dietary supplementation of 0.4%–0.6% chitosan oligosaccharide can enhance immunity and resistance to pathogens in grouper (*Epinephelus fuscoguttatus*) [123]. Galactomannan oligosaccharides (GMOS) increased the abundance of Bacteroidales, Lactobacillales, and Clostridiales in European sea bass when supplemented as feed additives [124]. As novel probiotics are identified in fish, the study of prebiotics targeting the growth of new probiotic species is warranted. For example, our work has demonstrated that mannose promotes the enrichment of *C. somerae* in fish, and a dietary supplementation of mannose exerts similar beneficial effects as *C. somerae* [7].

### 7.2. Other potential green inputs

A reasonable deduction is that it is possible to improve fish health by reducing harmful bacterial species in the fish gut microbiome. However, there is still a gap in research on the identification of harmful components in the fish microbiota. Although some opportunistic pathogens are considered to be harmful species in the fish microbiome, knowledge of their causal effects is still lacking. Nevertheless, strategies that target pathogens can be used for this purpose after identifying the harmful pathogens in the microbiome, such as quorum quenching techniques [125–127]. As mentioned above, it is also possible to regulate the intestinal microbiota by targeting host factors. Our previous work showed that extracellular polysaccharides of *Lactobacillus rhamnosus* can activate intestinal HIF-1α, increase the expression of antimicrobial peptides, and thus maintain microbial homeostasis in zebrafish [128]. Therefore, intestinal HIF-1α can be regarded as a target for microbial regulation, and the identification of other host factors will give rise to novel targets for the development of green inputs that improve microbial composition and homeostasis in fish.

## 8. Perspectives

Studies involving the intestinal microbiota of fish have dramatically increased in the past few years. However, many of these studies evaluate the microbial composition by means of 16S ribosomal RNA (rRNA) sequencing and present the results in a descriptive way. Studies on the functions of the intestinal microbiota have increased in recent years but are still relatively rare compared with the overall number of published papers involving the fish microbiota. The current gnotobiotic fish models await further improvement in order to be better used as a tool for research on the mechanisms of host-microbiota interaction in fish. Furthermore, to establish causal relationships about the bacterial species responsible for the function(s) of the microbiota, culturing the fish microbiota is another important subject for further investigation, and culturomic approaches that have been used in humans and other mammals should be adopted.

The gut microbiota could be used as an indicator for evaluating fish health. Furthermore, knowledge about the features of gut microbiome homeostasis and the health of fish can provide guides for how to regulate the intestinal microbiota in order to improve fish health and performance. We found that a higher/lower Fusobacteria:Proteobacteria ratio reflected the homeostasis/dysbiosis status of zebrafish microbiota, which corresponded to liver health [121]. In addition, our ongoing study on economic fish suggests that a higher (Fusobacteria + Firmicutes + Bacteroidetes):Proteobacteria ratio is correlated with fish health (unpublished data). However, a large research gap still remains in the current understanding of the structural features and signatures of gut microbiome homeostasis, health, and dysbiosis in fish. For this purpose, more comprehensive investigations about the ecological interactions in the fish microbiome should be conducted. The standardization of fish microbial profiles should also be considered [129], as this would minimize confounding factors and benefit the deduction of basic rules in terms of gut microbiome homeostasis and dysbiosis.

Research on the fish gut microbiota will give rise to more green inputs for aquaculture. Novel probiotics will be identified with the culturing of fish microbiota. Postbiotics and paraprobiotics derived from probiotics will also be developed [94,110]. Furthermore, the study of compounds and natural ingredients targeting beneficial and harmful bacteria or host factors that benefit microbial homeostasis will be a research direction that will lead to the development of green inputs for aquaculture. Overall, fish microbiota research can not only provide insights into the mechanism of host–microbiota interaction but also give rise to novel tools to promote fish health and the sustainability of the aquaculture industry.

## Compliance with ethics guidelines

Yinyin Luan, Ming Li, Wei Zhou, Yuanyuan Yao, Yalin Yang, Zhen Zhang, Einar Ringø, Rolf Erik Olsen, Jihong Liu Clarke, Shouqi Xie, Kangsen Mai, Chao Ran, and Zhigang Zhou declare that they have no conflict of interest or financial conflicts to disclose.

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