Host-Microbiota Interactions and Their Importance in Promoting Growth and Resistance to Opportunistic Diseases in Salmonids

Jeff Gauthier, Camille Lavoie, Steve J. Charette, and Nicolas Derome

Abstract Salmonids are second to carps as the most important group of farmed fish, with a total annual output of over 2 million tonnes. Intensive farming practices have been developed to maximize production but at the expense of exposing farmed fish to several simultaneous stressors including frequent handling procedures, overcrowding, and poor water quality. Sanitary, prophylactic, and curative measures in an intensive farming environment are commonly used to compensate for the immune impairment that results from an over-elicited stress response. This can disrupt global interactions between the host and its microbial flora (i.e., microbiota) that play a key role in maintaining fish health in the long term. The economic importance of salmonid fish calls for a better understanding of their host-microbiota interactions to develop therapeutic tools that are less damaging for the environment and human health as well as for the fish themselves. This chapter overviews the current knowledge on factors that alter salmonid microbiomes in aquaculture and discusses the state of the art on microbial profiling and modulation, as well as current research gaps and perspectives.

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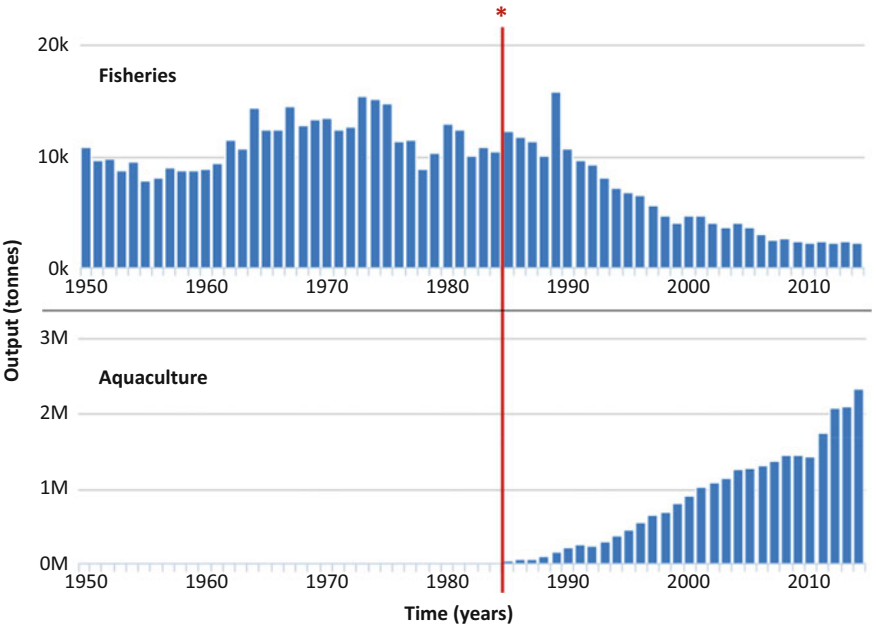

Fig. 1 Global production output for Atlantic salmon from 1950 to 2014. (asterisk) The red line indicates the tipping point (1982) from which aquaculture output started to surpass fisheries. Data from FAO Fish Stats (accessed July 4, 2018)

1 Introduction

1.1 Salmonid Aquaculture and Related Stressors

Since 2010, the global output of aquaculture reached 160 millions of tonnes, twice the amount produced by fisheries (FAO/OMS 2015). Of this number, salmonids are second to carps as the most important group of farmed fish, with a total output of over 2 million tonnes. Atlantic salmon (*Salmo salar*), which makes for two thirds of this market, has been since 1982 almost exclusively mass-produced through fish farming (Fig. 1).

This implies a strong pressure on fish farmers to keep up with high demand for this source of animal protein. Intensive farming practices have been developed to maximize production but at the expense of exposing farmed fish to several simultaneous stressors including frequent handling procedures, overcrowding, and poor water quality (Madaro et al. 2015). Unlike wild fish, captive fish cannot escape from those stressors. Even though stress is necessary to survive a danger or challenge, prolonged inescapable stress factors related to intensive rearing reduce the capacity of fish to maintain homeostasis, putting energy allocation required for reproduction, growth, and persistence on hold (Schreck 1982).

The reallocation of energy that occurs during the stress response is triggered by increased levels of the glucocorticoid hormone cortisol (Barton 2002). One of the major functions to be downregulated by elevated glucocorticoid levels is the immune system (Pickering and Pottinger 1985). Through inhibition of key transcription factors, cortisol effectively suppresses humoral factors involved in the inflammatory response and immune cell trafficking (Fast et al. 2008). Excess plasma cortisol has been shown to increase the susceptibility of brown trout (*Salmo trutta*), Atlantic salmon (*S. salar*), rainbow trout (*Oncorhynchus mykiss*), and numerous other hosts to bacterial, fungal, and parasitic diseases (Pickering and Duston 1982; Maule et al. 1989; Wilk et al. 1989; Johnson and Albright 1992).

When fish are exposed to persistent and inescapable stressors over a prolonged period of time, cortisol levels tend to remain elevated, thereby hindering the ability of stressed fish to revert back to a resting state. This was observed in Atlantic salmon, where fish exposed to handling stress had 25–75 mg/mL cortisol levels for up to 23 days post-challenge compared to near-zero levels in control groups (Madaro et al. 2015). In another study, head kidney macrophages from stressed Atlantic salmon (15 s out of water daily for 30 days) showed decreased survival when exposed to *Aeromonas salmonicida* (Fast et al. 2008).

Sanitary (e.g., egg disinfection), prophylactic (e.g., vaccination), and curative (e.g., antibiotherapy) measures in an intensive farming environment is commonly used in order to compensate for the immune impairment that results from an over-elicited stress response. When an infection occurs, it can be laborious and time-consuming to identify the strain that causes the disease. Consequently, antimicrobial agents having a broad spectrum (i.e., targeting a wide range of bacterial species) are prioritized. Although the bacterial strain that caused the infection may be correctly targeted, a wide range of other bacteria (including beneficial symbionts) are also affected. This can disrupt global interactions between the host and its microbial flora (i.e., microbiota) that play a key role in maintaining fish health in the long term.

1.2 Host-Microbiota Interactions and Their Involvement in Health

All animals live in close association with trillions of microbial cells. Their abundance is so important that they outnumber host cells by a 2:1 ratio (Sender et al. 2016). Up to 1.5% of an individual's biomass accounts for these microbes (Karlsson et al. 2013). Those constitute the host microbiota, i.e., the consortium of microbes residing on host surfaces (e.g., skin, intestines, etc.). In humans, the collective gene complement (i.e., the metagenome) of the microbiota may dwarf its host by a 150-fold factor in terms of unique functions (Qin et al. 2010). This vast gene repertoire assists the host by providing additional functions, such as metabolic pathways to digest otherwise indigestible compounds. As an example, humans cannot digest cellulose but gut bacteria in the large intestine can digest it into

short-chain fatty acids (SCFAs), which humans can process with their own enzymatic toolbox (Cummings 1984). Host microbiota also contributes to immunity through (1) direct antagonism toward pathogenic microorganisms (Cherrington et al. 1991; Hammami et al. 2013), (2) signaling to the immune system (Swiatczak and Cohen 2015), and (3) reducing the carrying capacity of the host for exogenous pathogens (Kamada et al. 2013).

Benefits provided by host-microbiota interactions are highly dependent on environmental and physiological parameters. For example, acute stress responses typically shut down digestive (Mayer 2000) and immune functions (Morey et al. 2015) to react to a life-threatening danger. Alterations of these functions change both the availability of certain nutrients for microbial symbionts and reactivity of the immune system toward them. As a result, stress indirectly alters microbiota composition and, thereby, the interactions with its host.

The economic importance of salmonid fish calls for a better understanding of their host-microbiota interactions to develop therapeutic tools that are less damaging for the environment and human health (Llewellyn et al. 2014). This chapter aims, on the one hand, to present an overview of the current knowledge on the taxonomic composition (i.e., diversity and structure) of salmonid microbiota and processes governing its assembly (ontogenesis). On the other hand, the state of the art on microbial profiling and modulation will be discussed, as well as current research gaps and perspectives.

2 An Overview of Salmonid Microbiomes

A special attention has been given to the microbiota of salmonids that are significantly important in aquaculture.

*2.1 Atlantic Salmon (*Salmo salar*)*

Atlantic salmon has the most extensively characterized microbiota of all salmonids to this present day, with 17 dedicated studies published between 2007 and 2018.[1] Most of this research focused on the skin and gut microbiota, including assessments of its response to migration, nutrition, antibiotherapy, and captivity (Navarrete et al. 2008; Gajardo et al. 2017; Dehler et al. 2017; He et al. 2018).

[1]PubMed search key: ("Atlantic salmon"[Title] and "microbiota"[Title]). Last accessed: August 10, 2018

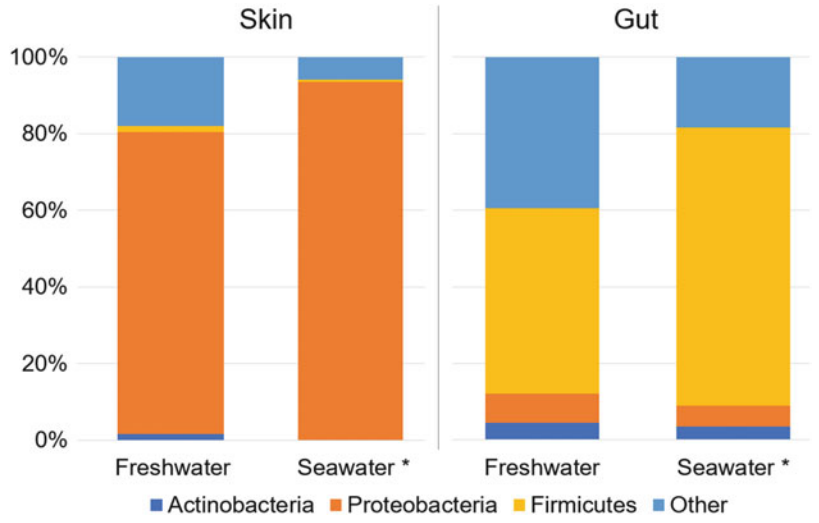

Fig. 2 Relative abundance of the dominant bacterial phyla in the Atlantic salmon skin and gut microbiota. *3 weeks post-transfer in seawater. Data from Lokesh and Kiron (2016) and Rudi et al. (2018)

2.1.1 Compositional Shifts During Freshwater-Seawater Migration

As other anadromous fish species, Atlantic salmon is exposed to two highly contrasted environments during its life cycle (i.e., freshwater and seawater), both of which differ greatly in terms of salinity, temperature, nutrient availability, and, potentially, environmental microbial exposure (Héry et al. 2014). Accordingly, major shifts in the abundance of dominant bacterial phyla were found in the skin and gut microbiota of Atlantic salmon before and after smoltification (Fig. 2). The most abundant phyla in skin and gut, respectively, *Proteobacteria* and *Firmicutes*, increase in abundance during freshwater to seawater transfers, while *Actinobacteria* decrease in both types of microbiota during this process (Lokesh and Kiron 2016; Rudi et al. 2018). However, the impact of this compositional shift on host physiology, and vice versa, remains unclear.

2.1.2 Influence of the Diet and Protein Sources

Wild Atlantic salmon feed exclusively on animal protein; juveniles start with zooplankton and feed on larger fish as they grow (Harvey et al. 2016). Accordingly, farmed salmon should be specifically fed with fishmeal as a primary source of animal protein. However, there is a growing pressure on the aquaculture industry to reduce the fishmeal content of feeds for improved sustainability and reduced cost (Rimoldi et al. 2018). Plant-based protein sources are an increasingly popular replacement for fishmeal (Newaj-Fyzul and Austin 2015). However, marine carnivorous fish have

Table 1 Shannon alpha diversity index of the distal gut microbiota from Atlantic salmon fed with diets of varying protein sources

Dietary protein sources (% $_{m/V}$)			MOS added[a]	Shannon index (mean ± SE)
Fishmeal	Soybean meal	Terrestrial animal meal		
40	0	12	+	2.33 ± 0.28
30	5	19	+	3.94 ± 0.46
18	10	29	+	2.78 ± 0.59
18	10	29	–	3.50 ± 0.45

[a]Diet supplemented with 0.2% mannan-oligosaccharide (MOS)

not evolved mechanisms to efficiently digest carbohydrates and non-nutritious compounds present in plant-based meals (Naylor et al. 2000). Soybean meal, one of the most promising alternatives to fishmeal (Herman and Schmidt 2016; Park et al. 2017), contains compounds that trigger inflammation in the distal intestine of salmonids (Heikkinen et al. 2006). Even though those inflammatory compounds can be removed by alcohol extraction, the resulting soybean protein concentrate (SPC) still alters the intestinal microbiota (Table 1). Nevertheless, fish fed with a SPC-rich diet supplemented with mannan-oligosaccharide (MOS) had an alpha diversity index more similar to fish fed exclusively with fish and terrestrial animal meals (Table 1). In another study, Atlantic salmon fed with either soybean meal, SPC, or guar meal had higher levels of lactic acid bacteria (LAB), as well as higher expression levels of proliferating cell nuclear antigen (PCNA). The cause-effect relationship between legume-based diets, LAB, and PCNA levels is still elusive, however (Gajardo et al. 2017).

2.1.3 Antibiotherapy

To our knowledge, few studies have addressed the impact of antibiotherapy on the Atlantic salmon microbiota. In 2017, a study investigated the impact of oxytetracycline (OTC), one of the most commonly used antibiotics against salmonid infectious diseases (Miranda and Zemelman 2002). OTC was administered daily in the form of medicated feed to salmon fingerlings. Microbiota composition was assessed by RFLP-PCR and sequencing of 16S rDNA amplicons. Whereas untreated microbiota was diverse and consisted mainly of *Pseudomonas*, *Acinetobacter*, *Bacillus*, *Flavobacterium*, *Psychrobacter*, *and Brevundimonas* spp., the microbiota of OTC-treated fish was dominated by *Aeromonas sobria* and *A. salmonicida.* Both species are known to harbor oxytetracycline resistance genes (Balassiano et al. 2007; Trudel et al. 2016). The latter is a well-known salmonid pathogen that causes furunculosis, a major opportunistic disease (Bullock et al. 1983). This study presented a textbook example of proliferation of opportunistic bacteria by collateral removal of competing microorganisms (see chapter "The Rise and Fall of Antibiotics in Aquaculture" for a detailed discussion on this topic). To our knowledge, no

other study investigated specifically how antibiotics impact the Atlantic salmon microbiota. In this regard, there is a significant knowledge gap regarding other common antimicrobials such as florfenicol (Nordmo et al. 1998) and sulfamethoxazole-trimethoprim (Kadlec et al. 2011).

2.1.4 Captivity

Even though the bulk of salmon aquaculture is intended to produce food, government-led programs were also introduced to restore endangered salmon populations in rivers (Province of Quebec, Canada, is a notable example). Most involve stocking rivers with hatchery-reared juveniles (usually 0+ or 1+ parrs). Even though stocking parrs is preferred to stocking captive adults because of the latter's low reproductive success, captive parrs do not survive as well in the wild as their wild-born counterparts. A 2018 study revealed a substantial mismatch between the microbiota of captive (meant for stocking) and wild parrs (Lavoie et al. 2018) sampled from two different rivers. Even though community composition from wild parrs was specific to the river, captive fish (born from wild breeders from either river) were not significantly differentiated despite their distinct genetic origin. Furthermore, their microbiota composition was highly distinct from their wild fish relatives. In addition, captive parrs' microbiota was dominated by *Firmicutes* (*Lactobacillaceae*), whereas wild parr's microbiota was enriched with *Proteobacteria* (*Enterobacteriaceae*). Those results were consistent with previous studies indicating that the microbiota composition is highly associated with the diet protein source (Desai et al. 2012; Gajardo et al. 2016). As such, captive parrs are fed with commercial pellets made from vegetable proteins, a great source of carbohydrates. The latter has been associated with an increase of lactic acid bacteria (LAB) such as *Lactobacillaceae* and contributes to the divergence between captive and wild parr's microbiota composition.

As well as highlighting the substantial contribution to diet and environmental conditions on the microbiota composition, this study also confirmed that the bacterial species richness (alpha diversity) can be associated with the selective pressure of an environment. In comparison to captive parrs, wild juveniles showed a much lower diversity index and a higher homogeneity within the individual's microbiota composition, suggesting that higher selective pressure translates into a more specialized microbiota composition (Derome et al. 2006).

Interestingly, some disparities were detected when studying the network interactions of taxa according to the parr's origin. For instance, a higher proportion of negative interactions was found within captive parr's microbiota (Fig. 3). Those results are of prime interest since it has been established that negative correlations can be associated with a higher dysbiosis index (Vázquez-Baeza et al. 2016). Overall, captivity and hatchery rearing highly contribute to the microbiota composition, even for parrs from the same genetic population. Studying microbial ecology in the aquaculture field is therefore totally pertinent for assessing the effect of

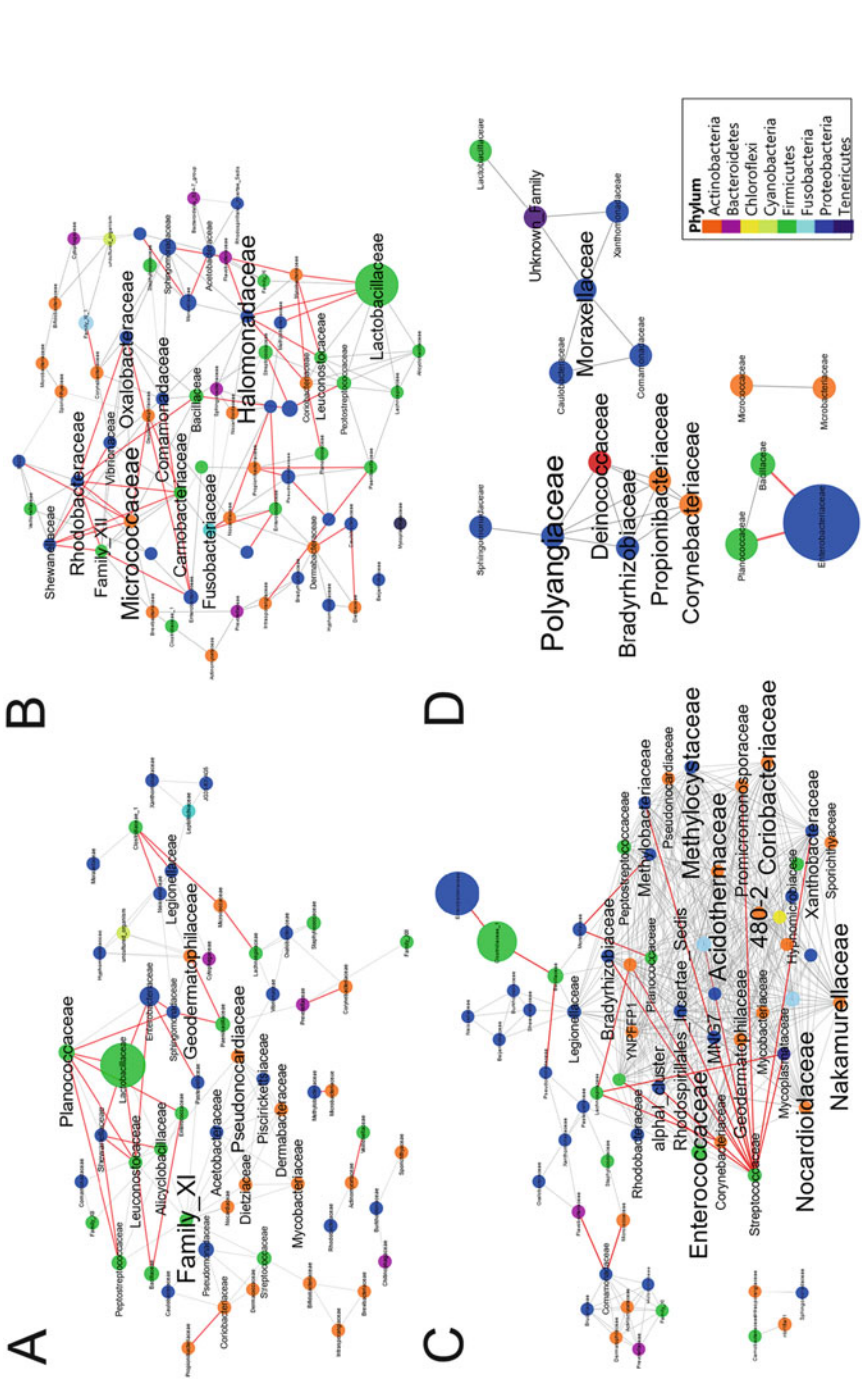

Fig. 3 Network analysis (Spearman correlation coefficient) of the microbiota at the family level depending on parrs' origin in two Canadian rivers. Node size is proportional to the relative abundance of each taxa, where low abundance taxa show more interactions than abundant taxa. Font size is proportional to the number of interactions for each taxa, illustrating keystone taxa for every environment. Negative correlations are illustrated by red edges. (**a**) Malbaie captive parrs; (**b**) Rimouski captive parrs; (**c**) Malbaie wild parrs; (**d**) Rimouski wild parrs. Data from Lavoie and Derome (2018, unpublished data)

captivity on physiology and microbiota, especially if reared fishes are meant to be released thereafter.

Since the diet of fishes appeared to be greatly associated with the host metabolism, stocking methods have been modified over time to mitigate the physiological mismatch of hatchery-reared fishes that are meant to be reintroduced in nature (Milot et al. 2013). For example, hatcheries are now raising Atlantic salmon juveniles until the alevin stage, which still have their unabsorbed yolk sac at the moment of stocking. However, hatchery rearing seems to have left a permanent imprint on the microbiota of stocked alevins, despite them being no longer exposed to an artificial environment and not being fed during the rearing (Lavoie and Derome 2018, unpublished data).

By analyzing the microbiota of wild and stocked juveniles that have been sampled in the river 4 months after stocking, differences between the microbiota of stocked and wild individuals are still highlighted. Overall, discrepancies are observed for the taxonomical composition of the microbiota as well as the diversity, suggesting a potential mismatch for metabolic functions. For instance, a higher diversity index is associated with stocked parrs' microbiota, indicating that the rearing conditions have a permanent effect on the structure of the microbiota. Even though the exact contribution of this mismatch on host fitness is unclear and deserves further investigation, hatchery rearing is proven to drive microbial processes at various levels. Acquiring a better understanding on how the microbiota is affected by the environment after stocking will certainly lead to the optimization of the conservation methods of endangered fish species.

2.2 *Rainbow Trout (*Oncorhynchus mykiss*)*

Rainbow trout is second to the Atlantic salmon as the most produced fish in salmonid aquaculture with 812,000 tonnes produced in 2014, which represents 35% of global Atlantic salmon production (FAO Fisheries and Aquaculture 2018). A total of 14 studies[2] on the rainbow trout microbiota were published from 2010 to 2018, most of which investigated the influence of diet on various physiological parameters. The remaining studies investigated the role of the microbiota in growth promotion and pathogen inhibition, as well as diet-immunity interactions and their impact on the microbiota. None of those studies addressed the impact of antibiotherapy on the microbiota composition and subsequent effects on fish health. Perhaps due to the relevance of this species in aquaculture, a special emphasis on the intestinal microbiota was found throughout most of the aforementioned studies.

[2]PubMed search key: ("rainbow trout"[Title] and "microbiota"[Title]). Last accessed: Dec 10, 2018

2.2.1 Influence of Nutrition

Farmed rainbow trout was found to possess a core gut microbiota of 52 bacterial lineages (Wong et al. 2013). This core gut microbiota was remarkably resilient to interindividual variation, diet, and rearing density changes, with no significant change in the abundance of bacterial classes. Nevertheless, the gut microbiota as a whole responds to diet composition.

One of the hot topics in rainbow trout aquaculture is the use of alternative (plant-based) protein sources to improve the sustainability of fish farming. However, those are quite rich in carbohydrates compared to rainbow trout's natural feed, which is very rich in protein (>40%) and poor in carbohydrates (<1%). A short hyperglucidic-hypoproteic stimulus (HHS) during early life stages was found to induce a long-term influence on the gut fungi (but not bacteria) profiles. Furthermore, it induced upregulation of glucose metabolism genes and downregulation of gluconeogenesis and amino acid catabolism genes in muscle tissue (Geurden et al. 2014). In the long term, HHS-treated fish did not differ in growth, feed intake, or efficiency of feed utilization. However, a significant effect on glucose homeostasis was observed. Up to 9 h after being fed the same commercial diet, HHS-treated fish had 1.5-fold higher glycemia than untreated fish. This hints to the possibility of nutritional programming as a way of optimizing the use of alternative plant-based feeds in fish farming.

The rainbow trout gut microbiota also responds to the inclusion of dietary additives. Supplementation with a plant essential oil mixture (MixOil) altered gut microbiota diversity indices and fillet quality metrics, but the link between those two remains unclear (Ceppa et al. 2018). The inclusion of organic acids in aquafeed was found to influence gut microbiota composition, but with unclear effects on host physiology (Jaafar et al. 2013).

2.2.2 Diet-Immunity Interactions

One of the main causes of mortality in rainbow trout aquaculture is *Yersinia ruckeri*, the causative agent of enteric redmouth disease (Tobback et al. 2007). The initial target organ for *Y. ruckeri* appears to be the gut (Méndez and Guijarro 2013). Accordingly, the administration of probiotic bacteria (via coated feed) enhanced resistance to this pathogen (Raida et al. 2003), but the mechanism of action was unclear.

In 2014, a Danish team investigated the missing link between microbiota, diet, and the immune response in fish challenged with *Y. ruckeri* (Ingerslev et al. 2014). Rainbow trout fry challenged by *Y. ruckeri* were split into two diet groups containing either (1) fishmeal + fish oil or (2) fishmeal/Pea meal (9:1) + rape seed oil. Microbiota composition was assessed by deep sequencing of the 16S rRNA gene, and immune gene expression was quantified by RT-qPCR. In summary, challenged fish fed with the marine-based diet had higher counts of *Yersinia* (as determined by

either bacteriology or 16S profiling) and had also increased expression levels of interleukins 1-beta and 2. The plant-based diet may have had a prebiotic effect by favoring the presence of taxa that are protective against *Y. ruckeri* (Ingerslev et al. 2014). However, post-infection cumulative survival did not significantly differ between challenged fish fed either diet.

2.2.3 Pathogen Inhibition

The total cultivable microbiota of Chilean farmed rainbow trout harbors lactic acid bacteria (LAB) in high abundance (Araújo et al. 2015). Of those, 71% (mostly *Lactococcus lactis* isolates) possess inhibitory activity against one or more of the following pathogens: *Lactococcus garvieae*, *Streptococcus iniae*, *Yersinia ruckeri*, *Aeromonas salmonicida*, and *Vibrio campbellii* (Araújo et al. 2015). Whether those LAB isolates do possess inhibitory activity in vivo remains to be investigated.

*2.3 Brook Charr (*Salvelinus fontinalis*)*

The brook charr microbiota remains largely mischaracterized, except for the skin mucus (SM) microbiota, for which response to intensive rearing conditions and symbiont-pathogen interactions (including interindividual variations) were investigated (Boutin et al. 2012, 2013a, b, 2014). To our knowledge, no published studies have yet discussed the impact of diet or therapeutic tools on both microbiota structure and brook charr physiology.

2.3.1 Microbiota Structure and the Stress Response

The brook charr SM microbiota is dominated by the bacterial phyla *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* (Boutin et al. 2013b). Their relative abundance shifts abruptly when fish are exposed to hypoxia and high-density stress (Fig. 4). In addition to those abundance shifts, strong co-occurrence patterns were found. Some co-occurring genera associated with opportunistic diseases (*Psychrobacter*, *Steroidobacter*, *Pseudomonas*, *Acinetobacter*, *Aeromonas*) were specific to stressed and dead fish, whereas others (*Sphingomonas*, *Methylobacterium*, *Propionibacterium*, and *Thiobacter)* were abundant only in unstressed fish (Boutin et al. 2013b). Beneficial bacteria tended to decrease in a colinear manner following a stress event, thus resulting in an empty niche for opportunistic pathogens, which accordingly tended to increase as co-abundant groups. The role of SM microbiota in preventing infections in its host might be more important than previously thought. Indeed, several endogenous strains, in addition to those isolated from the gut microbiota, have shown inhibitory effects against common brook charr pathogens (Table 2).

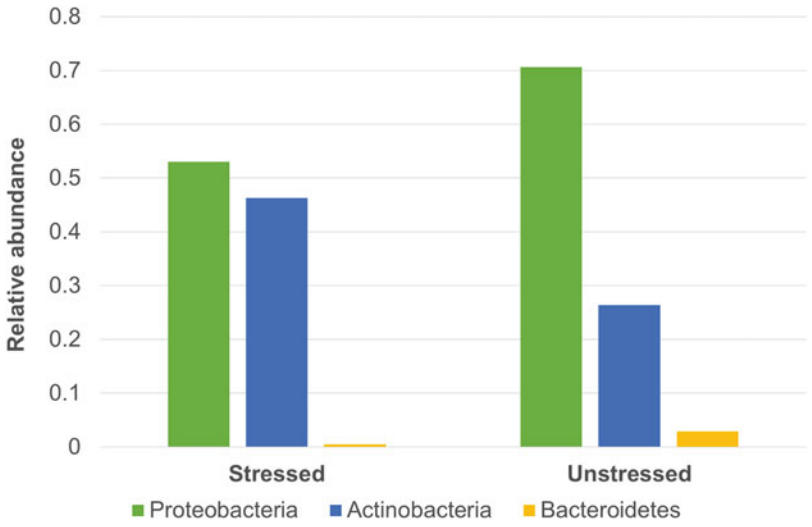

Fig. 4 Relative abundance shifts in the skin mucus microbiota of stressed versus unstressed brook charr. Stressed fish were exposed to high density (80 fishes in 10 L) until the oxygen concentration decreased to 3 mg/L (5 min). After stress exposure, fish were transferred in a new oxygenated tank to slowly recover. Data from Boutin et al. (2013b)

2.3.2 Symbiont-Based Therapeutic Tools Against Opportunistic Pathogens

An indigenous brook charr isolate from skin mucus (*Rhodococcus* sp. CPM5) decreased mortality due to pathogens *Flavobacterium psychrophilum* and *F. columnare* by 47% without disturbing the natural microbiota of skin mucus (Boutin et al. 2013a). Unexpectedly, it was not by recolonizing the skin mucus microbiota that CPM5 conferred its protective effect, but rather by colonizing the filtering mass of the recirculation system, where it may have had a positive impact on water quality, as *Flavobacterium* spp. were observed to be virtually absent from the circulating water in treated groups (Boutin et al. 2013a). In addition, CPM5-treated fish's water was dominated by *Sphingomonas* spp. unlike control tanks. Interestingly, *Sphingomonas* is the dominant bacterial genus in brook charr skin mucus. CPM5 may have indirectly improved resistance to flavobacteriosis by acting as a prebiotic for *Sphingomonas* spp., which in turn may have excluded *Flavobacterium* spp. from the surrounding water. This "symbiotic action at a distance" raised interesting questions regarding the nature of host-microbiota symbiotic relationships. Furthermore, it indicated that microbial symbionts may be recruited into novel ecological functions when readministered independently (Watson and Pollack 2001).

Other bacterial brook charr symbionts showed great promise as inhibitors of another major salmonid pathogen, *Aeromonas salmonicida* subsp. *salmonicida*

Table 2 Endogenous isolates from brook charr microbiota known to inhibit salmonid pathogens

Strain	Source	Origin	Known inhibitory effects			References
			Effective against	In vitro effect?[a]	In vivo effect?[b]	
Pseudomonas fluorescens ML11A	Skin mucus	Quebec, QC, Canada	*A. s. s.*[c]	Yes	NA	Gauthier (2016) and Gauthier et al. (2017a)
Pseudomonas fluorescens ML11B	Skin mucus	Quebec, QC, Canada	*A. s. s.*	Yes	NA	Gauthier (2016)
Pseudomonas fluorescens ML13	Skin mucus	Quebec, QC, Canada	*A. s. s.*	Yes	NA	
Aeromonas sobria TM12	Intestine	Kamouraska, QC, Canada	*A. s. s.*	Yes	NA	Gauthier et al. (2017b)
Aeromonas sobria TM18	Intestine	Kamouraska, QC, Canada	*A. s. s.*	Yes	NA	
Luteimonas sp. CP1	Skin mucus	Quebec, QC, Canada	*Flavobacterium columnare*	Yes	NA	Boutin et al. (2012)
Microbacterium sp. CP2	Skin mucus	Quebec, QC, Canada	*Flavobacterium psychrophilum*	Yes	NA	
Rhodococcus sp. CP3	Skin mucus	Quebec, QC, Canada	*Flavobacterium psychrophilum*	Yes	NA	
Microbacterium sp. CP4	Skin mucus	Quebec, QC, Canada	*Flavobacterium psychrophilum*	Yes	NA	
Rhodococcus sp. CP5	Skin mucus	Quebec, QC, Canada	*Flavobacterium columnare*	Yes	Yes	Boutin et al. (2012, 2013a)
Pseudomonas sp. CP6	Skin mucus	Quebec, QC, Canada	*Flavobacterium psychrophilum*	Yes	NA	Boutin et al. (2012)
Sphingopyxis sp. CP7	Skin mucus	Quebec, QC, Canada	*Flavobacterium psychrophilum*	Yes	NA	
Leucobacter sp. CP8	Skin mucus	Quebec, QC, Canada	*Flavobacterium columnare*	Yes	NA	
Dietzia sp. CP9	Skin mucus	Quebec, QC, Canada	*Flavobacterium psychrophilum*	Yes	NA	

[a]Evidence of in vitro inhibitory effect against pure pathogen cultures
[b]Successful decrease of mortality or morbidity when administered to challenged brook trout. *NA* not available
[c]*Aeromonas salmonicida* subsp. *salmonicida*

(*A. s. s.*). One of those, *Pseudomonas fluorescens* ML11A, was also recovered from skin mucus (Gauthier et al. 2017a) and exhibited a strong antagonistic effect across a wide range of *A. s. s.* from different geographical origins (Gauthier 2016). In addition, gut isolates belonging to the *Aeromonas sobria* (sensu stricto) species, TM12 and TM18, showed tremendous inhibitive properties against *A. s. s.* through inhibitory compound diffusion on agar (Gauthier et al. 2017b). Those two probionts

were shown to increase plasma lysozyme activity, respectively, by a 1.5- to 2-fold factor in in vivo preliminary experiments without *A. s. s.* challenge, suggesting a positive impact on host innate immunity (Gauthier et al. 2016, unpublished data). However, whether the inhibitive property of those probiont strains translates into a protective effect in vivo remains to be investigated.

3 An Overview of High-Throughput Methods and Their Contribution to Microbiota Studies

High-resolution study of the microbiota has been made possible with the advent of omics-based methods (genomics, transcriptomics, proteomics, etc.) following the emergence of high-throughput DNA sequencing during the late 2000s. This technological revolution made it possible to obtain several millions of nucleotide reads from tens to hundreds of samples at once, for costs that are orders of magnitude cheaper than Sanger sequencing for an equivalent amount of data (Vincent et al. 2017a). Knowing that a major fraction of microbial diversity is unculturable, high-throughput DNA sequencing technologies proved useful to the analysis of complex microbial assemblages. Several high-throughput sequencing methods have emerged, each offering distinct elements of information on the host-microbiota complex. For this reason, an integrative research strategy should ideally use a combination of these methods.

3.1 Whole-Genome Sequencing (for Specific Microbes)

Whole-genome shotgun (WGS) sequencing aims to obtain the whole DNA sequence of a specific organism. For individual microbes isolated from the microbiota, this method requires that the organism be in pure culture in order to avoid contaminating the sequence with exogenous DNA. Consequently, the DNA of individual nonculturable microbes cannot yet be sequenced independently (see paragraph below on metagenomics).

Briefly, a pure DNA extract of the organism is sheared into fragments of a few hundred base pairs long and is then sequenced using high-throughput technology, e.g., Illumina MiSeq (Tagini and Greub 2017). Typically, this process yields several hundred thousand DNA sequences (i.e., reads) that have to be assembled de novo (i.e., without prior knowledge on source DNA) to obtain the complete sequence of the organism's genome. In most genome sequencing projects, the assembly does not reach completion and yields tens to hundreds of larger chunks (contigs) that span from a few kilobases to a few megabases in length. In fact, since 2014, there are more genomes published as incomplete drafts than complete ones (Gauthier et al. 2018) since it does not interfere much with downstream analyses.

When a draft assembly is complete, gene sequences are then annotated by similarity searches against databases of known genes. Some examples of web services offering this analysis are the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al. 2016) and RAST (Overbeek et al. 2014). When gene annotation is complete, several downstream analyses can be applied: metabolic pathway reconstruction (Kanehisa et al. 2016), taxonomic assignment using average nucleotide identity (Konstantinidis and Tiedje 2005), phylogenomic inference (Delsuc et al. 2005), and virulence assessment (Chen et al. 2016) as well as antibiotic resistance gene prediction (Jia et al. 2017).

WGS sequencing was used, for example, to characterize the taxonomy and functions of individual bacterial symbionts in brook charr and rainbow trout which had potential as probiotic treatments against salmonid diseases (Boutin et al. 2012; Schubiger et al. 2015; Gauthier et al. 2017a, b), as well as pathogens (Reith et al. 2008; Rochat et al. 2017). However, WGS does not provide much insight on the higher organizational levels of the microbiota. The two following approaches (metabarcoding and metagenomics), respectively, address the following questions: (1) which microbes are involved and (2) how they contribute to the functional repertoire of the microbiome. Note that WGS data plays an important role in the annotation of functional data in metagenomics, as explained in the subsections below.

3.2 Metabarcoding (Who Is There?)

Metabarcoding is the massively parallel sequencing of a universal genetic marker to infer the taxonomic census of a community. It is currently the most common method in current microbiome studies, regardless of studied organisms (Garrido-Cardenas and Manzano-Agugliaro 2017; Mahato et al. 2017; Osman et al. 2018). The 16S ribosomal RNA gene is the most commonly used biomarker, due to its ubiquitous presence in bacterial, archaeal, and eukaryotic (organellar) genomes (Ju and Zhang 2015). Most, if not all, of the aforementioned salmonid-microbiota studies in Sect. 2 used 16S rRNA gene metabarcoding to assess microbiota composition.

First, DNA from the microbiota is extracted from host samples (e.g., skin mucus, gills, gut section). Then, a gene that is universally present across the widest range of organisms (a biomarker) is amplified by PCR. After this process, DNA "tags" are added to the amplified DNA products to allow "per sample" identification. PCR products are then sequenced simultaneously on a high-throughput apparatus (e.g., Illumina MiSeq or Ion Torrent PGM). There is a myriad of sample collection and DNA extraction methods that were developed in the last decade (Pollock et al. 2018), each with their own variability and biases for specific microbial groups. In a 2018 study, various combinations of gut sample collection, DNA extraction, and high-throughput sequencing were compared (Panek et al. 2018). However, optimization efforts (including the aforementioned study) have so far mostly been made for

human gut microbiota studies. No methodological analysis on salmonid sample processing (skin mucus, gills, gut) has yet been published.

After sequencing, several files are produced, each containing several tens of thousands of sequence reads from a specific sample. Several computer programs are available to process the raw reads, each contributing to one or more of the steps involved in the analysis (Table 3). Typically, reads will be clustered in operational taxonomic units (OTU) under the assumption that similar sequences belong to a single microbial lineage. OTUs are then quantified by counting the reads that were involved in their construction. Then, reads are compared against a sequence database of known organisms, e.g., Greengenes (DeSantis et al. 2006), RDP (Cole et al. 2014), or ARB-SILVA (Yilmaz et al. 2014), to determine the consensus taxonomy of each OTU. Finally, an "OTU table" is obtained, on which a plethora of quantitative methods can be used to identify differences in microbiota structure between treatments, conditions, or samples (Table 3).

In summary, (16S) metabarcoding ultimately attempts to correlate the microbiota taxonomic composition to treatments dispensed to a host or several health metrics (i.e., growth rate, size, blood cell count, plasma lysozyme activity). However, it does so without providing much information on the function of microbes that are present (Zepeda Mendoza et al. 2015). Functional roles may be either (1) inferred grossly by reviewing what is known about specific families or genera in the literature or (2) inferred systematically by using "metagenome prediction" software to link taxa to whole-genome data (Langille et al. 2013; Aßhauer et al. 2015). However, most metabarcoding methods resolve taxonomy up to the genus level, and the functional repertoire of species within a single genus can be tremendously variable. For example, the genus *Pseudomonas* encompasses a wide array of mutualistic, commensal, and pathogenic species living across different habitats (soil, water, or in association with animals or plants) (Silby et al. 2011). Unsurprisingly, about only 1% of all known *Pseudomonas* genes is shared among all of its known genomes (Freschi et al. 2018). Therefore, using metagenome prediction from metabarcoding may overgeneralize the attributes of a single genus.

3.3 Metagenomics (What Are They Doing?)

Metagenomics, in principle, is not that different from WGS sequencing from a methodological point of view. However, input samples will be DNA extracts of complex communities (e.g., gut tissue sections or skin mucus swabs) instead of a single organism's DNA, leading to the untargeted sequencing of all microorganism's genomic sequences present in a given sample, in addition to the host organism's genome (Quince et al. 2017). Due to the presence of DNA molecules from tens to thousands of different taxa in one sample, several hundred thousand fragments (contigs) per sample are typically obtained. Those contigs often correspond to individual gene sequences. Those are then annotated by homology search against known sequence databases, after which post-annotation analysis and visualization methods can be applied (Table 4). This methodological approach is therefore

Table 3 Major steps in a typical 16S amplicon sequence analysis pipeline, including key software involved

Step	Sub-step	Description	Example software
Data preprocessing	Quality filtering and trimming	Removing incorrectly called nucleotides in sequence reads, as well as reads of poor quality. In a trimming approach, reads are trimmed after quality reaches a certain threshold. This avoids making annotations based on erroneous data	Trimmomatic, sickle, QIIME, mothur, dada2
	Error learning	Certain computer programs use a prediction model to correct reads prior to downstream analyses. Though computationally intensive, this reduces data loss as low-quality parts of reads are corrected instead of simply being removed	dada2
	Sequence merging	Gene sequence fragments are often sequenced from both ends (i.e., paired-end), meaning that each molecule is associated with two reads. Each pair of reads providing from a single DNA fragment must be assembled together prior to continuing the analysis	pandaseq
Data processing	OTU clustering	Merged reads are clustered together, usually on the basis of an identity threshold (often >97%) assuming that similar sequences belong to the same taxonomic entity. Certain methods use exact matching but require the use of an error model (e.g., dada2)	QIIME, mothur, dada2
	Taxonomic assignment	The taxonomic ranks (kingdom, phylum, genus, species) of each OTU are obtained by the consensus of the annotation of all reads used to build it	QIIME, mothur, phyloseq
Statistical analysis	Alpha diversity	Within-sample diversity, i.e., a function of the number of OTUs present in a given condition or sample. For example, the Shannon diversity index measures both richness (the amount of species) and evenness (their distribution)	QIIME, mothur, phyloseq
	Beta diversity	Pairwise distance or dissimilarity between samples. For example, the Bray-Curtis dissimilarity index measures the ratio of unique species versus all species found in a pair of samples. Samples that have a Bray-Curtis index of 1 are entirely composed of mutually exclusive species	QIIME, mothur, phyloseq
	Differential abundance	Involves hypothesis testing to determine which taxa are differentially present in a pair of conditions (or samples)	DEseq2, edgeR (from phyloseq data)
	Co-abundance networks	Built from OTU correlation matrices, those allow the identification of taxa whose abundance is either colinear or mutually exclusive	phyloseq, igraph

Table 4 Major steps in a typical meta-(genomics, transcriptomics) analysis pipeline

Step	Sub-step	Description	Example software
Data preprocessing	Quality filtering and trimming	Removing incorrectly called nucleotides in sequence reads, as well as reads of poor quality. In a trimming approach, reads are trimmed after quality reaches a certain threshold. This avoids making annotations based on erroneous data	Trimmomatic, sickle, Trinity (for metatranscriptomics)
Data processing	De novo assembly	Reconstructing the DNA sequences in the input sample without prior knowledge. Due to the high complexity of microbial community samples, the output data is typically chunks (contigs) corresponding to gene sequences	IDBA-Meta, Ray Meta, SPADES, Trinity (for metatranscriptomics)
	Gene (or transcript) calling	Gene sequences found within contigs are clustered together using an identity threshold, assuming that highly similar sequences are homologous (i.e., code for the same kind of proteins). This process is homologous to the OTU clustering step in metabarcoding	FragGeneScan, Trinity (for metatranscriptomics)
	Annotation	Pairwise alignment of predicted genes against a database of known sequences. One can then predict the nature (function) of proteins encoded by those genes	BLAT, Diamond
Statistical analysis	Metabolic reconstruction	Using the annotation data, one can reconstruct metabolic pathways present in a sample and make comparisons of shared and unique steps between conditions or samples	BlastKOALA
	Differential abundance	Involves hypothesis testing to determine which genes are differentially present (or differentially expressed if metatranscriptomics) in a pair of conditions or samples	DEseq2, edgeR
	Co-abundance networks	Built from abundance correlation matrices, those allow the identification of genes (or transcripts) whose abundance is either colinear or mutually exclusive	phyloseq, igraph

promising to improve the management beneficial microbial functions in aquaculture, as evidenced in other research fields (Culligan et al. 2014). However, the high sequencing depth required to get enough coverage for microbial sequences, the inherent complexity of analyzing metagenomic data (e.g., the lack of functional annotation of most nonhuman microbial transcripts), as well as the required computational power and storage make this approach highly challenging.

Another challenge is that metagenomics gives insight on which gene functions are relevant in a biological system but does not predict their level of activity. Nevertheless, by sequencing host and bacterial messenger RNAs instead of total genomic DNA, one can indeed obtain a *metatranscriptome*, which can be annotated using similar methods; some assembly computer programs even allow the prediction of splicing variants resulting from the transcription of genes with intronic sequences (Haas et al. 2013). Moreover, the transcripts' abundance can be quantified (as it is proportional to the number of mapped reads) making it possible to determine the *level of expression* of a given gene (Bashiardes et al. 2016). Therefore, by allowing to quantify simultaneously both microbial and host tissue gene expression, this methodological approach is thus suitable to shed light on active host microbiota (including pathogens) functional interactions. Accordingly, metatranscriptomics, combined with metagenomic analysis, has shown that, in the human gut, a substantial fraction of microbial transcripts are differentially regulated relatively to their microbial genomic abundances (REF). Though promising, this technique has inherent challenges such as high per-sample cost, depletion of both eukaryotic and bacterial ribosomal RNA transcripts (~90–95% of a total RNA sample), and the lack of standard bioinformatics methods ensuring repeatability across studies (Martin et al. 2018). To our knowledge, no metatranscriptome data of a salmonid has yet been published.

4 Future Perspectives for Microbiota Modulation

4.1 Host-Microbiota Interactions in Light of the One Health Perspective

As previously mentioned in chapter "The Rise and Fall of Antibiotics in Aquaculture," an integrated view of biological systems is required to secure aquaculture production and ensure its sustainability. This is exemplified by the One Health perspective, which states that human, animal, and environmental health are co-dependent variables (Lebov et al. 2017). Therefore, acknowledging the contribution of the microbiota in animal health falls within this framework. Roles of the microbiota in salmonid health have been made increasingly clear throughout the last decade (see Sect. 2.2). Two relevant examples that would benefit from a holistic understanding are (1) the emergence of antibiotic resistance in salmonid pathogens, which is worsened by bacteria-to-bacteria gene transfer and the depletion of competing bacteria sensitive to antibiotics (Trudel et al. 2016), and (2) the impact of diet on the microbiota composition and its impact on immune function (see Sect. 2.2).

4.2 Fine-Grained Modulation Using Dietary Supplements

4.2.1 Probiotics

Probiotics are defined as a "live microbial culture added to feed or environment to increase viability of the host" (Gram and Ringø 2005). This positive effect on host physiology may originate from several mechanisms of action: (1) mechanisms targeting pathogens such as nutritional competition, diffusion of antimicrobial compounds, or competitive exclusion from epithelial surfaces (Bermudez-Brito et al. 2012; Kamada et al. 2013) and (2) mechanisms targeting the host itself, such as modulation of host immune signaling pathways (Kamada et al. 2013).

Although the specific mechanisms by which some probiotic strains exert their beneficial effects require further investigation, probiotic administration showed promising results on growth performance and general health of salmonid fish (Gatesoupe 2010). Some probiotic candidates showed great promise as prophylactic tools against opportunistic diseases (Boutin et al. 2012; Schubiger et al. 2015; Gauthier et al. 2017a, b). However, few of them, if any, have reached commercialization or even official approval for use in salmonid farming.

We may hope that, in the near future, probiotic administration will be guided by more and more thorough microbiota monitoring studies. For example, if a specific bacterial species is associated with increased immune function, then one or more isolates from this species could be administered to vulnerable fish as a prophylactic treatment. Perhaps microbial community assemblages that are reflective of a good health status will be engineered and administered as "microbiota transplants." The microbiota transplant strategy, for instance, is the most effective therapies (90% efficacy rate) against human nosocomial *Clostridium difficile* infections (Liubakka and Vaughn 2016).

However, the bioengineering of host-associated microbial communities is a complex task, as most of microbial diversity is unculturable using basic microbiological methods (Tanaka et al. 2014). Nevertheless, recent progress in human microbiota studies (Zihler Berner et al. 2013; Auchtung et al. 2015; Dostal et al. 2015) could pave the way toward similar approaches in salmonid health management. For instance, gut bioreactors, a special class of continuous-flow fermenters, proved to be an excellent method to cultivate complex human gut microbiota systems in vitro in highly controlled simulated settings (Macfarlane and Macfarlane 2007).

4.2.2 Prebiotics and Synbiotics

In addition to probiotics, prebiotics are dietary additives that are fermented by the gut microbiota into short-chain fatty acids (SCFAs), which are the main energy source for colonic epithelial cells. The SCFAs also modulate lipid synthesis (Marcil et al. 2002), stimulate the immune system and increase host resistance against pathogens

(Maslowski and Mackay 2010). Synbiotics are combinations of probiotics and prebiotics (Cerezuela et al. 2011). Synbiotics aim to simultaneously seed and maintain probiotic strains as dominant species in the gut. However, despite recent progress, there is limited information available on different aspects of synbiotics effects on fish (Cerezuela et al. 2011; Torrecillas et al. 2018), and their effect on the microbiota composition of salmonids is currently unknown.

4.2.3 Phage Therapy

Because phage particles are very specific to their bacterial hosts, they do not target both pathogens and the normal flora. Furthermore, phage particles replicate at the site of infection; thus curative doses can be fairly small. Moreover, although bacteria can become resistant to phages, these viral organisms can mutate and therefore evolve to counter phage-resistant bacteria (Matsuzaki et al. 2005), which synthetic antimicrobial treatments cannot do. The most important advantage of phages is that they might kill planktonic pathogens living in the surrounding water in addition to pathogens proliferating in carrier fish. Possible drawbacks of phage therapy include the possible transduction of virulence factors between bacteria; in addition, the vertebrate host may mount an immune response against the phage itself (see chapter "Would Bacteriophages Be a New Old Complement to Antibiotics in Aquaculture?" for a detailed discussion on phage therapy in salmonid aquaculture). Candidate phages that infect *A. s. s.* were isolated recently and opened the way to a broad-range treatment against multiple strains of this major salmonid pathogen (Vincent et al. 2017b). Other salmonid pathogens for which phage treatments are under development include *Flavobacterium psychrophilum* (Castillo et al. 2012; Madsen et al. 2013) and *F. columnare* (Prasad et al. 2011).

4.3 Coarse-Grained Modulation: The Case of K-Selection

In light of the One Health perspective, one may ponder over the efficacy of microbial management methods. Indeed, the strong demand for fish protein has resulted in a strong pressure on fish farmers to provide for an unprecedented increase of the human population (Duarte et al. 2009). Production strategies that may be efficient from an economical point of view (e.g., maximizing output at a minimal cost) may result in suboptimal rearing conditions such as overcrowding, hypoxia, and handling stress (Heikkinen et al. 2006). Disinfection methods and the aforementioned fine-grained microbiota management methods may help but may not address the root cause of certain diseases (i.e., poor rearing conditions). Current microbial management strategies aim to reduce the microbial load (e.g., surface disinfection of eggs and UV irradiation of incoming water) by assuming that fewer microbes translate into fewer risks of infectious disease. By doing so, those methods actually increase the water's carrying capacity for generalist opportunistic microbes (r-strategists)

whose life strategy is to proliferate as rapidly as possible, sometimes at the expense of their host (i.e., opportunistic pathogens) (Vadstein et al. 2018).

As a matter of fact, cod larvae (*Gadus morhua*) reared in recirculating aquaculture systems (RAS) had 72% higher survival rates than larvae reared in flow-through aquaculture (FTS) systems (Attramadal et al. 2014). RAS systems are specifically designed to promote the establishment of a complex microbial ecosystem that feeds of dissolved organic compounds and waste produced by the fish. Moreover, those systems are self-sustained (water is recirculating), resulting in a mature, specialist microbial community (K-strategists) and, therefore, low carrying capacity for opportunistic r-strategists. K-selection of microbial communities holds great promise as a microbiota management tool to promote fish health [for more details on K-selection, see chapter "Controlling Factors for Community Assembly in Developing Cod Larvae (*Gadus morhua*)"]. To our knowledge, research on K-selection of microbial communities in aquaculture has not yet been performed on salmonid fish.

4.4 Toward Real-Time Microbiota Monitoring

Novel DNA sequencing technologies have raised the bar in terms of throughput and scalability. For example, Oxford Nanopore has launched the MinION system, which allows amplicon and metagenome sequencing on a USB stick-side apparatus plugged on a laptop computer (Krehenwinkel et al. 2018). However, its high incorrect basecalling rate of 3%, which is equal to the well-adopted difference threshold for clustering OTUs, limits its usage in metabarcoding studies (Kerkhof et al. 2017). Because single-nucleotide differences are often critical to resolve distinct genera, it is imperative to distinguish biological sequence variation from amplicon sequencing errors (Callahan et al. 2016). With further improvements in sequencing accuracy and novel analysis pipelines, near-instant visualization and analysis of complex microbiome data from a laptop computer will perhaps be possible in the near future.

5 Conclusion

Since 2010, salmonids are second to carps as the most important group of farmed fish, with a total output of over 2 million tonnes. This results in a strong pressure on fish farmers to keep up with high demand for this source of animal protein. Intensive farming practices are commonly used in order to compensate for the immune impairment that results from an over-elicited stress response. This can disrupt global interactions between the host and its microbial symbionts (i.e., microbiota) that play a key role in maintaining fish health in the long term. This chapter presented an overview of the current knowledge on the taxonomic composition (i.e., diversity and structure) of salmonid microbiota and the state of the art on microbial profiling and

modulation, as well as current research gaps and perspectives. A special attention has been given to the microbiota of salmonids that are significantly important in aquaculture.

- Atlantic salmon has the most extensively characterized microbiota of all salmonids to this present day, with 17 dedicated studies published between 2007 and 2018. Most of this research focused on the skin and gut microbiota, including assessments of its response to migration, nutrition, antibiotherapy, and captivity.
- Rainbow trout is the second most produced salmonid fish in salmonid aquaculture and is also the second most studied in terms of host-microbiota interactions. A total of 14 studies investigated the influence of diet, growth promotion, and pathogen inhibition, as well as diet-immunity interactions and their impact on the rainbow trout microbiota. However, none of those studies addressed the impact of antibiotherapy on the microbiota composition and subsequent effects on fish health.
- The brook charr microbiota remains largely mischaracterized, except for the skin mucus microbiota, for which response to intensive rearing conditions and symbiont-pathogen interactions (including interindividual variations) were investigated. To our knowledge, no published studies have yet discussed the impact of diet or therapeutic tools on both microbiota structure and brook charr physiology, though ongoing studies are currently underway.

High-resolution study of the microbiota has been made possible with the advent of high-throughput DNA sequencing during the late 2000s. Several methods have emerged, each offering distinct elements of information on the host-microbiota complex.

- Whole-genome shotgun (WGS) sequencing aims to obtain the whole DNA sequence of a specific organism.
- Metabarcoding is the massively parallel sequencing of a universal genetic marker to infer the taxonomic census of a community. It is currently the most common method in modern microbiome studies, regardless of which organisms are studied.
- Metagenomics is the whole-genome shotgun sequencing of total DNA extracts from complex communities instead of a single organism's DNA, leading to the untargeted sequencing of all microorganisms' genomic sequences present in a given sample, in addition to the host organism's genome.

These methods allowed the study of salmonid microbiota from various levels of organization, i.e., from an individual microbe's genome to the global functional interactions between host and the hundreds of microbial symbionts. Several health-promoting and therapeutic applications have benefitted from those omics-based methods. Whole-genome sequencing allows a thorough characterization of some bacterial probiotic candidates, as well as bacteriophages, that show great promise as prophylactic tools against opportunistic diseases. Metabarcoding and metagenomics allow modelling the microbiota structure as a function of health and treatment parameters. The latter are particularly useful in monitoring difference in microbiota

composition across conditions that tremendously differ between each other (e.g., flow-through aquaculture vs recirculating aquaculture systems).

However, there are significant knowledge gaps in how salmonid-microbiota systems are affected as a whole by antibiotherapy, diet, and rearing conditions. The cause-effect relationships between treatments (or conditions), differential microbiota composition, and host physiology often remain unresolved in most studies. In addition, there are great inherent difficulties associated with the analysis of such a complex multivariate system as the microbiota, coupled with the lack of methodological consensus between studies. Perhaps the implementation of "good practices" and new technological advances should help resolving the complexity of host-microbiota systems to the benefit of salmonid aquaculture. Finally, there is growing awareness that fish and their rearing environments are complex ecosystems and, accordingly, that the well-being of both fish and microbiota must be considered when developing therapeutic tools or intensive rearing protocols.

References

Araújo C, Muñoz-Atienza E, Nahuelquín Y et al (2015) Inhibition of fish pathogens by the microbiota from rainbow trout (*Oncorhynchus mykiss*, Walbaum) and rearing environment. Anaerobe 32:7–14. https://doi.org/10.1016/j.anaerobe.2014.11.001

Aßhauer KP, Wemheuer B, Daniel R, Meinicke P (2015) Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics 31:2882–2884. https://doi.org/10.1093/bioinformatics/btv287

Attramadal KJK, Truong TMH, Bakke I et al (2014) RAS and microbial maturation as tools for K-selection of microbial communities improve survival in cod larvae. Aquaculture 432:483–490. https://doi.org/10.1016/j.aquaculture.2014.05.052

Auchtung JM, Robinson CD, Britton RA (2015) Cultivation of stable, reproducible microbial communities from different fecal donors using minibioreactor arrays (MBRAs). Microbiome 3. https://doi.org/10.1186/s40168-015-0106-5

Balassiano IT, Bastos Mdo C, Madureira DJ et al (2007) The involvement of *tetA* and *tetE* tetracycline resistance genes in plasmid and chromosomal resistance of *Aeromonas* in Brazilian strains. Mem Inst Oswaldo Cruz 102:861–866. https://doi.org/10.1590/S0074-02762007005000121

Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr Comp Biol 42:517–525. https://doi.org/10.1093/icb/42.3.517

Bashiardes S, Zilberman-Schapira G, Elinav E (2016) Use of metatranscriptomics in microbiome research. Bioinform Biol Insights 10:19–25. https://doi.org/10.4137/BBI.S34610

Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S et al (2012) Probiotic mechanisms of action. Ann Nutr Metab 61:160–174. https://doi.org/10.1159/000342079

Boutin S, Bernatchez L, Audet C, Derôme N (2012) Antagonistic effect of indigenous skin bacteria of brook charr (*Salvelinus fontinalis*) against *Flavobacterium columnare* and *F. psychrophilum*. Vet Microbiol 155:355–361. https://doi.org/10.1016/j.vetmic.2011.09.002

Boutin S, Audet C, Derôme N (2013a) Probiotic treatment by indigenous bacteria decreases mortality without disturbing the natural microbiota of *Salvelinus fontinalis*. Can J Microbiol 59:662–670

Boutin S, Bernatchez L, Audet C, Derôme N (2013b) Network analysis highlights complex interactions between pathogen, host and commensal microbiota. PLoS One 8:e84772. https://doi.org/10.1371/journal.pone.0084772

Boutin S, Sauvage C, Bernatchez L et al (2014) Inter individual variations of the fish skin microbiota: host genetics basis of mutualism? PLoS One 9:e102649. https://doi.org/10.1371/journal.pone.0102649

Bullock G, Cipriano R, Snieszko S (1983) Furunculosis and other diseases caused by *Aeromonas salmonicida*. US Fish & Wildlife Publications, Washington, DC

Callahan BJ, McMurdie PJ, Rosen MJ et al (2016) DADA2: high resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869

Castillo D, Higuera G, Villa M et al (2012) Diversity of *Flavobacterium psychrophilum* and the potential use of its phages for protection against bacterial cold water disease in salmonids. J Fish Dis 35:193–201. https://doi.org/10.1111/j.1365-2761.2011.01336.x

Ceppa F, Faccenda F, Filippo CD et al (2018) Influence of essential oils in diet and life-stage on gut microbiota and fillet quality of rainbow trout (*Oncorhynchus mykiss*). Int J Food Sci Nutr 69:318–333. https://doi.org/10.1080/09637486.2017.1370699

Cerezuela R, Meseguer J, Esteban M (2011) Current knowledge in synbiotic use for fish aquaculture: a review. J Aquac Res Dev 1:1–7

Chen L, Zheng D, Liu B et al (2016) VFDB 2016: hierarchical and refined dataset for big data analysis—10 years on. Nucleic Acids Res 44:D694–D697. https://doi.org/10.1093/nar/gkv1239

Cherrington CA, Hinton M, Pearson GR, Chopra I (1991) Short-chain organic acids at pH 5.0 kill *Escherichia coli* and *Salmonella* spp. without causing membrane perturbation. J Appl Bacteriol 70:161–165

Cole JR, Wang Q, Fish JA et al (2014) Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res 42:D633–D642. https://doi.org/10.1093/nar/gkt1244

Culligan EP, Sleator RD, Marchesi JR, Hill C (2014) Metagenomics and novel gene discovery: promise and potential for novel therapeutics. Virulence 5:399–412. https://doi.org/10.4161/viru.27208

Cummings JH (1984) Cellulose and the human gut. Gut 25:805–810. https://doi.org/10.1136/gut.25.8.805

Dehler CE, Secombes CJ, Martin SAM (2017) Seawater transfer alters the intestinal microbiota profiles of Atlantic salmon (*Salmo salar* L.). Sci Rep 7:13877. https://doi.org/10.1038/s41598-017-13249-8

Delsuc F, Brinkmann H, Philippe H (2005) Phylogenomics and the reconstruction of the tree of life. Nat Rev Genet 6:361–375. https://doi.org/10.1038/nrg1603

Derome N, Duchesne P, Bernatchez L (2006) Parallelism in gene transcription among sympatric lake whitefish (*Coregonus clupeaformis Mitchill*) ecotypes. Mol Ecol 15:1239–1249. https://doi.org/10.1111/j.1365-294X.2005.02968.x

Desai AR, Links MG, Collins SA et al (2012) Effects of plant-based diets on the distal gut microbiome of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 350–353:134–142. https://doi.org/10.1016/j.aquaculture.2012.04.005

DeSantis TZ, Hugenholtz P, Larsen N et al (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069–5072. https://doi.org/10.1128/AEM.03006-05

Dostal A, Lacroix C, Bircher L et al (2015) Iron modulates butyrate production by a child gut microbiota in vitro. MBio 6:e01453–e01415. https://doi.org/10.1128/mBio.01453-15

Duarte CM, Holmer M, Olsen Y et al (2009) Will the oceans help feed humanity? https://doi.org/10.1525/bio.2009.59.11.8. http://www.bioone.org/doi/abs/10.1525/bio.2009.59.11.8. Accessed 18 Dec 2018

FAO Fisheries & Aquaculture (2018) FAO fisheries & aquaculture – cultured aquatic species information programme – *Oncorhynchus mykiss* (Walbaum, 1792). FAO Fisheries &

Aquaculture. http://www.fao.org/fishery/culturedspecies/Oncorhynchus_mykiss/en. Accessed 18 Dec 2018

FAO/OMS (2015) An overview of Atlantic salmon, its natural history, aquaculture, and genetic engineering. http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/VeterinaryMedicineAdvisoryCommittee/ucm222635.htm#Section_3:_Aquaculture. Accessed 15 Jun 2015

Fast MD, Hosoya S, Johnson SC, Afonso LOB (2008) Cortisol response and immune-related effects of Atlantic salmon (*Salmo salar* Linnaeus) subjected to short- and long-term stress. Fish Shellfish Immunol 24:194–204. https://doi.org/10.1016/j.fsi.2007.10.009

Freschi L, Vincent AT, Jeukens J et al (2018) The *Pseudomonas aeruginosa* pan-genome provides new insights on its population structure, horizontal gene transfer and pathogenicity. Genome Biol Evol. https://doi.org/10.1093/gbe/evy259

Gajardo K, Rodiles A, Kortner TM et al (2016) A high-resolution map of the gut microbiota in Atlantic salmon (*Salmo salar*): a basis for comparative gut microbial research. Sci Rep 6:30893. https://doi.org/10.1038/srep30893

Gajardo K, Jaramillo-Torres A, Kortner TM et al (2017) Alternative protein sources in the diet modulate microbiota and functionality in the distal intestine of Atlantic Salmon (*Salmo salar*). Appl Environ Microbiol 83. https://doi.org/10.1128/AEM.02615-16

Garrido-Cardenas JA, Manzano-Agugliaro F (2017) The metagenomics worldwide research. Curr Genet 63:819–829. https://doi.org/10.1007/s00294-017-0693-8

Gatesoupe FJ (2010) Chapter 32 – Probiotics and other microbial manipulations in fish feeds: prospective health benefits. In: Watson RR, Preedy VR (eds) Bioactive foods in promoting health. Academic Press, Boston, pp 541–552

Gauthier J (2016) Développement d'outils thérapeutiques à base de probiotiques endogènes contre la furonculose (*Aeromonas salmonicida*) chez l'Omble de fontaine d'aquaculture (Phase 1 – in vitro)

Gauthier J, Charette SJ, Derome N (2017a) Draft genome sequence of *Pseudomonas fluorescens* ML11A, an endogenous strain from Brook Charr with antagonistic properties against *Aeromonas salmonicida subsp. salmonicida*. Genome Announc 5. https://doi.org/10.1128/genomeA.01716-16

Gauthier J, Vincent AT, Charette SJ, Derome N (2017b) Strong genomic and phenotypic heterogeneity in the *Aeromonas sobria* species complex. Front Microbiol 8. https://doi.org/10.3389/fmicb.2017.02434

Gauthier J, Vincent AT, Charette SJ, Derome N (2018) A brief history of bioinformatics. Brief Bioinform. https://doi.org/10.1093/bib/bby063

Geurden I, Mennigen J, Plagnes-Juan E et al (2014) High or low dietary carbohydrate:protein ratios during first-feeding affect glucose metabolism and intestinal microbiota in juvenile rainbow trout. J Exp Biol 217:3396–3406. https://doi.org/10.1242/jeb.106062

Gram L, Ringø E (2005) Prospects of fish probiotics. In: Holzapfel WH, Naughton PJ (eds) Microbial ecology of the growing animal. Elsevier, Amsterdam, pp 379–417

Haas BJ, Papanicolaou A, Yassour M et al (2013) De novo transcript sequence reconstruction from RNA-Seq: reference generation and analysis with Trinity. Nat Protoc 8. https://doi.org/10.1038/nprot.2013.084

Hammami R, Fernandez B, Lacroix C, Fliss I (2013) Anti-infective properties of bacteriocins: an update. Cell Mol Life Sci 70:2947–2967. https://doi.org/10.1007/s00018-012-1202-3

Harvey AC, Solberg MF, Troianou E et al (2016) Plasticity in growth of farmed and wild Atlantic salmon: is the increased growth rate of farmed salmon caused by evolutionary adaptations to the commercial diet? BMC Evol Biol 16:264. https://doi.org/10.1186/s12862-016-0841-7

He X, Chaganti SR, Heath DD (2018) Population-specific responses to interspecific competition in the gut microbiota of two Atlantic Salmon (*Salmo salar*) populations. Microb Ecol 75:140–151. https://doi.org/10.1007/s00248-017-1035-6

Heikkinen J, Vielma J, Kemiläinen O et al (2006) Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout

(*Oncorhynchus mykiss*). Aquaculture 261:259–268. https://doi.org/10.1016/j.aquaculture.2006.07.012

Herman EM, Schmidt MA (2016) The potential for engineering enhanced functional-feed soybeans for sustainable aquaculture feed. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.00440

Héry M, Volant A, Garing C et al (2014) Diversity and geochemical structuring of bacterial communities along a salinity gradient in a carbonate aquifer subject to seawater intrusion. FEMS Microbiol Ecol 90:922–934. https://doi.org/10.1111/1574-6941.12445

Ingerslev H-C, Strube ML, Jørgensen L v G et al (2014) Diet type dictates the gut microbiota and the immune response against *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol 40:624–633. https://doi.org/10.1016/j.fsi.2014.08.021

Jaafar RM, Kania PW, Larsen AH et al (2013) Gut microbiota changes in rainbow trout, *Oncorhynchus mykiss* (Walbaum), during organic acid feed supplementation and *Yersinia ruckeri* infection. J Fish Dis 36:599–606. https://doi.org/10.1111/jfd.12047

Jia B, Raphenya AR, Alcock B et al (2017) CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res 45:D566–D573. https://doi.org/10.1093/nar/gkw1004

Johnson SC, Albright LJ (1992) Effects of cortisol implants on the susceptibility and the histopathology of the responses of naive coho salmon *Oncorhynchus kisutch* to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). Dis Aquat Org 14:195–205

Ju F, Zhang T (2015) 16S rRNA gene high-throughput sequencing data mining of microbial diversity and interactions. Appl Microbiol Biotechnol 99:4119–4129. https://doi.org/10.1007/s00253-015-6536-y

Kadlec K, von Czapiewski E, Kaspar H et al (2011) Molecular basis of sulfonamide and trimethoprim resistance in fish-pathogenic *Aeromonas* isolates. Appl Environ Microbiol 77:7147–7150. https://doi.org/10.1128/AEM.00560-11

Kamada N, Chen GY, Inohara N, Núñez G (2013) Control of pathogens and pathobionts by the gut microbiota. Nat Immunol 14:685–690. https://doi.org/10.1038/ni.2608

Kanehisa M, Sato Y, Morishima K (2016) BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. J Mol Biol 428:726–731. https://doi.org/10.1016/j.jmb.2015.11.006

Karlsson F, Tremaroli V, Nielsen J, Bäckhed F (2013) Assessing the human gut microbiota in metabolic diseases. Diabetes 62:3341–3349. https://doi.org/10.2337/db13-0844

Kerkhof LJ, Dillon KP, Häggblom MM, McGuinness LR (2017) Profiling bacterial communities by MinION sequencing of ribosomal operons. Microbiome 5:116. https://doi.org/10.1186/s40168-017-0336-9

Konstantinidis KT, Tiedje JM (2005) Genomic insights that advance the species definition for prokaryotes. Proc Natl Acad Sci U S A 102:2567–2572. https://doi.org/10.1073/pnas.0409727102

Krehenwinkel H, Pomerantz A, Henderson JB et al (2018) Nanopore sequencing of long ribosomal DNA amplicons enables portable and simple biodiversity assessments with high phylogenetic resolution across broad taxonomic scale. bioRxiv 358572. https://doi.org/10.1101/358572

Langille MGI, Zaneveld J, Caporaso JG et al (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 31:814–821. https://doi.org/10.1038/nbt.2676

Lavoie C, Courcelle M, Redivo B, Derome N (2018) Structural and compositional mismatch between captive and wild Atlantic salmon (*Salmo salar*) parrs' gut microbiota highlights the relevance of integrating molecular ecology for management and conservation methods. Evol Appl 11:1671–1685. https://doi.org/10.1111/eva.12658

Lebov J, Grieger K, Womack D et al (2017) A framework for one health research. One Health 3:44–50. https://doi.org/10.1016/j.onehlt.2017.03.004

Liubakka A, Vaughn BP (2016) *Clostridium difficile* infection and fecal microbiota transplant. AACN Adv Crit Care 27:324–337. https://doi.org/10.4037/aacnacc2016703

Llewellyn MS, Boutin S, Hoseinifar SH, Derome N (2014) Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. Front Microbiol 5:207. https://doi.org/10.3389/fmicb.2014.00207

Lokesh J, Kiron V (2016) Transition from freshwater to seawater reshapes the skin-associated microbiota of Atlantic salmon. Sci Rep 6:19707. https://doi.org/10.1038/srep19707

Macfarlane GT, Macfarlane S (2007) Models for intestinal fermentation: association between food components, delivery systems, bioavailability and functional interactions in the gut. Curr Opin Biotechnol 18:156–162. https://doi.org/10.1016/j.copbio.2007.01.011

Madaro A, Olsen RE, Kristiansen TS et al (2015) Stress in Atlantic salmon: response to unpredictable chronic stress. J Exp Biol 218:2538–2550. https://doi.org/10.1242/jeb.120535

Madsen L, Bertelsen SK, Dalsgaard I, Middelboe M (2013) Dispersal and survival of *Flavobacterium psychrophilum* phages in vivo in rainbow trout and in vitro under laboratory conditions: implications for their use in phage therapy. Appl Environ Microbiol 79:4853–4861. https://doi.org/10.1128/AEM.00509-13

Mahato NK, Gupta V, Singh P et al (2017) Microbial taxonomy in the era of OMICS: application of DNA sequences, computational tools and techniques. Antonie Van Leeuwenhoek 110:1357–1371. https://doi.org/10.1007/s10482-017-0928-1

Marcil V, Delvin E, Seidman E et al (2002) Modulation of lipid synthesis, apolipoprotein biogenesis, and lipoprotein assembly by butyrate. Am J Physiol Gastrointest Liver Physiol 283:G340–G346

Martin TC, Visconti A, Spector TD, Falchi M (2018) Conducting metagenomic studies in microbiology and clinical research. Appl Microbiol Biotechnol 102:8629–8646. https://doi.org/10.1007/s00253-018-9209-9

Maslowski KM, Mackay CR (2010) Diet, gut microbiota and immune responses. Nat Immunol 12:5–9

Matsuzaki S, Rashel M, Uchiyama J et al (2005) Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. J Infect Chemother 11:211–219

Maule AG, Tripp RA, Kaattari SL, Schreck CB (1989) Stress alters immune function and disease resistance in Chinook salmon (*Oncorhynchus tshawytscha*). J Endocrinol 120:135–142

Mayer EA (2000) The neurobiology of stress and gastrointestinal disease. Gut 47:861–869. https://doi.org/10.1136/gut.47.6.861

Méndez J, Guijarro JA (2013) In vivo monitoring of *Yersinia ruckeri* in fish tissues: progression and virulence gene expression. Environ Microbiol Rep 5:179–185. https://doi.org/10.1111/1758-2229.12030

Milot E, Perrier C, Papillon L et al (2013) Reduced fitness of Atlantic salmon released in the wild after one generation of captive breeding. Evol Appl 6:472–485. https://doi.org/10.1111/eva.12028

Miranda CD, Zemelman R (2002) Bacterial resistance to oxytetracycline in Chilean salmon farming. Aquaculture 212:31–47. https://doi.org/10.1016/S0044-8486(02)00124-2

Morey JN, Boggero IA, Scott AB, Segerstrom SC (2015) Current directions in stress and human immune function. Curr Opin Psychol 5:13–17. https://doi.org/10.1016/j.copsyc.2015.03.007

Navarrete P, Mardones P, Opazo R et al (2008) Oxytetracycline treatment reduces bacterial diversity of intestinal microbiota of Atlantic salmon. J Aquat Anim Health 20:177–183. https://doi.org/10.1577/H07-043.1

Naylor RL, Goldburg RJ, Primavera JH et al (2000) Effect of aquaculture on world fish supplies. Nature 405:1017–1024. https://doi.org/10.1038/35016500

Newaj-Fyzul A, Austin B (2015) Probiotics, immunostimulants, plant products and oral vaccines, and their role as feed supplements in the control of bacterial fish diseases. J Fish Dis 38:937–955. https://doi.org/10.1111/jfd.12313

Nordmo, Riseth H, Varma et al (1998) Evaluation of florfenicol in Atlantic salmon, *Salmo salar* L.: efficacy against furunculosis due to *Aeromonas salmonicida* and cold water vibriosis due to *Vibrio salmonicida*. J Fish Dis 21:289–297. https://doi.org/10.1046/j.1365-2761.1998.00106.x

Osman M-A, Neoh H-M, Ab Mutalib N-S et al (2018) 16S rRNA gene sequencing for deciphering the colorectal cancer gut microbiome: current protocols and workflows. Front Microbiol 9:767. https://doi.org/10.3389/fmicb.2018.00767

Overbeek R, Olson R, Pusch GD et al (2014) The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res 42:D206–D214. https://doi.org/10.1093/nar/gkt1226

Panek M, Paljetak HČ, Barešić A et al (2018) Methodology challenges in studying human gut microbiota – effects of collection, storage, DNA extraction and next generation sequencing technologies. Sci Rep 8:5143. https://doi.org/10.1038/s41598-018-23296-4

Park H, Weier S, Razvi F et al (2017) Towards the development of a sustainable soya bean-based feedstock for aquaculture. Plant Biotechnol J 15:227–236. https://doi.org/10.1111/pbi.12608

Pickering AD, Duston J (1982) Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. J Fish Biol 23:163–175

Pickering AD, Pottinger TG (1985) Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. J Fish Biol 27:611–619. https://doi.org/10.1111/j.1095-8649.1985.tb03206.x

Pollock J, Glendinning L, Wisedchanwet T, Watson M (2018) The madness of microbiome: attempting to find consensus "best practice" for 16s microbiome studies. Appl Environ Microbiol 84:e02627–e02617. https://doi.org/10.1128/AEM.02627-17

Prasad Y, Arpana, Kumar D, Sharma AK (2011) Lytic bacteriophages specific to *Flavobacterium columnare* rescue catfish, *Clarias batrachus* (Linn.) from columnaris disease. J Environ Biol 32:161–168

Qin J, Li R, Raes J et al (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65. https://doi.org/10.1038/nature08821

Quince C, Walker AW, Simpson JT et al (2017) Shotgun metagenomics, from sampling to analysis. Nat Biotechnol 35:833–844. https://doi.org/10.1038/nbt.3935

Raida MK, Larsen JL, Nielsen ME, Buchmann K (2003) Enhanced resistance of rainbow trout, *Oncorhynchus mykiss* (Walbaum), against *Yersinia ruckeri* challenge following oral administration of *Bacillus subtilis* and *B. licheniformis* (BioPlus2B). J Fish Dis 26:495–498. https://doi.org/10.1046/j.1365-2761.2003.00480.x

Reith ME, Singh RK, Curtis B et al (2008) The genome of *Aeromonas salmonicida subsp. salmonicida* A449: insights into the evolution of a fish pathogen. BMC Genomics 9:427. https://doi.org/10.1186/1471-2164-9-427

Rimoldi S, Terova G, Ascione C et al (2018) Next generation sequencing for gut microbiome characterization in rainbow trout (*Oncorhynchus mykiss*) fed animal by-product meals as an alternative to fishmeal protein sources. PLoS One 13:e0193652. https://doi.org/10.1371/journal.pone.0193652

Rochat T, Barbier P, Nicolas P et al (2017) Complete genome sequence of *Flavobacterium psychrophilum* strain OSU THCO2-90, used for functional genetic analysis. Genome Announc 5. https://doi.org/10.1128/genomeA.01665-16

Rudi K, Angell IL, Pope PB et al (2018) Stable core gut microbiota across the freshwater-to-saltwater transition for farmed Atlantic Salmon. Appl Environ Microbiol 84:e01974–e01917. https://doi.org/10.1128/AEM.01974-17

Schreck CB (1982) Stress and rearing of salmonids. Aquaculture 28:241–249. https://doi.org/10.1016/0044-8486(82)90026-6

Schubiger CB, Orfe LH, Sudheesh PS et al (2015) Entericidin is required for a probiotic treatment (*Enterobacter* sp. strain C6-6) to protect trout from cold-water disease challenge. Appl Environ Microbiol 81:658–665. https://doi.org/10.1128/AEM.02965-14

Sender R, Fuchs S, Milo R (2016) Revised estimates for the number of human and bacteria cells in the body. bioRxiv 036103. https://doi.org/10.1101/036103

Silby MW, Winstanley C, Godfrey SAC et al (2011) *Pseudomonas* genomes: diverse and adaptable. FEMS Microbiol Rev 35:652–680. https://doi.org/10.1111/j.1574-6976.2011.00269.x

Swiatczak B, Cohen IR (2015) Gut feelings of safety: tolerance to the microbiota mediated by innate immune receptors. Microbiol Immunol 59:573–585. https://doi.org/10.1111/1348-0421.12318

Tagini F, Greub G (2017) Bacterial genome sequencing in clinical microbiology: a pathogen-oriented review. Eur J Clin Microbiol Infect Dis 36:2007–2020. https://doi.org/10.1007/s10096-017-3024-6

Tanaka T, Kawasaki K, Daimon S et al (2014) A hidden pitfall in the preparation of agar media undermines microorganism cultivability. Appl Environ Microbiol 80:7659–7666. https://doi.org/10.1128/AEM.02741-14

Tatusova T, DiCuccio M, Badretdin A et al (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569

Tobback E, Decostere A, Hermans K et al (2007) *Yersinia ruckeri* infections in salmonid fish. J Fish Dis 30:257–268. https://doi.org/10.1111/j.1365-2761.2007.00816.x

Torrecillas S, Rivero-Ramírez F, Izquierdo MS et al (2018) Feeding European sea bass (*Dicentrarchus labrax*) juveniles with a functional synbiotic additive (mannan oligosaccharides and *Pediococcus acidilactici*): an effective tool to reduce low fishmeal and fish oil gut health effects? Fish Shellfish Immunol 81:10–20. https://doi.org/10.1016/j.fsi.2018.07.007

Trudel MV, Vincent AT, Attéré SA et al (2016) Diversity of antibiotic-resistance genes in Canadian isolates of *Aeromonas salmonicida* subsp. *salmonicida*: dominance of pSN254b and discovery of pAsa8. Sci Rep 6. https://doi.org/10.1038/srep35617

Vadstein O, Attramadal KJK, Bakke I, Olsen Y (2018) K-selection as microbial community management strategy: a method for improved viability of larvae in aquaculture. Front Microbiol 9. https://doi.org/10.3389/fmicb.2018.02730

Vázquez-Baeza Y, Hyde ER, Suchodolski JS, Knight R (2016) Dog and human inflammatory bowel disease rely on overlapping yet distinct dysbiosis networks. Nat Microbiol 1:16177. https://doi.org/10.1038/nmicrobiol.2016.177

Vincent AT, Derome N, Boyle B et al (2017a) Next-generation sequencing (NGS) in the microbiological world: how to make the most of your money. J Microbiol Methods 138:60–71. https://doi.org/10.1016/j.mimet.2016.02.016

Vincent AT, Paquet VE, Bernatchez A et al (2017b) Characterization and diversity of phages infecting *Aeromonas salmonicida* subsp. *salmonicida*. Sci Rep 7:7054. https://doi.org/10.1038/s41598-017-07401-7

Watson RA, Pollack JB (2001) Symbiotic composition and evolvability. In: Kelemen J, Sosík P (eds) Advances in artificial life. Springer, Berlin, pp 480–490

Wilk R, Andersen K, Uglenes I, Egidius E (1989) Cortisol-induced increase in susceptibility of Atlantic salmon, *Salmo salar*, to *Vibrio salmonicida*, together with effects on the blood cell pattern. Aquaculture 83:201–215

Wong S, Waldrop T, Summerfelt S et al (2013) Aquacultured rainbow trout (*Oncorhynchus mykiss*) possess a large core intestinal microbiota that is resistant to variation in diet and rearing density. Appl Environ Microbiol 79:4974–4984. https://doi.org/10.1128/AEM.00924-13

Yilmaz P, Parfrey LW, Yarza P et al (2014) The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic Acids Res 42:D643–D648. https://doi.org/10.1093/nar/gkt1209

Zepeda Mendoza ML, Sicheritz-Pontén T, Gilbert MTP (2015) Environmental genes and genomes: understanding the differences and challenges in the approaches and software for their analyses. Brief Bioinform 16:745–758. https://doi.org/10.1093/bib/bbv001

Zihler Berner A, Fuentes S, Dostal A et al (2013) Novel Polyfermentor intestinal model (PolyFermS) for controlled ecological studies: validation and effect of pH. PLoS One 8:e77772. https://doi.org/10.1371/journal.pone.0077772