



Gut Microbiota of *Salmo trutta fario* and *Oncorhynchus mykiss*: Implications for Fish Health and Aquaculture Management

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Abstract

This review compares the gut microbiota of two economically significant freshwater fish species from India's Himalayas, *Salmo trutta fario* and *Oncorhynchus mykiss*. Knowing the composition and variety of gut microbiota in these two species is crucial to their health and productivity. Recent sequencing advances have helped to find and characterize different microbial communities in numerous habitats, including fish gut microbiota. The gut microbiota of these two fish has been compared using Himalayan literature. Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria dominate both species' gut microbiota, but their relative abundance differs. *S. trutta fario* has a more metabolically varied gut microbiota than *O. mykiss*. Scientific literature reveals that diet, water quality, seasonality, and host genetics influence the fish gut microbiome. Nutrition greatly affects the intestinal microbiome of animals. This study examines how the gut microbiota affects these fish's immune systems and probiotics' ability to boost their health and performance. This review reveals how changing the gut microbiota of these species might increase their health and production. In order to enhance the health of these commercially important fish species, there is a need for more research to understand the complex interaction between gut microbiota, host genetics, and environmental factors.

Keywords: Gut Microbiota; Probiotics; Microbial Communities; *Salmo trutta fario*; *Oncorhynchus mykiss*; Himalayan Region; India

Abbreviations: GI: Gastrointestinal; GALT: Gut Associated Lymphoid Tissue.

Introduction

The gut microbiota is made up of microorganisms that live on body surfaces and cavities. Commensal or mutualistic bacteria alter host biology [1]. Gut flora affects vertebrate digestive health. Allochthonous and autochthonous gut microbiota exist. Allochthonous bacteria are foreign to the

host ecosystem, while autochthonous bacteria are native. These bacteria form long-term relationships with the host. They shield hosts from pathogens. Host-related factors impact the fish gut microbiota and can benefit or harm the host. The gut microbiome balances fish Metabolism, digestion, and immunity is controlled. Fish health, behavior, and growth depend on the gut microbiota [2,3]. Culture-oriented methods, which underestimate microbial diversity, dominate fish intestinal microbiota research [4-9]. Culture-based microbiota function studies don't explain

in vivo processes. Environmental factors, host genetics, developmental stage, and immunological conditions affect the fish gut microbiome. Foregut and hindgut microbial compositions differ [10-17]. Proteobacteria, Firmicutes, and Bacteroidetes dominate most fish gastrointestinal tracts. Actinobacteria, Fusobacteria, Bacilli, Clostridia, and Verrucomicrobia dominate fish gut microbes. Development, digestion, immunology, and disease resistance depend on the fish gut microbiota [18]. The gut microbiome affects fish behavior, digestion, and immunity.

Food, environment, and host development affect gut microbiota. Fish guts include pathogenic, mutualistic, and benign microorganisms. These species affect host immunity and physiology. Host size, developmental stage, nutrition, environment, and season affect fish gut microbiota [19-21]. Aquaculture needs hybrid fish. The gut microbiota affects growth, behavior, digestion, and immunity. Pathogenic, mutualistic, and benign commensal microorganisms influence fish gut physiological functioning. Fish gut microorganisms depend on environment, trophic level, eating behavior, and host-specific traits.

The gut microbiota impacts host fish development, metabolism, feeding behavior, and immune response [22-24]. Fish intestines have 107–111 microorganisms per gram. Digestive enzymes and water quality can affect gut flora. Due to AMR bacteria and hard-to-treat fish diseases, gut microbes may help fish as probiotics. Understanding fish gut microbiomes helps aquaculture managers improve fish health and well-being [24,25]. Metagenomics and next-generation sequencing enable ambient sample microbial genome analysis, transforming fish gut microbiota research. Brown trout's endogenous autochthonous gut microbiota is unknown; however, its allochthonous microbiome has been studied. Effective management requires understanding gut microorganisms' role in host physiology.

Composition of Fish Gut Microbiome

Microorganisms are present in various fish organs, including the skin, digestive tract, and internal organs. The gut microbiota, which is influenced by factors such as environmental conditions, hereditary patterns, developmental stages, nutrition, and diet composition, is highly diverse. Significant differences in the composition of gut microbiota have been reported between marine and freshwater fish, fresh water species having a higher abundance of *Acinetobacter* sp., *Aeromonas* sp., *Flavobacterium* sp., *Lactococcus* sp., *Pseudomonas* sp., *Bacteroides* sp., *Clostridium* sp., *Fusobacterium* sp., and members of *Enterobacteriaceae* [18,26-29]. In freshwater fish, carnivores and omnivores have shorter gut lengths and lower short-chain fatty acids (SCFAs) than marine fish due to unassimilable algal constituents.

Enzyme-producing bacteria have been identified in the fish gut microbiota, with the two main phyla being Bacteroidetes and Firmicutes in mammals and Proteobacteria in fish. In freshwater and seawater fish, the dominant phyla were Proteobacteria and Firmicutes, with the dominant genus including *Citrobacter* sp., *Exiguobacterium* sp., *Pseudomonas* sp., *Lactococcus* sp., *Acinetobacter* sp., *Anoxybacillus* sp., *Escherichia coli*, and *GeoBacillus* sp. *Oncorhynchus* sp., Firmicutes play a significant role in fish probiotic properties and energy reabsorption. The presence of similar bacterial phyla, regardless of taxonomic position or geographical location of the fish, indicates the vital role of the microbiome in host functions such as nutrient absorption, digestion, and immune response [30-33]. Microbial diversity in *Salmo* sp. and *Oncorhynchus* sp.

Fish gastrointestinal (GI) microbiota has been studied for over a century by several pioneers. However, the majority of research into gut microbial communities focuses on mammals, despite fish representing the greatest species diversity among vertebrates [6]. To comprehend the co-evolution of gut microbes and vertebrates, broad fish analyses are crucial. Studies have evaluated the gut microbiota of various fish species, such as zebrafish, guppy, and rainbow trout. In addition, they have evaluated economically valuable aquatic animals, including carp, Atlantic salmon, sturgeon, and Atlantic cod. Nonetheless, these studies provide only limited insight into the composition of the gut microbiota in fish and co-evolutionary patterns [8,17,31,34-37]. In recent years, attempts have been made to reveal the structure of the intestinal microbiome of a few fish species that inhabit a wide range of habitats. However, knowledge of microbiota variations in fish is incomplete, and it remains uncertain whether structural microbiome alterations found in diseases are epiphenomenal or causal.

Fish are the first vertebrate group with full capacities to mount disease resistance strategies through adaptive and innate immune mechanisms. Therefore, microbiome research in teleost fish lags behind higher taxa. However, our understanding of fish microbiota variations is still evolving [38-41]. Microbial communities colonize virtually every surface of the host organism exposed to the external environment. These communities include members of the prokaryota, eukaryota, and viruses, which together provide an enormous enzymatic capacity and play a key role in controlling many aspects of host physiology. However, the core microbiota is instantly altered, resulting in dysbiosis. Such dysbiosis represents the state in which the microbiota's ecological balance is critically disturbed, triggering perturbations in the meta-community structure that may damage some of the less represented beneficial species, thereby producing pathological states at any developmental stage of the host [42-44].

In antibiotic-induced dysbiosis, the microbiota's functional aspects are affected, including the microbiota's ability to break down fibers and starches into absorbable short-chain fatty acids (SCFAs), resulting in high levels of undigested carbohydrates that trigger a pathological state in the host [44]. Over the past few years, the fundamental role played by the microbiota in the induction, knowledge, and function of the vertebrate immune system has revolutionized the field of immunology, ranging from fish to humans [45,46]. Thus, it is now widely accepted that the microbiome benefits vertebrates in maintaining overall health. In fish, the interrelations between both entities are even more complex since microbes and animals share their outer environment, which is characterized by a high load of organic material that directly supports microbial growth [27,47].

Most aquaculture probiotics include lactic acid bacteria

and *Bacillus* sp. Isolated from mammals or terrestrial environments [48]. Although several studies have shown that exogenous bacteria benefit fish, many use lactic acid bacteria, or *Bacillus* sp. conclusive or showed no beneficial effect on the host [49,50]. Studies have examined the microbial community association with the fish intestines of *Salmo trutta fario* and *Oncorhynchus mykiss* in the Himalayan region of India. This is to understand their gut microbiota composition and co-evolution patterns. These studies have shed light on the functional diversity of the gut microbiota of these fish species and their associated environmental and host factors. A comparative analysis of the gut microbiota of these two fish species in the Himalayan region of India may provide new insights into their ecological niches, as shown in Table 1, nutritional physiology, and evolutionary history [8,37,51].

S No.	Isolate Bacteria	<i>Oncorhynchus mykiss</i>	<i>Salmo trutta fario</i>	References
1	Gram negative aerobic motile rods			
(i)	<i>Alcaligenes</i> sp., <i>Pseudomonas</i> sp. and <i>Shewanella</i> sp.	Present	Absent	Zhang, et al.
2	Gram negative aerobic coccobacilli and rods			
(i)	<i>Acinetobacter</i> sp., <i>Moraxella</i> sp., and psychrobacter	Absent	Present	Bøggwald, et al.
(ii)	<i>Enterobacteriaceae</i> (<i>Citrobacter</i> sp., <i>Enterobacter</i> sp., <i>Edwardsiella</i> sp., <i>Hafnia</i> sp., <i>Klebsiella</i> sp., <i>Proteus</i> sp., and <i>Providencia</i> sp.);	Present	Absent	Komaroff, et al.
(iii)	Micrococcaceae (<i>Kocuria</i> <i>Macarococcus</i> sp., <i>Planococcus</i> sp., and <i>Staphylococcus</i> sp.)	Present	Absent	Bøggwald, et al.
(iv)	<i>Lactococcus</i> sp.	Absent	Absent	Xia, et al.
(v)	<i>Lactobacillus</i> sp.	Present	Present	Pelto, et al.
(vi)	<i>Micrococcu</i> sp. <i>Bacillus</i> sp.	Absent	Present	
(vii)	Proterobacteria and fusobacteria	Present	Present	
(viii)	Fermicutes and Bacteroids (diet dependent)	Absent	Present	Miettinen, et al.

Table 1: Showing guts microbiome diversity in *Salmo* sp. and *Oncorhynchus* sp.

In mammalian hosts, butyric acid plays a crucial role in providing energy to gastrointestinal cells. It exhibits various health benefits, such as enhancing mucus production, acting as an anti-carcinogen and anti-inflammatory agent, and regulating satiation. Butyrate is observed in the gut of both herbivorous and omnivorous fish, but not in carnivorous species due to their low carbohydrate diets. Studies have shown that butyric acid can inhibit potential pathogens in freshwater fish and is sold as a food additive to promote fish health and growth. However, trials using blends of sodium

butyrate and other additives have not succeeded. Further research should explore the role of fusobacteria in the gut microbiota of fish species [52-58].

Importance of Fish Gut Microbiota

The gut microbiota is considered an "extra organ" that plays a crucial role in intestinal development, physiology, growth, and overall health. Studies have revealed that the gut microbiota regulates feeding, digestion, metabolic processes,

and immune responses. This helps maintain energy homeostasis in the body. The fish gut microbiota plays a crucial role in several aspects of fish biology [19-21,59].

Studies have shown that the gut microbiota plays a significant role in host growth and development, behavior, and immune system function. Animal guts provide vital functions that cannot be performed by the host itself. The metabolic role of the gut microbiome has gained attention, especially with the refinement of high-throughput sequencing and computational tools. Dysbiosis can lead to various diseases, whereas a state of “normobiosis” dominated by beneficial microorganisms promotes host health [60].

The gut microbiota of aquatic vertebrates synthesizes vitamins and amino acids. This synthesis is positively correlated with anaerobic bacteria abundance in the genera *Bacteroides* sp. and *Clostridium* sp. in Nile tilapia.

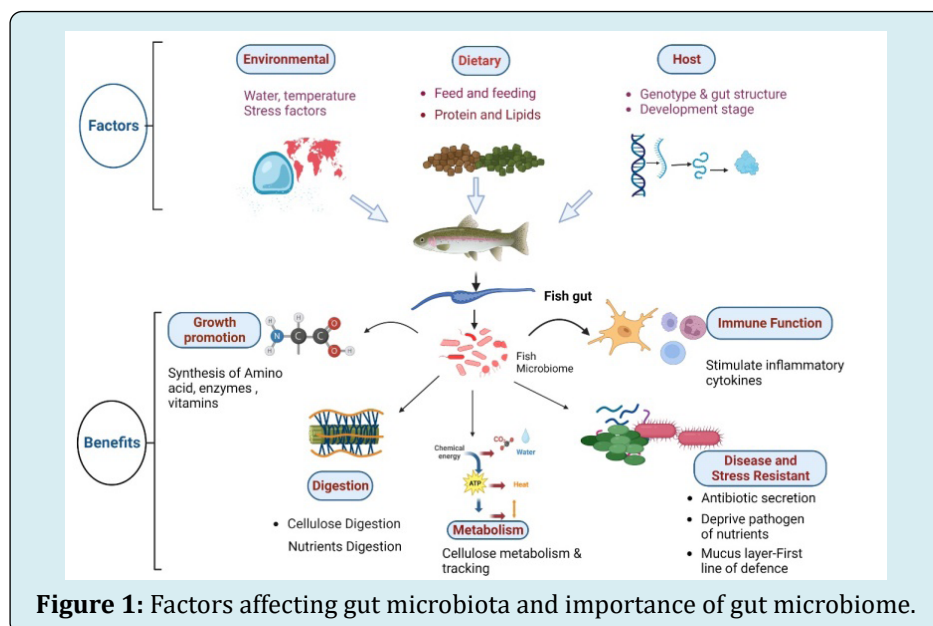
The gut microbiota plays a vital role in maintaining fish health in aquaculture, as it protects fish against infections caused by waterborne pathogens and opportunistic commensals. Diversity and stability of the gut microbiota are crucial to maintaining a healthy gut microbiome. This can be achieved through probiotic bacteria, such as *Bacillus* sp. and *Lactobacillus* sp. that stimulate the expression of inflammatory cytokines, increase mucus layer production, and enhance phagocytic activity [61-64].

Manipulating the gut microbiota in fish can be achieved through various methods, including dietary proteins, lipids, probiotics, and prebiotics. Dietary proteins and lipids modulate the gut microbial composition by providing

suitable substrates for bacterial growth, while probiotics and prebiotics selectively ferment ingredients that result in specific changes in the gastrointestinal microbiota, thereby conferring health benefits to the host [65,66].

Salmo trutta fario and *Oncorhynchus mykiss*, which are significant fish species, have microbial communities in their guts, predominantly consisting of bacteria. Microbial communities' composition and diversity are influenced by habitat, feeding habits, and physiological conditions. Potentially pathogenic bacteria have also been reported in the gut microbiota of these fish species. Probiotics have been studied as a potential tool to modulate the endogenous gut microbiota and improve brown trout growth performance, immunity, and disease resistance [67].

Few beneficial effects of gut microbiota are conferred on host fish, and such impacts can be systematically classified into biological and immunological impacts. These functions of bacteria in fish GI tracts are discussed on the basis of studies carried out in this sector. The importance of gut microbiota can be seen in Figure 1. The gut microbiota's physiological impact on the host fish is perceived through a number of interactions between metabolic ability and the host metabolism of the gut microbiota. This relationship is defined by the failure of host fish to break down certain components of food, such as xenobiotic compounds or cellulose. These are used as a medium of energy by the host gut microbiota of fish, and the metabolites generated from this are used as an energy source by the fish. Another component that contributes to the physiological effect on the fish gut microbiota is the synthesis or part of the synthesis of an essential supplement like a vitamin [9].



The presence of native Gastrointestinal (GI) microbiota in fish has been recognized, though little is known about the bacterial communities and their establishment, diversity, and most importantly, their role in fish nutrition and health [68,69].

Role of Fish Gut Microbiota in Disease Resistance

The gut microbiota is crucial for the development of the fish's immune system and optimal nutrient absorption. Beneficial bacterial strains have been developed for the treatment of microorganism-induced bacterial diseases. The isolation of *Lactococcus lactis* from marine fish, which produces bacteriocin nisin that inhibits *Lactococcus garvieae*, highlights its potential as a preventative strategy for lactococcosis. Similarly, bacteria isolated from the gut of a deep-sea shark had antagonistic activity in the fish gut. The gastrointestinal tract is the main site for interactions between environmental microorganisms and the host. The mucus layer provides the first line of defense against pathogenic challenges by secreting various protective and antimicrobial substances [70,71].

Role of Fish Gut Microbiota in Digestion

Intestinal microflora enzymes may help digest cellulose and other substrates poorly absorbed by mammals [72]. Fish intestines are colonized by many heterophilic aerobic and anaerobic microorganisms. The gut microbiota provides vitamins, amino acids, and enzymes by breaking down food [73]. Digestive tract anatomy determines gut bacteria communities.

There is a large and diverse enzymatic potential in gut bacteria that may interfere with a host animal's metabolism. Aquatic bacteria may affect the fish gut microbiome [74]. Tilapia (*Oreochromis mossambica*), Chinese grass carp (*Ctenopharyngodon idella*), and common carp (*Cyprinus carpio*) digestive tract microbes show cellulolytic and amylolytic activities [75,76]. Studies confirmed the existence of an enzyme-producing microbial population in fish gut and demonstrated that the bacterial load in feeds affects intestinal microflora by showing that amylolytic bacterial populations consisting of *Bacillus* spp. are significantly present in the digestive tracts of *L. bata* and *C. mrigala*, respectively [75,77].

Fish digestive system enzyme-producing microbes can be employed as probiotics in fish diets, especially larval ones. Probiotics are used by isolating helpful intestinal bacteria from adult animals and adding large amounts to the feed of young animals of the same species [78,79]. Commercial aquaculture requires probiotics for creating diets at larval stages to limit feed preparation expenses. Fish lack cellulase,

yet their digestive systems include aerobic and facultative anaerobic bacteria communities that break down plant food [75,76,80].

Impact of Fish Gut Microbiota on Host

Gnotobiotic studies also indicate GI microbe involvement in fish immunity and xenobiotic metabolism. Microbial regulation of glycoprotein production in the GI tract is reported in *Dicentrarchus* sp. [81]. The GI microbes play a critical role in the development and maturation of GALT (gut-associated lymphoid tissue), which in turn mediates a variety of host immune functions [82]. A complex and integrated interaction between the epithelium, immune components in the mucosa, and microbes is responsible for the development and maturation of the gut-associated immune system of the host. Gnotobiotic studies in different animal models also support this notion [83]. Several mechanisms are proposed for the GI bacteria's involvement in GALT development. Bacteria could stimulate B cell proliferation in GALT through a classical antigen-specific immune response like protein A of *Staphylococcus aureus* and protein L of *Peptostreptococcus magnus* [84,85].

The gut-associated lymphoid tissue (GALT) must develop mechanisms to distinguish between potentially pathogenic microorganisms and commensal microbiota to decide whether to show tolerance or induce an immune response. This is because the intestinal microbiota is constantly in direct contact with the gut mucosa. The mucosal immunity system regulates cells like phagocytes and lymphocytes found in GALT, resulting in powerful immune responses against dangerous foreign pathogens. This is done by coordinating, targeting, and mounting quickly for pathogen presentation and destruction. Normal intestinal mucosal colonization has a positive impact on immune regulatory processes, and the disruption of these processes by an unbalanced microbiota may aid in illnesses [86,87].

Impact of Fish Gut Microbiota on Host Nutrient Metabolism

The gut microbiota of hosts is involved in nutrient metabolism, especially cholesterol metabolism and tracking. It has been reported that the gnotobiotic *Danio* sp. larvae failed in the uptake of protein macromolecules, with a significant difference in the levels of farnesyl diphosphate synthetase and apolipoprotein B compared with conventional larvae [88]. Moreover, the microbial upregulation of apolipoprotein B, which plays a pivotal role in intra- and extracellular cholesterol tracking, and the downregulation of liver-specific cholesterol 7 α -hydrolase, which catalyzes the first step in cholesterol catabolism and bile acid biosynthesis, indicate the microbial modulation of cholesterol metabolism and

tracking [88,89].

The gut microbial population represents a very significant and diversified enzymatic potential. The enzymatic mass present in the digestive tract might interfere with a major part of the host animal's metabolism. Microflora present in the fish digestive tract grow upon the food absorbed by the host animal, digestive secretions, and fragments scaled off the mucosal epithelium [73]. Cellulolytic bacteria present in the fish digestive tract influence metabolism, and a correlation exists between the degree of cellulolytic activity and feeding habits.

Impact of Fish Gut Microbiota on Host Health

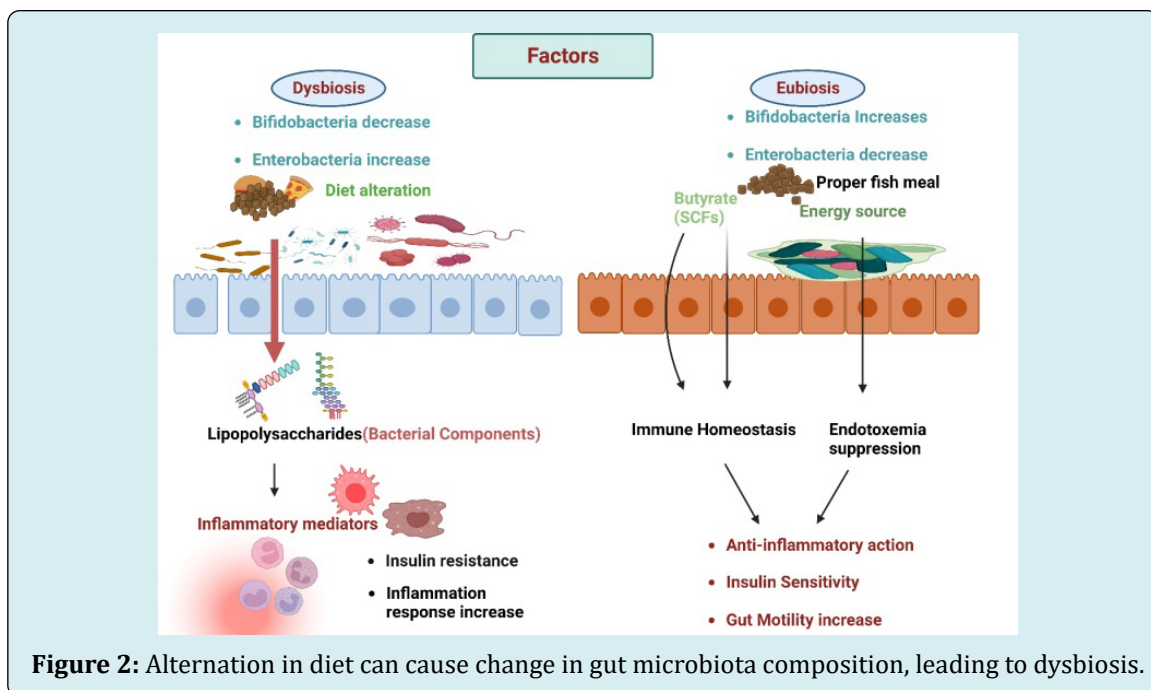
For some time, it was thought that the longevity of hosts was connected with the activity of the gut microbiota [73]. The GI microbiota in endothermic animals facilitates digestion and protects against infections [90]. By depriving invasive pathogens of nutrients and secreting antibiotic chemicals, these gut microorganisms can defend the host. Fish have a variety of microorganisms in their intestines that can thwart diseases. Whereas Sugita, et al. discovered that 3.2% of GI bacterial isolates in freshwater fish were effective against *Aeromonas* sp. [73], they discovered that 2.7% of GI

bacteria in freshwater fish inhibited various pathogens.

A host's gut typically contains a diverse population of commensal, pathogenic, and non-pathogenic bacteria that can have a substantial impact on the overall health of the host and susceptibility to disease. In the intestine of a healthy animal, certain bacteria are permanent and others are transitory. The host microbiota and regulatory system are in balance. However, if this equilibrium is upset, a number of bacteria in the transitory stage can cause fatal illnesses [91,92].

Impact of Fish Gut Microbiota on Host Nutrient Metabolism

The composition and diversity of the gut microbiome of fish are influenced by various host-associated factors, such as genetics, age, diet, and environment, as shown in Figure 1. Alterations in diet can cause changes in the gut microbiota composition, leading to dysbiosis characterized by an overgrowth of harmful microorganisms [93], as shown in Figure 2. A series of exogenous and endogenous factors can affect the establishment and nature of microbial composition in fish GI tracts. Fish developmental stage depends on [91,94,95], gut structure [95], and the surrounding environment, like ambient water temperature.



Biotic (genotype, physiological status, pathobiology, and lifestyle) and abiotic (environmental) factors may affect the fish gut microbiota and influence its composition and diversity, as well as its function and metabolic activity, like feeding, growth, energy storage, and the health of the fish

[96]. Studies have shown that microbes in water affect fish gut microbiota [97,98]. Two main factors influencing grass carp (*Ctenopharngodon* sp.) GI microbiota is water and sediment. Reported that the composition of intestinal lactic acid bacteria (LAB) varied with seasons in four species of fish,

namely silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*), catfish channel (*Ictalurus punctatus*), and deep-bodied crucian carp (*Carassius cuvieri*) [99]. The intestinal microbiota of fish from estuarine environments is closer to that of freshwater fish. In contrast, the intestinal microbiota of fish from mixed salinity ecosystems is more similar to a saltwater fish's intestinal microbiota. Nevertheless, it is difficult to evaluate the exact role of salinity in forming the intestinal microbiota as they used different fish from freshwater or marine water [99]. Recently revealed the gut microbiota composition of Nile tilapia born salinized. The study results showed that in high-salinity conditions, the abundance of *Devosia* and *Pseudomonas* species increased. *Cetobacterium* sp. is more common [99]. Feeding habit is also an important factor influencing GI microbial diversity and is increasing among carnivores, omnivores, and herbivores [51,100-102].

Stress factors can significantly affect the GI microbiota [96]. When different types of chemicals, antibiotics, and pollutants like pesticides, herbicides, and insecticides enter the digestive tract of an aquatic animal, they can drastically affect the composition of the dominant GI microbiota and may lead to the elimination of species from the whole microbial community [103]. Feed and feeding conditions considerably influence the composition of the GI microbiota of fish [104], and during the larval stage, the gut microbial flora changes rapidly with respect to feeding.

Conclusion

The gut microbiota of fish, especially salmonids such as brown trout and rainbow trout, has significant roles in the development, digestion, immunity, and disease resistance of the gastrointestinal tract. Although there have been extensive investigations into the allochthonous microbiota, there is a dearth of information on the autochthonous gut microbiota of these species. Further research is necessary to fully understand the impact of gut microbiota on fish health and identify potential strategies to modulate it to improve growth performance, immunity, and disease resistance. These factors are influenced by various host-associated factors, and disturbances in the gut microbiota can lead to dysbiosis and various diseases. Although the comparison of fish gut microbiota with mammalian gut microbiota reveals fascinating associations, research on fish gut microbiota is still in its infancy. *Salmo trutta fario* and *Oncorhynchus mykiss*, both native to the Himalayan belt, have been found to harbor bacterial communities in their guts, with their composition and diversity being influenced by various factors. Further research on the gut microbiota of these fish species can provide valuable insights into their ecology, physiology, and health. The gut microbiome plays a crucial role in regulating various physiological processes in fish,

including digestion, metabolism, and immune response. The gut microbiota community in fish changes with the host's developmental stage, size, and environmental factors, such as habitat, diet, and season. Understanding the fish gut microbiome can help develop effective strategies to promote the health and growth of fish in aquaculture and conserve the aquatic ecosystem [105-107]. The recent emergence of metagenomics and next-generation sequencing techniques has revolutionized fish gut microbiota research. Studies on *Salmo trutta fario* and *Oncorhynchus mykiss* gut microbiota have shown that the composition of the gut microflora varies according to diet, habitat, geographical location, and season. Further research is necessary to fully understand the dynamics of fish gut microbiota and their impact on fish health. The gut microbiota plays a critical role in the physiology and health of fish. Research has shown that freshwater fish have a unique gut microbial composition due to their environmental conditions. Understanding the role of gut microbiota in fish is essential to developing effective strategies for their management and improving their health and well-being. However, the current understanding of the intestinal microbiota of fish is limited due to culture-based approaches. Gut microbiota composition in fish can vary depending on environmental conditions, host genetics, developmental stage, and immune status. Furthermore, microbial composition in different regions along the GI tract also differs, with foregut communities being significantly different from hindgut communities. The gut-associated microbes might have potential beneficial or harmful effects on the host, and maintaining a healthy microbiome is essential for optimal fish health.

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Competing Interest

The authors declare no completing interest.

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