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REVIEW ARTICLE

# Systematic literature review identifying bacterial constituents in the core intestinal microbiome of rainbow trout (*Oncorhynchus mykiss*)

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## Abstract

Fish aquaculture has become the fastest growing sector in global food production. Thus, ensuring the sustainability of aquaculture practices is of the utmost importance. Studies in higher vertebrates (i.e. mammals) have demonstrated the role of the host microbiome in physiological processes from nutrient acquisition to pathogen protection. Therefore, analysis of fish microbiomes is an important factor to consider with regard to overall animal health and welfare. Rainbow trout (*Oncorhynchus mykiss*) are an economically valued fish cultured worldwide. Several studies have identified microbial constituents inhabiting the intestinal tract of rainbow trout. To better elucidate some of the core constituents of the rainbow trout intestinal microbiome, this systematic literature review analysed the relative abundance results from 25 articles published on the rainbow trout intestinal microbiome from 2017 to 2021. Bacteria classified within the phyla Firmicutes and Proteobacteria were observed in every study. At the family level, *Lactobacillaceae* was consistently observed. Additionally, bacteria in the Actinobacteria, Bacteroides, and Tenericutes phyla were identified in at least 50% of the studies. Interestingly, *Mycoplasma* spp. were occasionally the most dominant organisms present in the microbiome. Overall, the results here identify bacteria that are commonly found members of the rainbow trout intestinal microbiome.

**KEYWORDS**
16S rRNA, aquaculture, Firmicutes, gastrointestinal microbiome, Proteobacteria

## 1 | INTRODUCTION

Expansion of the human population has led to an increased demand for aquatic animal food production. Because freshwater and marine fish provide a substantial source of animal protein in the human diet (Food and Agriculture Organization of the United Nations, 2020; Godfray et al., 2010), the need for this specific food product has risen sharply. Traditional sources of fish (i.e. wild-caught fisheries) are overexploited and unable to keep up with the current rate of human seafood consumption (Godfray et al., 2010; Ye & Gutierrez, 2017). The

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aquaculture industry can alleviate some of the strain on depleted natural fisheries by providing an alternative fish source. In fact, aquaculture has become the fastest growing food industry in the animal production sector (Troell et al., 2014). Because of its rapid growth, more focus has been on increasing this industry's sustainability. One way in which this is being accomplished is by using alternative diet supplements, as discussed below, to reduce reliance on fish meal, the main ingredient of aquaculture fish feeds. Fish meal is often sourced from overexploited captured fisheries (Klinger & Naylor, 2012; Naylor et al., 2009). Moving forward, another major avenue of research in the aquaculture industry is related to maintaining its sustainability by ensuring the health and welfare of farmed fish. The microbiomes inhabiting fish aid the animal in multiple ways and can be a good indicator for overall health of the animals (Banerjee & Ray, 2017; de Bruijn et al., 2018; Desai et al., 2012; Legrand et al., 2020; Tarnecki et al., 2017). With the introduction of next-generation sequencing (NGS) and improved bioinformatic methods, a growing number of studies have been performed to analyse the fish microbiome. One species of fish associated with an extensive number of 16S rRNA gene-based microbiome investigations, rainbow trout (*Oncorhynchus mykiss*), was selected as the focus of this systematic literature review.

Rainbow trout (*O. mykiss*) are typically carnivorous fish, though they can consume plant protein-based diets (Craft et al., 2016) and effectively utilise dietary carbohydrates (Balasubramanian et al., 2016; Geurden et al., 2007). They dwell in freshwater ecosystems, though anadromous stock (living in marine environments, but spawning in freshwater) exist as steelhead trout, and they are endemic to the Pacific Northwest of North America (Hinshaw et al., 2004). Farming of rainbow trout began relatively recently (compared to other species like Nile tilapia [Popma & Masser 1999]), around the late 19th century (Hardy 2002). However, their incorporation in the aquaculture industry worldwide has led to rainbow trout currently being one of the top 15 most farmed fish (Food and Agriculture Organization of the United Nations, 2020). This increased farming potential for rainbow trout has led to increased human consumption and a subsequent rise in demand (Hinshaw et al., 2004). The expansion of rainbow trout farming has helped to elucidate optimal farming parameters. For example, these fish grow best at colder water temperatures (∼15°C) and require exposure to ample aeration (Hardy, 2002). Despite past research that examined the role of the microbiome of rainbow trout on the health and welfare of the animal, additional analyses are still needed to improve farming methods in the future.

Communities of microorganisms that inhabit a particular environmental niche, including on or in animals, comprise the microbiome (Alexander, 1971). Like higher vertebrates (i.e. mammals), fish contain various tissue-specific microbiomes, such as the microbiomes associated with the skin and gills (Larsen et al., 2013; Legrand et al., 2018; Rosado et al., 2019a; Xu et al., 2013) or the gastrointestinal tract (Clements et al., 2014; Tarnecki et al., 2017; Wang et al., 2018). These microorganisms provide several key benefits for its fish hosts. For instance, the bacteria present in the intestinal tract microbiome play an important role in maintaining energy homeostasis (Butt & Volkoff, 2019; Nicholson et al., 2012). Host digestive processes are also aided by the ability of the microbiome to facilitate nutrient acquisition from the diet and provide essential nutrients (e.g. vitamins) for the host (Chatterjee et al., 2017; Ghanbari et al., 2015; Llewellyn et al., 2014; Nicholson et al., 2012; Tarnecki et al., 2017). During host immune system development, the microbiome helps guide the ability to recognise self from non-self and modulate immune system regulation (Brugman et al., 2018; Kelly & Salinas, 2017). Lastly, the microbiome of fish confers some protection against fish pathogens, such as *Aeromonas* spp. (Nya & Austin, 2009; Park et al., 2017), *Streptococcus agalactiae* (Silva et al., 2020), *Flavobacterium* spp. (Mohammed & Arias, 2015; Nematollahi et al., 2003), and *Yersinia ruckeri* (Ingerslev et al., 2014), by occupying space, competing for nutrients, and in some cases directly inhibiting their growth (Chatterjee et al., 2017; Hai 2015).

Exogenous factors influence the dynamics of fish colonisation by microbes. In contrast to most mammals, fish are constantly and directly exposed to environmental microorganisms in the aquatic environment. Because of their immersion in the water column, the microbiomes of fish are strongly affected by changes in temperature (Element et al., 2020; Huyben et al., 2018), salinity (Element et al., 2020; Zhang et al., 2016), and, specifically important for aquaculture-raised animals, stocking density (Du et al., 2019; Parma et al., 2020). This aquatic, high-density lifestyle creates more opportunities for exposure to pathogens, which can be associated with altered fish microbiomes (Kelly & Salinas, 2017; Rosado et al., 2019b). The aquaculture industry, like other agricultural and livestock sectors in food production, has historically employed the use of antibiotics in order to not only control pathogens, but promote growth. The use of prophylactic antibiotics is partly to blame for the increased prevalence of antibiotic-resistant pathogens (Cabello, 2006). Antibiotics can also have a major impact on the microbiomes of fish and ultimately risk the health of the fish stocks (Cabello, 2006; Carlson et al., 2015; He et al., 2017; Schmidt et al., 2017). To provide a viable alternative to prophylactic antibiotic administration, which is still done in some regions outside of the United States, an increasing amount of research has been conducted on the efficacy of probiotics (live microbes) and prebiotics (compounds metabolised by microbes) in promoting a healthy microbiome. For instance, these dietary supplements have the capability to support healthy fish physiology and microbiomes while also inhibiting pathogen proliferation (Al-Hisnawi et al., 2019; Dawood et al., 2020; Giorgia et al., 2018; Hooshyar et al., 2020; Mora-Sánchez et al., 2020; Park et al., 2017; Zabidi et al., 2021). Dietary changes especially affect the inhabitants of the gastrointestinal tract. One such change has been aimed at reducing reliance on the unsustainable use of fishmeal by supplementing or outright replacing it with alternative protein sources (Olsen & Hasan, 2012; Turchini et al., 2019). Through these efforts, many studies have also investigated the impact of alternative feeds on the fish microbiome such as plant-based alternatives (Blaufuss et al., 2020; Estruch et al., 2015; Lim & Lee, 2009) and insect meals (Huyben et al., 2019; Rimoldi et al., 2021; Terova et al., 2021) among other sources (Gasco et al., 2018; Rimoldi et al., 2018; Tlusty et al., 2017).

Fish microbiomes are also impacted by intrinsic physiological factors. The divergence between the microbiomes of fish associated with different trophic levels (i.e. carnivores and herbivores) may be related to the evolutionary development of the two types of fish and may even involve some co-speciation (Groussin et al., 2017; Phillips et al., 2012). Unlike herbivorous and omnivorous fish species, the intestinal tracts of carnivorous fish, like trout, are less complex morphologically, being shorter in length and straighter in comparison to other fish (Al-Hisnawi, 1947; Elliott & Bellwood, 2003). In fact, it has been observed that the intestinal tract of rainbow trout is more microbially homogenous than other mucosal sites (Lowrey et al., 2015). Development of the intestinal tract as the fish matures also has a significant impact on the structure of the microbial communities; the colonising members of the microbiome can shift depending on the stage of animal development (Li et al., 2017). Though less studied than host age, host genetics and phylogeny have also been implicated as possible drivers in the selection of the microbiome inhabitants (Tzeng et al., 2015; Liu et al., 2016).

Regardless of the factors influencing the rainbow trout microbiome, there are some members of the microbial community that are consistently present (Dehler et al., 2017; Gajardo et al., 2016; Ricaud et al., 2018; Roeselers et al., 2011; Tarnecki et al., 2017; Wong et al., 2013). This core set of microbes could illustrate the 'healthy' microbiome and therefore be used to establish targets for beneficial manipulation. Therefore, identifying the core set of microbes that inhabit the intestinal tract of rainbow trout will be helpful in advancing the aquaculture-raised rainbow trout industry. This systematic review compiles an overview of the recent pertinent literature in the field to identify members of the rainbow trout intestinal microbiome that are consistently present.

## 2 | METHODS

### 2.1 | Article inclusion criteria

This systematic review was accomplished by analysing microbiome data from articles published between January 2017 and July 2021 that utilised 16S rRNA gene-based approaches. The search was performed on Google Scholar with inclusion of the key terms 'rainbow trout' and 'microbiome' for English language articles. Moreover, reviewed papers were selected such that a variety of testing variables (e.g. diet, location, etc.) were included as part of the analysis (Table 1). Papers that did not include a clear description of the relative abundances of individual phyla were filtered out for the quantitative analysis reported in Table 2.

### 2.2 | Core constituent analyses

The taxonomic data were analysed using Microsoft Excel v.2203 (Microsoft). Control and treatment animals within a given study were analysed as a group together, since the goal was to identify commonalities rather than differences between the groups. The frequency of each phylum observed, out of the total 25 papers analysed, was reported as a percentage. No minimum relative abundance value was used as a criterion to omit phyla. Instead, a 'phylum-positive' identification was defined by a simple observation of an individual phylum within the referenced study; bacterial phyla associated with rainbow trout intestines were reported here when observed in the reviewed paper. Variable parameters impacting the microbiome as reported in each study, including different treatment groups (e.g. diet), were recorded (Table 1). The effect of tissue type (see below) was analysed in more detail. Briefly, the 'total' category is defined by the percentage of papers reporting phyla that were associated with either digesta (i.e. faeces) or mucosa out of the 25 total papers; therefore, a positive result reported for the 'total' only needed to be present in at least one of the tissues. Conversely, the tissue-specific percentages represent that the frequency a particular phylum was identified in a tissue type (i.e. digesta, mucosa, or the combination of digesta plus mucosa at the point of genomic DNA extraction) out of the total number of papers that analysed the particular tissue of interest.

Of the 25 total papers included for this systematic review, 10 reported the relative abundances of intestinal phyla in clearly formatted numerical formats. The relative abundance values are defined as the number of sequences associated with a specific taxon normalised to the total number of sequences within a sample (represented as a percentage of the total sequences). These reported relative abundances corresponding to each phylum were averaged together between different studies regardless of tissue type. For studies that investigated more than one treatment, the relative abundances associated with each treatment were averaged together prior to inclusion within the full dataset reported here. The standard error mean (SEM) represents the error associated with the averaged relative abundances corresponding to the number of papers that observed the phylum of interest.

Not all articles reviewed here reported the family-level (or lower classification) taxonomy. Family-level analysis was accomplished by calculating the frequency with which a particular family was observed out of the total number of papers that identified its associated parent phylum classification (i.e. phylum-positive). Tissue-specific analysis is also reported as either the total (representing an identification in either tissue, digesta, or mucosa), digesta, mucosa, or the combination of digesta and mucosa (tissues combined prior to genomic DNA extractions).

## 3 | RESULTS AND DISCUSSION

### 3.1 | Identifying bacterial members of the core intestinal microbiome

Studies of fish intestinal tracts analysed in this systematic literature review tested the impact of various parameters including diet, pathogens, host genetics, environment, and host age on the gastrointestinal microbiome of rainbow trout (Table 1). These studies encompassed locations around the globe with fish originating from several different farmed sources. The location of the study did not seem to impact the microbiome composition in that different study sites were found to yield similar microbiome composition, whereas geographically similar sites yielded different microbiome compositions, often being influenced by diet and/or animal age. However, regardless of the variables, a core set of bacteria appeared to be consistently present within

**TABLE 1** Parameters specified in referenced studies.

| Reference | GI tissue type[a] | Study variables | | | GI site[b] | Trout culturing system | Final fish weight | 16S rRNA gene hypervariable region | Feed | Sampling depth | Study water temperature | Geographic location |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Diet | Pathogen | Other | | | | | | | | |
| Huyben et al., 2019 | Combo | Diet | | | Hind-gut | Flow-through | 74–82 g | V4 | In-house | 12 | 10.9 ± 0.4°C | Sweden |
| Lyons et al., 2017a* | Combo | Diet | | | Hind-gut | Flow-through | 117–137 g | V4 | Hellenic Centre for Marine Research (Greece) | 12 | 14 ± 1°C | United Kingdom |
| Lyons et al., 2017b* | Combo | | | Exploratory | Hind-gut | Farm/flow-through | 99 g[c]/191 g[d] (aquarium) | V4 | Not specified | 12 (farm); 9 (flow-though) | 11.8°C | Scotland |
| Michl et al., 2017* | Combo | Diet | | Age | Whole tract | RAS[e] | 1–4 g | V6–V8 | In-house | 15 | 11.6 ± 1.1°C | Germany |
| Parshukov et al., 2019 | Combo | | Pathogen | | Whole tract | Farm | 240–850 g | V3–V4 | Veronesi (Italy) | 3–8 | 15.4 ± 0.5°C (July),<br>15.3 ± 1.1°C (August),<br>14.2 ± 0.04°C (September) | Russia |
| Rimoldi et al., 2018 | Combo | Diet | | | Whole tract | Flow-through | 252–298 g | V3–V4 | Naturalleva (Italy) | 4 | 12.5 ± 0.3°C | Italy |
| Rimoldi et al., 2021* | Combo | Diet | | | Whole tract | Flow-through | Not specified | V4 | In-house | 8 | 13 ± 1°C | Italy |
| Brown et al., 2019 | Mucosa | | Pathogen | Genetics | Mid-gut | Tanks | 194 g | V1–V3 | CSF, Inc. (USA) | 10 | 14.5°C | West Virginia, USA |
| Etyemez Büyükdeveci et al., 2018 | Mucosa | Diet | | | Whole tract | Pond | 171–186 g | V4 | Skretting Optima Trout 3P (Turkey) | 6 | 14–18°C | Turkey |
| Farzad et al., 2021 | Mucosa | Diet | | | Mid-gut | RAS | 115–122 g | V4 | Finfish Gold, Ziegler (USA) | 6 | 17.1 ± 0.78°C | Virginia, USA |
| Gatesoupe et al., 2018* | Mucosa | Diet | | | Hind-gut | Tanks/farm | 230–345 g | V3–V4[f] | In-house | 11–13 | 6.5–8°C (Period 1),<br>17–18°C (Period 2) | France |
| Gonçalves & Gallardo-Escárate, 2017* | Mucosa | Diet | | | Whole tract | RAS | Not specified | V4 | Ewos (Chile) | 4-6 | 13 ± 0.5°C | Chile |
| Hines et al., 2021 | Mucosa | Diet | | | Mid-gut | RAS | 180–250 g | V4 | Finfish, Ziegler (USA) and in-house | 4 | 14–15°C | Virginia, USA |
| Mora-Sánchez, et al., 2020 | Mucosa | Diet | Pathogen | | Whole tract | Tanks | 36–39 g | Not specified | Inicio Plus 887, BioMar (Spain) | 4 | Not specified | Spain |
| Pérez-Sánchez et al., 2020* | Mucosa | Diet | Pathogen | | Not specified | Not specified | 44–48 g | V1–V3 | Aquasoja (Portugal) | 4 | Not specified | Spain |

(Continues)

**TABLE 1** (Continued)

| Reference | GI tissue type[a] | Study variables: Diet | Pathogen | Other | GI site[b] | Trout culturing system | Final fish weight | 16S rRNA gene hypervariable region | Feed | Sampling depth | Study water temperature | Geographic location |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Terova et al., 2021* | Mucosa | Diet | | | Whole tract | Flow through | 312–353 g | V3–V4 | SPAROS LDA (Portugal) and Ÿnsect (France) | 6 | 13 ± 1°C | Italy |
| Al-Hisnawi et al., 2019 | Mucosa Digesta | Diet | | | Hind-gut | RAS | Not specified | V1–V2 | Ewos Sigma 50 (Chile) | 3 | 14.7 ± 0.6°C | United Kingdom |
| Betiku et al., 2018* | Mucosa Digesta | Diet | | Environment | Not specified | RAS/flow-through | 103–123 g | V3–V4 | In-house | 3 | 15°C (RAS), 14.2–15.5°C (flow-through) | Montana, USA |
| Huyben et al., 2018 | Mucosa Digesta | Diet | | | Hind-gut | Flow-through | Not specified | V4 | In-house | 3 | 11.4 ± 0.3°C (cold), 18 ± 0.3°C (warm) | Sweden |
| Blaufuss et al., 2020 | Digesta | Diet | | | Hind-gut | RAS | 406–488 g | V3–V4 | In-house | 9 | 15°C | Idaho, USA |
| Ceppa et al., 2018 | Digesta | Diet | | Age | | Tanks | 1050 g | V1–V3 | VITA and ECOFISH, Veronesi (Italy) | 3 (initial timepoints) 6 (final timepoint) | 12.57 ± 0.78°C | Italy |
| Pelusio et al., 2020 | Digesta | Diet | | Environment | Hind-gut | RAS | 300–320 g | V3–V4 | Ecofish 4, Veronesi SpA (Italy) | 12[g] (day 82) 6[g] (day 89) | 15 ± 1°C | Italy |
| Ricaud et al., 2018* | Digesta | | | Genetics | Mid- and hind-gut | Tanks | 87–90 g | V3–V4 | T-2P Omega (France) | 10 | Not specified | France |
| Terova et al., 2019 | Digesta | Diet | | | Whole tract | Flow-through | 216–223 g | V3–V4 | In-house | 6 | 13 ± 1°C | Italy |
| Yildirimer & Brown, 2018 | Digesta | | | Environment | Whole tract | Raceway | Not specified | V4 | Not specified | 10 | Not specified | Northwest, USA |

*Note*: An asterisk (*) denotes which study results were used for the relative abundance analysis reported in Table 2.

[a] Gastrointestinal (GI) tissue type utilised for microbiome analysis. Combination of mucosa and digesta (combo); mucosa and digesta separately analysed as indicated.

[b] Intestinal site isolated for microbiome analysis.

[c] Farmed animals.

[d] Flow-through tank-raised animals.

[e] Recirculating Aquaculture System (RAS).

[f] DNA product was reverse-transcribed from 16 rRNA.

[g] Each sample represents the pool of 5 fish per tank.

**TABLE 2** Average phyla-level relative abundances.

| Phylum | Mean relative abundance (%)[a] | Reported papers[b] |
|---|---|---|
| Acidobacteria | 2.00 ± 0.00 | 2 |
| Actinobacteria | 6.28 ± 3.66 | 8 |
| Bacteroidetes | 5.62 ± 3.76 | 7 |
| Firmicutes | 18.7 ± 4.10 | 10 |
| Fusobacteria | 14.3 ± 12.7 | 6 |
| Proteobacteria | 29.0 ± 7.49 | 10 |
| Spirochaetes | 5.94 ± 4.01 | 3 |
| Tenericutes | 46.5 ± 15.7 | 6 |

[a] Error is represented by the mean relative abundances of that particular phylum across the specified number of 'reported papers' ± SEM.
[b] Number of studies wherein the phylum-level relative abundances were clearly reported (out of 10 total papers).

the trout gastrointestinal tract in every study. Bacteria belonging to the phyla Firmicutes and Proteobacteria were always present in the trout intestinal microbiome (Figure 1). Also identified in a majority of the studies (>50% of the 25 studies investigated here) were the phyla Actinobacteria, Bacteroidetes, and Tenericutes.

Although bacteria in the phyla Firmicutes and Proteobacteria were present in every study, the relative abundances of each phylum can be vastly different depending on the study. Most studies indicated the microbiome is dominated by these two phyla, while other studies found these two phyla to exist in much lower abundances relative to other more dominant organisms such as Fusobacteria and Tenericutes (Brown et al., 2019; Farzad et al., 2021; Hines et al., 2021; Huyben et al., 2018; Lyons et al., 2017a, 2017b; Mora-Sánchez et al., 2020; Terova et al., 2021). On average, bacteria classified as Firmicutes accounted for ∼19% of the relative abundances and bacteria classified as Proteobacteria accounted for ∼29% of the relative abundances (Table 2).

## 3.2 | Firmicutes

Firmicutes are a phylum of bacteria that typically have a low G+C (guanine and cytosine nucleotide) content in their genomes and most, but not all of them, have a Gram-positive cell wall structure as part of their inherent physiology. The family *Lactobacillaceae* is one of the most common constituents of the Firmicutes phylum observed (Figure 2). Overall, the family *Lactobacillaceae* is more commonly identified within the trout digesta, largely defining the allochthonous microbiome (transient bacteria), as opposed to the mucosal layer (Figure 2). This heterogenic group of bacteria includes several species that can provide probiotic effects for their fish hosts (Claesson et al., 2007). Specifically, some *Lactobacillus* species confer immune-stimulatory and antioxidant properties for fish (Adeshina et al., 2020; Dawood et al., 2020; Hooshyar et al., 2020; Pérez-Sánchez et al., 2011). Two other common Firmicutes families, *Streptococcaceae* and *Clostridiaceae*, were also more prevalent in the digesta than mucosa of the rainbow trout (Figure 2). The *Streptococcaceae* family contains several fish-specific pathogens including the *Lactococcus garvieae* analysed by Pérez-Sánchez et al. (2020). This group may also encompass opportunistic pathogens that are otherwise common constituents of the rainbow trout microbiome. It is possible the constituents belonging to the *Lactobacillaceae* family may be acting as probiotics to inhibit further proliferation by opportunistic pathogens such as those in the *Streptococcaceae* family (Harikrishnan et al., 2011; Heo et al., 2013; Kim et al., 2019; Son et al., 2009). Similar to members of the *Streptococcaceae* family, constituents belonging to the *Clostridiaceae* family represent several higher vertebrate pathogens occasionally associated with an increased level of microbiome dysbiosis and disease (Muñiz Pedrogo et al., 2019; Picchianti-Diamanti et al., 2018; Scarpa et al., 2011). The role of *Clostridiaceae* in the internal microbiome of trout is not well known; however, some strains have actually been shown to act as probiotics for the fish host (de Bruijn et al., 2018; Sakai et al., 1995).

## 3.3 | Proteobacteria

Proteobacteria are a major phylum of Gram-negative bacteria. Within Proteobacteria, the most common families identified in the rainbow trout intestine were *Enterobacteriaceae*, *Pseudomonadaceae*, and *Moraxellaceae* (Figure 2). The family *Enterobacteriaceae* is a broadly encompassing group of bacteria that includes pathogens like *Yersinia ruckeri* and *Aeromonas* spp. and commensal organisms like *Escherichia coli* (Conway & Cohen, 2015). One major benefit posed by *Enterobacteriaceae* members is the production of short-chain fatty acids via sugar fermentation similar to *Clostridiaceae* members (Gottschalk, 1986; Wüst et al., 2009). Certain species in the *Enterobacteriaceae* family can act as protective organisms against pathogenic infections in trout (Schubiger et al., 2015). The families *Pseudomonadaceae* and *Moraxellaceae* include several fish pathogens (Austin and Austin, 2012), notably those of the *Acinetobacter* genus within *Moraxellaceae* (Rossau et al., 1991). Though *Acinetobacter* was identified as being present in the study, the cultured fish did not exhibit disease caused by these organisms. A protective system generated by lactic acid bacteria, similar to their antagonistic nature against *Streptococcaceae*, may be inhibiting excessive proliferation by these potentially pathogenic bacterial families.

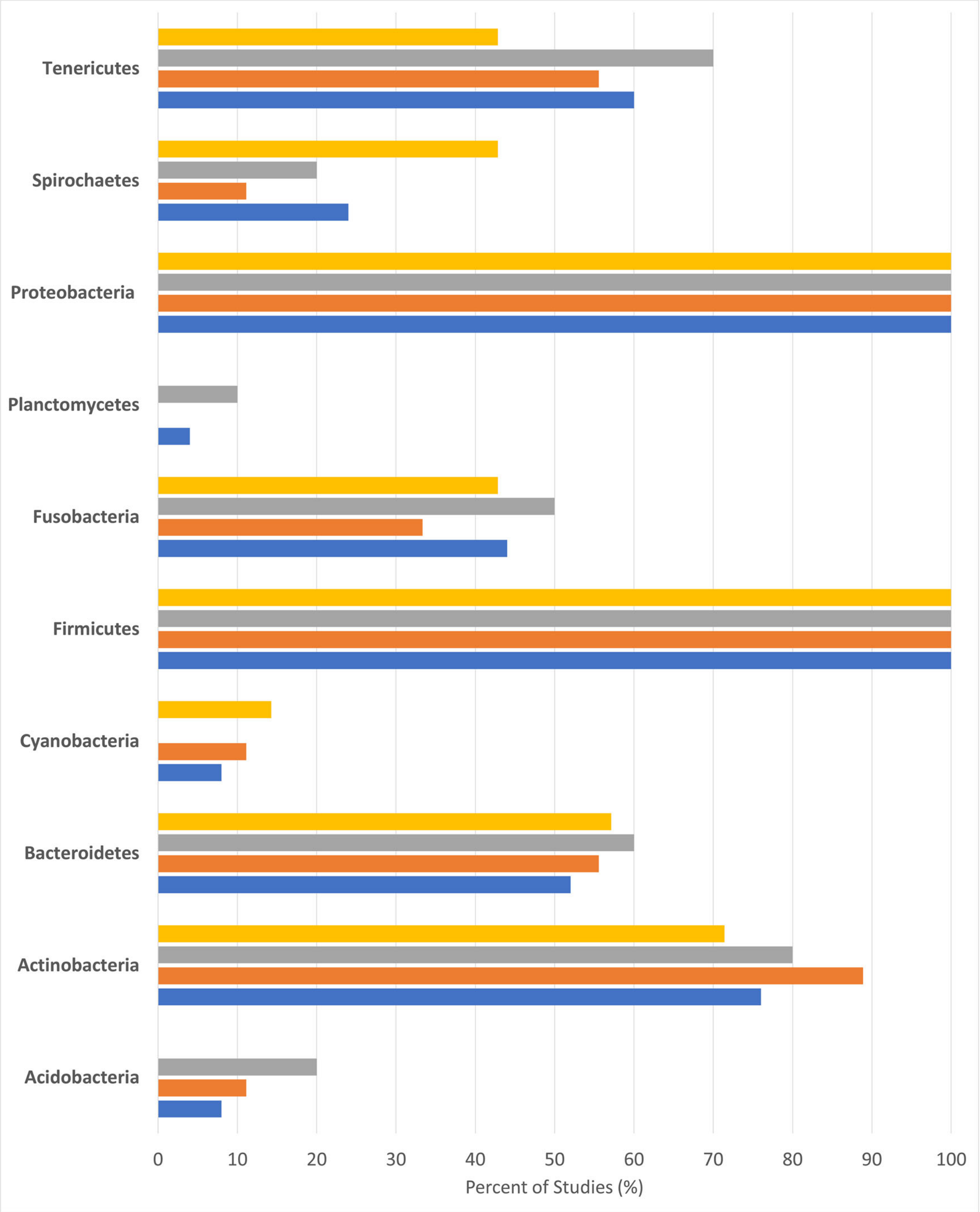

**FIGURE 1** Common phyla present in rainbow trout gut microbiomes. Bars represent the percent frequency of positive phylum identification in fish tissue samples on a per study basis. Positive phylum identifications (phylum-positive) denote the phylum was observed in each study, regardless of relative abundance, in the particular tissue. Frequencies are measured as the percent of phyla present within all papers used for this systematic review (blue; *N* = 25), papers that analysed the digesta (orange; *N* = 9), papers that analysed the mucosa (grey; *N* = 10), or papers that analysed the combination of both mucosa and digesta (yellow; *N* = 7).

### 3.4 | Actinobacteria

The phylum Actinobacteria is composed of mostly Gram-positive bacteria that primarily occupy soil and water environments. Though not found in every study, bacteria belonging to the phylum Actinobacteria were identified in at least 75% of the studies reviewed (Figure 1). Actinobacteria are also typically present at lower relative abundances compared to the more common Firmicutes and Proteobacteria (Table 2). However, its levels vary at different stages in the life cycle of rainbow trout. Specifically, Actinobacteria appear to dominate the microbiome of trout during the early life stages compared to fish during adulthood wherein a much smaller relative abundance is observed

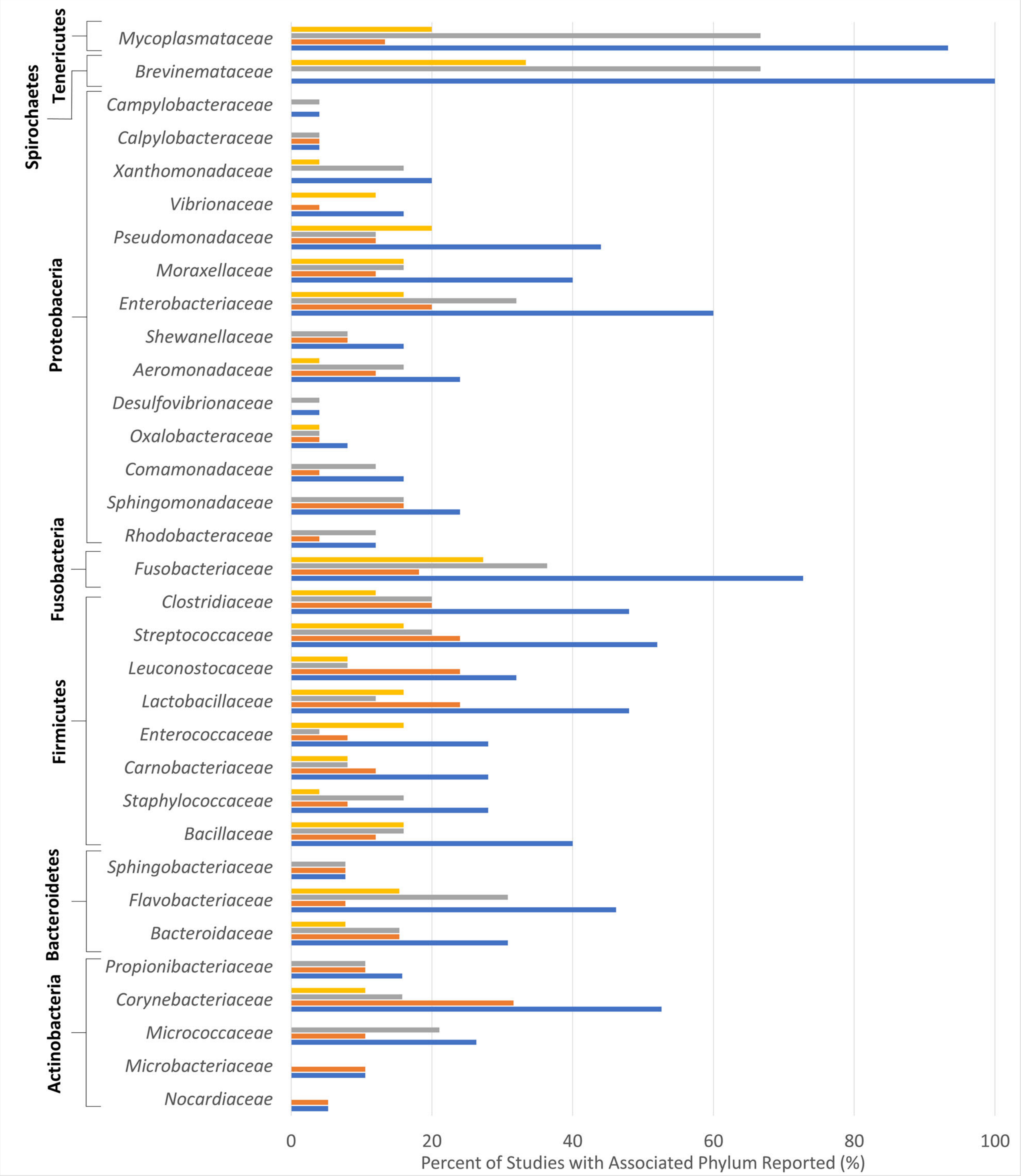

**FIGURE 2** Common bacterial families identified in the core phyla of rainbow trout gut microbiomes. Bars represent the percent frequency of family presence in fish tissue samples. Positive phylum identifications (phylum-positive) denote the phylum was observed in each study, regardless of relative abundance, in the particular tissue. Frequencies are defined as the percentage of phylum-positive papers wherein a family is identified. Colours represent the percentage of all phylum-positive papers used for this systematic review (blue; $N = 25$), phylum-positive papers that analysed the digesta (orange; $N = 9$), phylum-positive papers that analysed the mucosa (grey; $N = 10$), or phylum-positive papers that analysed the combination of both mucosa and digesta (yellow; $N = 7$).

(Ceppa et al., 2018; Piazzon et al., 2019). Therefore, age appears to be a driving factor that influences the selection of microbial constituents throughout the life of the fish.

The most commonly identified Actinobacteria family, *Corynebacteriaceae* (Figure 2), encompasses bacteria prevalent in the microflora of fish species including salmonids (Hartviksen et al., 2014; Izvekova, Izvekov & Plotnikov, 2007; Ruohonen et al., 2014). Though it consists of human and fish pathogens such as *Corynebacterium diphtheriae*, the studies used for the taxonomic analysis in this systematic review did not indicate any disease associated with infection by *Corynebacterium* spp.

### 3.5 | Bacteroidetes

Bacteroidetes are non-spore-forming, anaerobic or aerobic, rod-shaped bacteria found in a variety of environments such as soil and marine water environments, as well as the microbiome of animals and humans. Bacteria associated with the Bacteroidetes phylum were identified in at least 50% of the studies analysed in this systematic review and are typically in lower relative abundances compared to Firmicutes, Proteobacteria, and Actinobacteria (Figure 1; Table 2). The dominant Bacteroidetes family, *Flavobacteriaceae* (Figure 2), includes important fish pathogens such as *Flavobacterium columnare* and *Flavobacterium psychrophilum* (Mohammed & Arias, 2015; Nematollahi et al., 2003). *Flavobacterium psychrophilum* may pose a serious issue due to the prevalence of antibiotic resistance (Dalsgaard & Madsen, 2000), which, when compounded with the high frequency of identification in trout microbiomes, indicates the importance of controlling this opportunistic pathogen.

### 3.6 | Tenericutes

The Tenericutes phylum is dominated by bacteria associated with the *Mycoplasmataceae* family, a group of bacteria lacking cell walls (Figure 2), per the reviewed studies. However, it should be noted that *Mycoplasmataceae* has recently been reclassified within the Firmicutes phylum (Parks et al., 2018). Tenericutes was present in at least 60% of the papers analysed here (Figure 1). When present, this group of bacteria typically dominates the microbiome compared to the other organisms (Table 2). *Mycoplasma*, a common genera within the *Mycoplasmataceae* family, can sometimes dominate the trout intestinal microbiome (Al-Hisnawi et al., 2019; Brown et al., 2019; Farzad et al., 2021; Lyons et al., 2017a, 2017b). Uniquely, this group of bacteria are usually associated with a host due to their increased vulnerability resulting from the lack of a cell wall (Razin, 1967). This phenomenon is further validated by the results presented here indicating a higher abundance of *Mycoplasmataceae* found in the autochthonous microbiome (mucosal adherent bacteria) versus transient digesta microbiome present in trout faeces (Figure 2). Within fish, *Mycoplasma* spp. are increasingly being identified as commensal organisms instead of pathogens, though some species such as *Mycoplasma mobile* are known to cause disease (Stadtländer & Kirchhoff, 1990; Stadtländer et al., 1995). Further studies are necessary to further characterise the role of this bacterium within rainbow trout.

### 3.7 | Microbial populations associated with the gastrointestinal mucosa and digesta

Though distinct from external tissues, the microbial communities may be relatively homogenous along the gastrointestinal tract (Lowrey et al., 2015). This relative microbial homogeneity is due, in part, to a relatively shorter and linear intestinal tract with consistent resting levels of effectors (i.e. intestinal ions and pH), regardless of intestinal topography (Bucking & Wood, 2009; Fard, Weisheit and Poynton, 2007; Lowrey et al., 2015). However, within the gastrointestinal tract, some members of the microbiome colonise the mucosal epithelial layer and are considered adherent, whereas others are transient as they are sloughed off into the lumen and/or are contained within the faecal material that comprises the digesta. The sampling method within the gastrointestinal tract (i.e. digesta only, mucosa only, or intestinal mucosa combined with digesta) (Table 1) had a direct impact on the organisms that were observed in the microbiome. Notably, most of the taxa were identified across all sample types, though some patterns were apparent. For instance, Firmicutes tended to be identified more often in the digesta than mucosa (Figure 1). On the other hand, Proteobacteria were identified more in the mucosa than the digesta. *Mycoplasmataceae* were reported in both digesta and mucosa types, but this family of bacteria was present in the mucosa five times more often than in the digesta (Figure 2). Taxa that are more common in the digesta but not in the mucosa, such as *Lactobacillaceae*, *Leuconostocaceae*, and *Corynebacteriaceae*, indicate transient organisms, including potential *Lactobacillus* probiotics, that may be unable to colonise the host's mucosa (O'Toole & Cooney, 2008; Walter, 2008). Conversely, the taxa present to a high degree in the mucosa but not in the digesta (e.g. *Flavobacteriaceae*, *Brevinemataceae*, *Mycoplasmataceae*, and *Enterobacteriaceae*) indicate adherent organisms that likely colonised the host's intestinal mucosa and thus are able to persist, despite otherwise detrimental conditions such as pH fluctuations (Banerjee & Ray, 2017). However, it is important to note that the increased abundance of a particular organism within the mucosa does not necessarily guarantee long-term mucosal colonisation, likely due to competitive exclusion (Frese et al., 2012). The sloughing of dead bacterial cells may contribute to these taxa reported from both sample types (Ventura et al., 2009).

### 3.8 | Limitations of methods used to assess microbiome constituents and future approaches

Microbiome analysis based on sequencing of the 16S rRNA gene has continued to provide a metric to analyse microbial populations independent of culture-based techniques. With the advent of NGS, high-throughput 16S rRNA gene-based studies have generated a plethora of microbiome data. The majority of studies reviewed here utilised Illumina MiSeq NGS, which generates short-read sequences. These short-read sequences are useful for comparing reads aligned to the shorter sequences available within the hypervariable regions of the 16S rRNA gene. Each hypervariable region presents differing resolutions between bacterial taxa. Because of these differential resolutions, it is important to choose the most appropriate target region for microbial community analysis. A standard method proposed by the Earth Microbiome Project (Thompson et al., 2017) involves targeting the fourth hypervariable region (V4). Though most of the studies here also targeted the V4 region, about half also included the V3 region during amplification. Inclusion of the V3–V4 data may represent a higher resolution bias resulting from the more precise primer sets used (Almeida et al., 2018; Fuks et al., 2018). However, any introduced biases would be more apparent at lower taxonomic classes (i.e. genus and species levels). A potential solution to overcome biases introduced using individual hypervariable regions and even utilisation of the full 16S rRNA gene (e.g. bias introduced by copy number) for microbial community analyses could be the implementation of metagenomic sequencing (e.g. Rasmussen et al., 2022). While the costs and specific experience required for running metagenomic analyses are greater than that of 16S-based community analyses, the additional insights gained from metagenomics can greatly increase taxonomic classification confidence. Potentially, a more precise bacterial function within the microbiome can be inferred by successfully identifying bacteria at the species and even strain level. Ideally, microbial community studies should begin to incorporate metagenomics as the expense of these more robust analyses decreases and the number of available applications increases.

Assigning taxonomic information to sequences also inherently introduces bias (Mysara et al., 2017; Schmidt et al., 2015), though this bias reduces with the inclusion and verification of more reference genomes. The majority of studies used for this systematic review analysed microbiome data by assigning taxonomy to artificially created operational taxonomic units (OTUs). Most of the OTUs here were defined by 97% similarities. As sequencing technologies continue to advance with reduction of inherent errors and bioinformatic tools develop around increasing the confidence of filtered sequences (e.g. DADA2 [Callahan et al., 2016]), the frequency of implementing OTU clustering will continue to decrease. Additional work in this field would also be benefitted by the consistent publication of taxonomic relative abundance tables for ease of comparison.

### 3.9 | Concluding thoughts

All studies used for this systematic review analysed the microbiomes of rainbow trout raised in aquaculture. As shown here, a core set of intestinal microbial constituents exists independent of study variables including age, specific intestinal sampling site, and geographical location. However, it would be informative to investigate how culturing rainbow trout affects the microbiome compared to wild-caught fish and if this impacts the members of the core microbiome. Additional analyses to define the core microbiome can involve identifying taxa shared between testing groups based upon more advanced criteria than the simple presence or absence of taxa (e.g. abundance-based, persistence, connectivity [Shade & Handelsman, 2012]). However, the methods used in this systematic review have enabled the elucidation of some commonly found bacterial constituents of the aquaculture-raised rainbow trout intestinal microbiome.

## AUTHOR CONTRIBUTIONS

**Ian HInes**: Conceptualisation; data curation; formal analysis; investigation; visualisation; writing—original draft; writing—review and editing. **Maggie Marshall**: Data curation; formal analysis; investigation; writing—review and editing. **Stephen A. Smith**: Conceptualisation; writing—review and editing. **David Kuhn**: Conceptualisation; writing—review and editing. **Ann Stevens**: Conceptualisation; project administration; supervision; writing—original draft; writing—review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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## REFERENCES

Adeshina, I., Abubakar, M.I.-O. & Ajala, B.E. (2020) Dietary supplementation with *Lactobacillus acidophilus* enhanced the growth, gut morphometry, antioxidant capacity, and the immune response in juveniles of the common carp, *Cyprinus carpio*. *Fish Physiology and Biochemistry*, 46, 1375–1385.

Alexander, M. (1971) *Microbial ecology*. New York: John Wiley & Sons, Inc.

Al-Hisnawi, A., Rodiles, A., Rawling, M.D., Castex, M., Waines, P., Gioacchini, G., et al. (2019) Dietary probiotic *Pediococcus acidilactici* MA18/5 M modulates the intestinal microbiota and stimulates intestinal immunity in rainbow trout (*Oncorhynchus mykiss*). *Journal of the World Aquaculture Society*, 50, 1133–1151.

Al-Hisnawi, A.H. (1947) The feeding habits and the morphology of the alimentary tract of some teleosts living in the neighbourhood of the Marine Biological Station, Ghardaga, Red Sea. *Publications of the Marine Biological Station, Ghardaqa (Red Sea)*, 5, 1–61.

Almeida, A., Mitchell, A.L., Tarkowska, A. & Finn, R.D. (2018) Benchmarking taxonomic assignments based on 16S rRNA gene profiling of the microbiota from commonly sampled environments. *Gigascience*, 7, 1–10.

Austin, B. & Austin, D.A. (2012) *Pseudomonadaceae* representatives. In: Austin, B. & Austin, D. A. (Eds.) *Bacterial fish pathogens: disease of farmed and wild fish*. Dordrecht: Springer, pp. 341–356.

Balasubramanian, MN., Panserat, S., Dupont-Nivet, M., Quillet, E., Montfort, J., Le Cam, A. et al. (2016) Molecular pathways associated with the nutritional programming of plant-based diet acceptance in rainbow trout following an early feeding exposure. *BMC Genomics*, 17, 1–20.

Banerjee, G. & Ray, A.K. (2017) Bacterial symbiosis in the fish gut and its role in health and metabolism. *Symbiosis*, 72, 1–11.

Betiku, OC., Yeoman, CJ., Gaylord, T.G, Americus, B., Olivo, S., Duff, G.C. et al. (2018) Water system is a controlling variable modulating bacterial diversity of gastrointestinal tract and performance in rainbow trout. *PLoS ONE*, 13, e0195967.

Blaufuss, P.C., Bledsoe, J.W., Gaylord, T.G, Sealey, W.M., Overturf, K.E. & Powell, M.S. (2020) Selection on a plant-based diet reveals changes in oral tolerance, microbiota and growth in rainbow trout (*Oncorhynchus mykiss*) when fed a high soy diet. *Aquaculture*, 525, 735287.

Brown, R.M., Wiens, G.D. & Salinas, I. (2019) Analysis of the gut and gill microbiome of resistant and susceptible lines of rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology*, 86, 497–506.

Brugman, S., Ikeda-Ohtsubo, W., Braber, S., Folkerts, G., Pieterse, C.M.J. & Bakker, P.A.H.M. (2018) A comparative review on microbiota manipulation: lessons from fish, plants, livestock, and human research. *Frontiers in Nutrition*, 5, 80.

Bucking, C. & Wood, C.M. (2009) The effect of postprandial changes in pH along the gastrointestinal tract on the distribution of ions between the solid and fluid phases of chyme in rainbow trout. *Aquaculture Nutrition*, 15, 282–296.

Butt, R.L. & Volkoff, H. (2019) Gut microbiota and energy homeostasis in fish. *Front Endocrinol*, 10, 9.

Cabello, F.C. (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology*, 8, 1137–1144.

Callahan, B.J., Mcmurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13, 581–583.

Carlson, J.M., Hyde, E.R., Petrosino, J.F., Manage, A.B.W. & Primm, T.P. (2015) The host effects of *Gambusia affinis* with an antibiotic-disrupted microbiome. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 178, 163–168.

Ceppa, F., Faccenda, F., De Filippo, C., Albanese, D., Pindo, M., Martelli, R. et al. (2018) Influence of essential oils in diet and life-stage on gut microbiota and fillet quality of rainbow trout (*Oncorhynchus mykiss*). *International Journal of Food Science and Nutrition*, 69, 318–333.

Chatterjee, S., Datta, S., Sharma, S. Tiwari, S. & Gupta, D.K. (2017) Health and environmental applications of gut microbiome: a review. *Ecological Chemistry and Engineering S*, 24, 467–482.

Claesson, M.J., Van Sinderen, D. & O'toole, PW. (2007) The genus *Lactobacillus* – a genomic basis for understanding its diversity. *FEMS Microbiology Letters*, 269, 22–28.

Clements, K.D., Angert, E.R., Montgomery, W.L. & Choat, J.H. (2014) Intestinal microbiota in fishes: what's known and what's not. *Molecular Ecology*, 23, 1891–1898.

Conway, T. & Cohen, P.S. (2015) Commensal and pathogenic *Escherichia coli* metabolism in the gut. *Microbiology Spectrum*, 3, MBP–0006–2014.

Craft, C.D., Ross, C., Sealey, W.M., Gaylord, T.G., Barrows, FT., Fornshell, G. et al. (2016) Growth, proximate composition, and sensory characteristics of rainbow trout *Oncorhynchus mykiss* consuming alternative proteins. *Aquaculture*, 459, 223–231.

Dalsgaard, I. & Madsen, L. (2000) Bacterial pathogens in rainbow trout, *Oncorhynchus mykiss* (Walbaum), reared at Danish freshwater farms. *Journal of Fish Diseases*, 23, 199–209.

Dawood, M.A.O., Moustafa, E.M., Elbialy, Z.I., Farrag, F., Lolo, E.E.E., Abdel-Daim, H.A. et al. (2020) *Lactobacillus plantarum* L-137 and/or $\beta$-glucan impacted the histopathological, antioxidant, immune-related genes and resistance of Nile tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila*. *Research in Veterinary Science*, 130, 212–221.

de Bruijn, I., Liu, Y., Wiegertjes, G.F. & Raaijmakers, J.M. (2018) Exploring fish microbial communities to mitigate emerging diseases in aquaculture. *FEMS Microbiology Ecology*, 94, 161.

Dehler, C.E., Secombes, C.J. & Martin, S.A.M. (2017) Environmental and physiological factors shape the gut microbiota of Atlantic salmon parr (*Salmo salar* L.). *Aquaculture*, 467, 149–157.

Desai, A.R., Links, M.G., Collins, S.A., Mansfield, G.S., Drew, M.D., Van Kessel, A.G. et al. (2012) Effects of plant-based diets on the distal gut microbiome of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 350-353, 134–142.

Du, F., Li, Y., Tang, Y., Su, S., Yu, J., Yu, F. et al. (2019) Response of the gut microbiome of *Megalobrama amblycephala* to crowding stress. *Aquaculture*, 500, 586–596.

Element, G., Engel, K., Neufeld, J.D., Casselman, J.M., Coeverden De Groot, P., Greer, C.W. et al. (2020) Seasonal habitat drives intestinal microbiome composition in anadromous Arctic char (*Salvelinus alpinus*). *Environmental Microbiology*, 22, 3112–3125.

Elliott, J.P. & Bellwood, D.R. (2003). Alimentary tract morphology and diet in three coral reef fish families. *Journal of Fish Biology*, 63, 1598–1609.

Estruch, G., Collado, M.C., Peñaranda, D.S., Tomás Vidal, A., Jover Cerdá, M., Pérez Martínez, G. et al. (2015) Impact of fishmeal replacement in diets for gilthead sea bream (*Sparus aurata*) on the gastrointestinal microbiota determined by pyrosequencing the 16S rRNA gene. *PLoS ONE*, 10, e0136389.

Etyemez Büyükdeveci, M., Balcázar, J.L., Demirkale, İ. & Dikel, S. (2018) Effects of garlic-supplemented diet on growth performance and intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 486, 170–174.

Fard, M., Weisheit, C. & Poynton, S.L. (2007) Intestinal pH profile in rainbow trout *Oncorhynchus mykiss* and microhabitat preference of the flagellate *Spironucleus salmonis* (Diplomonadida). *Diseases of Aquatic Organisms*, 76, 241–249.

Farzad, R., Kuhn, D.D., Smith, S.A., O'Keefe, S.F., Hines, I.S., Bushman, T.J. et al. (2021) Effects of selenium-enriched prebiotic on the growth performance, innate immune response, oxidative enzyme activity and microbiome of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 531, 735980.

Food and Agriculture Organization of the United Nations (FAO). (2020) *The State of World Fisheries and Aquaculture 2020*. FAO.

Frese, S.A., Hutkins, R.W. & Walter, J. (2012) Comparison of the colonization ability of autochthonous and allochthonous strains of lactobacilli in the human gastrointestinal tract. *Adv Microbiol*, 02, 399–409.

Fuks, G., Elgart, M., Amir, A., Zeisel, A., Turnbaugh, P.J., Soen, Y. et al. (2018) Combining 16S rRNA gene variable regions enables high-resolution microbial community profiling. *Microbiome*, 6, 1–13.

Gajardo, K., Rodiles, A., Kortner, T.M., Krogdahl, Å., Bakke, A.M., Merrifield, D.L. et al. (2016) A high-resolution map of the gut microbiota in Atlantic salmon (*Salmo salar*): a basis for comparative gut microbial research. *Scientific Reports*, 6, 30893.

Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T. et al. (2018) Fishmeal alternative protein sources for aquaculture feeds. In: Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T. et al. (Eds.) *Feeds for the aquaculture sector*. Cham: Springer, pp. 1–28.

Gatesoupe, F.-J., Fauconneau, B., Deborde, C., Madji Hounoum, B., Jacob, D., Moing, A., et al. (2018) Intestinal microbiota in rainbow trout, Oncorhynchus mykiss, fed diets with different levels of fish-based and plant ingredients: a correlative approach with some plasma metabolites. *Aquaculture Nutrition*, 24, 1563–1576.

Geurden, I., Aramendi, M., Zambonino-Infante, J. & Panserat, S. (2007) Early feeding of carnivorous rainbow trout (*Oncorhynchus mykiss*) with a hyperglucidic diet during a short period: effect on dietary glucose utilization in juveniles. *Am J Physiol – Regul Integr Comp Physiol*, 292, R2275–R2283.

Ghanbari, M., Kneifel, W. & Domig, K.J. (2015) A new view of the fish gut microbiome: advances from next-generation sequencing. *Aquaculture*, 448, 464–475.

Giorgia, G., Elia, C., Andrea, P., Cinzia, C., Stefania, S., Ana, R. et al. (2018) Effects of lactogen 13, a new probiotic preparation, on gut microbiota and endocrine signals controlling growth and appetite of *Oreochromis niloticus* juveniles. *Microbial ecology*, 76, 1063–1074.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F. et al. (2010) Food security: the challenge of feeding 9 billion people. *Science*, 327, 812–818.

Gonçalves, A.T. & Gallardo-Escárate, C. (2017) Microbiome dynamic modulation through functional diets based on pre- and probiotics (mannan-oligosaccharides and *Saccharomyces cerevisiae*) in juvenile rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Microbiology*, 122, 1333–1347.

Gottschalk, G. (1986) Regulation of bacterial metabolism. In: Gottschalk, G. (Ed.) *Bacterial metabolism*. New York: Springer-Verlag, pp. 178–207.

Groussin, M., Mazel, F., Sanders, J.G., Smillie, C.S., Lavergne, S., Thuiller, W. et al. (2017) Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. *Nature Communications*, 8, 1–12.

Hai, N.V. (2015) The use of probiotics in aquaculture. *Journal of Applied Microbiology*, 119, 917–935.

Hardy, R.W. (2002) Rainbow trout, *Oncorhynchus mykiss*. In: Webster, C.D. & Lim, C. (Eds.) *Nutrient requirements and feeding of finfish for aquaculture*. Wallingford, UK: CABI Publishing, pp. 184–202.

Harikrishnan, R., Kim, M.-C., Kim, J.-S., Balasundaram, C. & Heo, M.-S. (2011) Probiotics and herbal mixtures enhance the growth, blood constituents, and nonspecific immune response in *Paralichthys olivaceus* against *Streptococcus parauberis*. *Fish & Shellfish Immunology*, 31, 310–317.

Hartviksen, M., Bakke, A.M., Vecino, J.G., Ringø, E. & Krogdahl, Å. (2014) Evaluation of the effect of commercially available plant and animal protein sources in diets for Atlantic salmon (*Salmo salar* L.): digestive and metabolic investigations. *Fish Physiology and Biochemistry*, 40, 1621–1637.

He, S., Wang, Q., Li, S., Ran, C., Guo, X., Zhang, Z. et al. (2017) Antibiotic growth promoter olaquindox increases pathogen susceptibility in fish by inducing gut microbiota dysbiosis. *Science China Life Sciences*, 60, 1260–1270.

Heo, W.-S., Kim, Y.-R., Kim, E.-Y., Bai, S.C. & Kong, I.-S. (2013) Effects of dietary probiotic, *Lactococcus lactis* subsp. lactis I2, supplementation on the growth and immune response of olive flounder (*Paralichthys olivaceus*). *Aquaculture*, 376-379, 20–24.

Hines, I.S., Ferguson, C.S., Bushman, T.J., Gatlin, D.M., Jensen, R.V., Smith, S.A. et al. (2021) Impact of a yeast-based dietary supplement on the intestinal microbiome of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture Research*, 52, 1594–1604.

Hinshaw, J.M., Fornshell, G. & Kinnunen, R. (2004) *A profile of the aquaculture of trout in the United States*. Report for USDA Risk Management Agency, Federal Crop Insurance Corporation, through Mississippi State University.

Hooshyar, Y., Abedian Kenari, A., Paknejad, H. & Gandomi, H. (2020) Effects of *Lactobacillus rhamnosus* ATCC 7469 on different parameters related to health status of rainbow trout (*Oncorhynchus mykiss*) and the protection against *Yersinia ruckeri*. *Probiotics and antimicrobial proteins*, 12, 1370–1384.

Huyben, D., Sun, L., Moccia, R., Kiessling, A., Dicksved, J. & Lundh, T. (2018) Dietary live yeast and increased water temperature influence the gut microbiota of rainbow trout. *Journal of Applied Microbiology*, 124, 1377–1392.

Huyben, D., Vidaković, A., Werner Hallgren, S. & Langeland, M. (2019) High-throughput sequencing of gut microbiota in rainbow trout (*Oncorhynchus mykiss*) fed larval and pre-pupae stages of black soldier fly (*Hermetia illucens*). *Aquaculture*, 500, 485–491.

Ingerslev, H.-C., Strube, M.L., Jørgensen, L.V.G., Dalsgaard, I., Boye, M. & Madsen, L. (2014) Diet type dictates the gut microbiota and the immune response against *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology*, 40, 624–633.

Izvekova, G.I., Izvekov, E.I. & Plotnikov, A.O. (2007) Symbiotic microflora in fishes of different ecological groups. *Izvestiia Akademii Nauk Seriia Biologicheskaia*, 34, 728–737.

Kelly, C. & Salinas, I. (2017) Under pressure: interactions between commensal microbiota and the teleost immune system. *Frontiers in immunology*, 8, 559.

Kim, J., Lee, K.W., Jeong, H.S., Rahman Ansary, M.W., Kim, H.S., Kim, T. et al. (2019) Oral administration effect of yacon, ginger and blueberry on the growth, body composition and plasma chemistry of juvenile olive flounder (*Paralichthys olivaceus*) and immunity test against *Streptococcus iniae* compared to a commercial probiot. *Aquaculture Reports*, 15, 100 212.

Klinger, D. & Naylor, R. (2012) Searching for solutions in aquaculture: charting a sustainable course. *Annual Review of Environment and Resources*, 37, 247–276.

Larsen, A., Tao, Z., Bullard, S.A. & Arias, C.R. (2013) Diversity of the skin microbiota of fishes: evidence for host species specificity. *FEMS Microbiology Ecology*, 85, 483–494.

Legrand, T.P.R.A., Catalano, S.R., Wos-Oxley, M.L., Stephens, F., Landos, M., Bansemer, M.S. et al. (2018) The inner workings of the outer surface: skin and gill microbiota as indicators of changing gut health in yellowtail kingfish. *Frontiers in Microbiology*, 8, 1–17.

Legrand, T.P.R.A., Wynne, J.W., Weyrich, L.S. & Oxley, A.P.A. (2020) A microbial sea of possibilities: current knowledge and prospects for an improved understanding of the fish microbiome. *Reviews in Aquaculture*, 12, 1101–1134.

Li, X., Zhou, L., Yu, Y., Ni, J., Xu, W. & Yan, Q. (2017) Composition of gut microbiota in the gibel carp (*Carassius auratus gibelio*) varies with host development. *Microbial Ecology*, 74, 239–249.

Lim, S.-J. & Lee, K.-J. (2009) Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. *Aquaculture*, 290, 283–289.

Liu, H., Guo, X., Gooneratne, R., Lai, R., Zeng, C., Zhan, F. et al. (2016) The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. *Scientific Reports*, 6, 24340.

Llewellyn, M.S., Boutin, S., Hoseinifar, S.H. & Derome, N. (2014) Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. *Frontiers in Microbiology*, 5, 1–17.

Lowrey, L., Woodhams, D.C., Tacchi, L. & Salinas, I. (2015) Topographical mapping of the rainbow trout (*Oncorhynchus mykiss*) microbiome reveals a diverse bacterial community with antifungal properties in the skin. *Applied and Environmental Microbiology*, 81, 6915–6925.

Lyons, P.P., Turnbull, J.F., Dawson, K.A. & Crumlish, M. (2017a) Effects of low-level dietary microalgae supplementation on the distal intestinal microbiome of farmed rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research*, 48, 2438–2452.

Lyons, P.P., Turnbull, J.F., Dawson, K.A. & Crumlish, M. (2017b) Phylogenetic and functional characterization of the distal intestinal microbiome of rainbow trout *Oncorhynchus mykiss* from both farm and aquarium settings. *Journal of Applied Microbiology*, 122, 347–363.

Michl, S.C., Ratten, J.-M., Beyer, M., Hasler, M., Laroche, J., Schulz, C. (2017) The malleable gut microbiome of juvenile rainbow trout (*Oncorhynchus mykiss*): diet-dependent shifts of bacterial community structures. *PLoS ONE*, 12, e0177735.

Mohammed, H.H. & Arias, C.R. (2015) Potassium permanganate elicits a shift of the external fish microbiome and increases host susceptibility to columnaris disease. *Veterinary Research*, 46, 1–13.

Mora-Sánchez, B., Balcázar, J.L. & Pérez-Sánchez, T. (2020) Effect of a novel postbiotic containing lactic acid bacteria on the intestinal microbiota and disease resistance of rainbow trout (*Oncorhynchus mykiss*). *Biotechnology Letters*, 42, 1957–1962.

Muñiz Pedrogo, D.A., Chen, J., Hillmann, B., Jeraldo, P., Al-Ghalith, G., Taneja, V. et al. (2019) An increased abundance of *Clostridiaceae* characterizes arthritis in inflammatory bowel disease and rheumatoid arthritis: a cross-sectional study. *Inflammatory Bowel Diseases*, 25, 902-913.

Mysara, M., Vandamme, P., Props, R., Kerckhof, F.-M., Leys, N., Boon, N. et al. (2017) Reconciliation between operational taxonomic units and species boundaries. *FEMS Microbiology Ecology*, 93, 1–12.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P. et al. (2009) Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 15103–15110.

Nematollahi, A., Decostere, A., Pasmans, F. & Haesebrouck, F. (2003) *Flavobacterium psychrophilum* infections in salmonid fish. *Journal of Fish Diseases*, 26, 563–574.

Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W. et al. (2012) Host-gut microbiota metabolic interactions. *Science*, 336, 1262–1267.

Nya, E.J. & Austin, B. (2009) Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, 32, 963–970.

Olsen, R.L. & Hasan, M.R. (2012) A limited supply of fishmeal: impact on future increases in global aquaculture production. *Trends in Food Science & Technology*, 27, 120–128.

O'Toole, P.W. & Cooney, J.C. (2008) Probiotic bacteria influence the composition and function of the intestinal microbiota. *Interdiscip Perspect Infect Dis* 2008, 1–9.

Park, Y., Lee, S., Hong, J., Kim, D., Moniruzzaman, M. & Bai, S.C. (2017) Use of probiotics to enhance growth, stimulate immunity and confer disease resistance to *Aeromonas salmonicida* in rainbow trout (*Oncorhynchus mykiss*). *Aquac Res*, 48, 2672–2682.

Parks, D.H., Chuvochina, M., Waite, D.W., Rinke, C., Skarshewski, A., Chaumeil, P.-A. et al. (2018) A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nature Biotechnology*, 36, 996.

Parma, L., Pelusio, N.F., Gisbert, E., Esteban, M.A., D'amico, F., Soverini, M. et al. (2020) Effects of rearing density on growth, digestive conditions, welfare indicators and gut bacterial community of gilthead sea bream (*Sparus aurata*, L. 1758) fed different fishmeal and fish oil dietary levels. *Aquaculture*, 518, 734854.

Parshukov, A.N., Kashinskaya, E.N., Simonov, E.P., Hlunov, O.V., Izvekova, G.I., Andree, K.B. et al. (2019) Variations of the intestinal gut microbiota of farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), depending on the infection status of the fish. *Journal of Applied Microbiology*, 127, 379–395.

Pelusio, N.F., Rossi, B., Parma, L., Volpe, E., Ciulli, S., Piva, A. et al. (2020) Effects of increasing dietary level of organic acids and nature-identical compounds on growth, intestinal cytokine gene expression and gut microbiota of rainbow trout (*Oncorhynchus mykiss*) reared at normal and high temperature. *Fish & Shellfish Immunology*, 107, 324–335.

Pérez-Sánchez, T., Balcázar, J.L., García, Y., Halaihel, N., Vendrell, D., De Blas, I. et al. (2011) Identification and characterization of lactic acid bacteria isolated from rainbow trout, *Oncorhynchus mykiss* (Walbaum), with inhibitory activity against *Lactococcus garvieae*. *Journal of Fish Diseases*, 34, 499–507.

Pérez-Sánchez, T., Mora-Sánchez, B., Vargas, A. & Balcázar, J.L. (2020) Changes in intestinal microbiota and disease resistance following dietary postbiotic supplementation in rainbow trout (*Oncorhynchus mykiss*). *Microbial Pathogenesis*, 142, 104060.

Phillips, C.D., Phelan, G., Dowd, S.E., McDonough, M.M., Ferguson, A.W., Delton Hanson, J. et al. (2012) Microbiome analysis among bats describes influences of host phylogeny, life history, physiology and geography. *Molecular Ecology*, 21, 2617–2627.

Piazzon, M.C., Naya-Català, F., Simó-Mirabet, P., Picard-Sánchez, A., Roig, F.J., Calduch-Giner, J.A. et al. (2019) Sex, age, and bacteria: how the intestinal microbiota is modulated in a protandrous hermaphrodite fish. *Frontiers in Microbiology*, 10, 2512.

Picchianti-Diamanti, A., Panebianco, C., Salemi, S., Sorgi, M., Di Rosa, R., Tropea, A. et al. (2018) Analysis of gut microbiota in rheumatoid arthritis patients: disease-related dysbiosis and modifications induced by etanercept. *International Journal of Molecular Sciences*, 19, 2938.

Popma, T. & Masser, M. (1999) *Tilapia life history and biology*. Southern Regional Aquaculture Center.

Rasmussen, J.A., Villumsen, K.R., Ernst, M., Hansen, M., Forberg, T., Gopalakrishnan, S. et al. (2022) A multi-omics approach unravels metagenomic and metabolic alterations of a probiotic and synbiotic additive in rainbow trout (*Oncorhynchus mykiss*). *Microbiome*, 10, 1–19.

Razin, S.M. (1967) Mycoplasmas. In: Baron, S. (Ed.) *Nature*, vol. 213. 4th edition. Galveston, TX: University of Texas Medical Branch at Galveston, pp. 116–117.

Ricaud, K., Rey, M., Plagnes-Juan, E., Larroquet, L., Even, M., Quillet, E. et al. (2018) Composition of intestinal microbiota in two lines of rainbow trout (*Oncorhynchus mykiss*) divergently selected for muscle fat content. *Open Microbiol J*, 12, 308–320.

Rimoldi, S., Antonini, M., Gasco, L., Moroni, F. & Terova, G. (2021) Intestinal microbial communities of rainbow trout (*Oncorhynchus mykiss*) may be improved by feeding a *Hermetia illucens* meal/low-fishmeal diet. *Fish Physiology and Biochemistry*, 47, 365–380.

Rimoldi, S., Terova, G., Ascione, C., Giannico, R. & Brambilla, F. (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.

Roeselers, G., Mittge, E.K., Stephens, W.Z., Parichy, D.M., Cavanaugh, C.M., Guillemin, K. et al. (2011) Evidence for a core gut microbiota in the zebrafish. *The ISME Journal*, 5, 1595–1608.

Rosado, D., Pérez-Losada, M., Severino, R., Cable, J. & Xavier, R. (2019a) Characterization of the skin and gill microbiomes of the farmed seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*). *Aquaculture*, 500, 57–64.

Rosado, D., Xavier, R., Severino, R., Tavares, F., Cable, J. & Pérez-Losada, M. (2019b) Effects of disease, antibiotic treatment and recovery trajectory on the microbiome of farmed seabass (*Dicentrarchus labrax*). *Scientific Reports*, 9, 1–11.

Rossau, R., Van Landschoot, A., Gillis, M. & De Ley, J. (1991) Taxonomy of *Moraxellaceae* fam. nov., a new bacterial family to accommodate the genera *Moraxella*, *Acinetobacter*, and *Psychrobacter* and related organisms. *International Journal of Systematic Bacteriology*, 41, 310–319.

Ruohonen, K., Kettunen, A., Wadsworth, S., Hartviksen, M., Vecino, J.L.G., Ringø, E., et al. (2014) Alternative dietary protein sources for Atlantic salmon (*Salmo salar* L.) effect on intestinal microbiota, intestinal and liver histology and growth. *Aquac Nutr*, 20, 381–398.

Sakai, M., Yoshida, T., Atsuta, S. & Kobayashi, M. (1995) Enhancement of resistance to vibriosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum), by oral administration of *Clostridium butyricum* bacterin. *Journal of Fish Diseases*, 18, 187–190.

Scarpa, M., Grillo, A., Pozza, A., Faggian, D., Ruffolo, C., Scarpa, M. et al. (2011) TLR2 and TLR4 up-regulation and colonization of the ileal mucosa by *Clostridiaceae* spp. in chronic/relapsing pouchitis. *Journal of Surgical Research*, 169, e145–e154.

Schmidt, T.S.B., Matias Rodrigues, J.F. & von Mering, C. (2015) Limits to robustness and reproducibility in the demarcation of operational taxonomic units. *Environmental Microbiology*, 17, 1689–1706.

Schmidt, V., Gomez-Chiarri, M., Roy, C., Smith, K. & Amaral-Zettler, L. (2017) Subtle microbiome manipulation using probiotics reduces antibiotic-associated mortality in fish. *mSystems*, 2, e00133–17.

Schubiger, C.B., Orfe, L.H., Sudheesh, P.S., Cain, K.D., Shah, D.H. & Call, D.R. (2015) Entericidin is required for a probiotic treatment (*Enterobacter* sp. Strain C6-6) to protect trout from cold-water disease challenge. *Applied and Environmental Microbiology*, 81, 658–665.

Shade, A. & Handelsman, J. (2012) Beyond the Venn diagram: the hunt for a core microbiome. *Environmental Microbiology*, 14, 4–12.

Silva, B.R.D.S., Derami, M.S., Paixão, D.A., Persinoti, G.F., Dias Da Silveira, W. & Maluta, R.P. (2020) Comparison between the intestinal microbiome of healthy fish and fish experimentally infected with *Streptococcus agalactiae*. *Aquac Res*, 51, 3412–3420.

Son, V.M., Chang, C.-C., Wu, M.-C., Guu, Y.-K., Chiu, C.-H. & Cheng, W. (2009) Dietary administration of the probiotic, *Lactobacillus plantarum*, enhanced the growth, innate immune responses, and disease resistance of the grouper *Epinephelus coioides*. *Fish & Shellfish Immunology*, 26, 691–698.

Stadtlander, C. & Kirchhoff, H. (1990) Gill organ culture of rainbow trout, *Salmo gairdneri* Richardson: an experimental model for the study of pathogenicity of *Mycoplasma mobile* 163 K. *Journal of Fish Diseases*, 13, 79–86.

Stadtländer, C.T.K.-H., Lotz, W., Körting, W. & Kirchhoff, H. (1995) Piscine gill epithelial cell necrosis due to *Mycoplasma mobile* strain 163 K: comparison of in-vivo and in-vitro infection. *Journal of Comparative Pathology*, 112, 351–359.

Tarnecki, A.M., Burgos, F.A., Ray, C.L. & Arias, C.R. (2017) Fish intestinal microbiome: diversity and symbiosis unravelled by metagenomics. *Journal of Applied Microbiology*, 123, 2–17.

Terova, G., Gini, E., Gasco, L., Moroni, F., Antonini, M. & Rimoldi, S. (2021) Effects of full replacement of dietary fishmeal with insect meal from *Tenebrio molitor* on rainbow trout gut and skin microbiota. *Journal of Animal Science and Biotechnology*, 12, 1–14.

Terova, G., Rimoldi, S., Ascione, C., Gini, E., Ceccotti, C. & Gasco, L. (2019) Rainbow trout (*Oncorhynchus mykiss*) gut microbiota is modulated by insect meal from *Hermetia illucens* prepupae in the diet. *Reviews in Fish Biology and Fisheries*, 29, 465–486.

Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., Locey, K.J. et al. (2017) A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, 551, 457–463.

Tlusty, M., Rhyne, A., Szczebak, J.T., Bourque, B., Bowen, J.L., Burr, G. et al. (2017) A transdisciplinary approach to the initial validation of a single cell protein as an alternative protein source for use in aquafeeds. *PeerJ*, 5, e3170.

Troell, M., Naylor, R.L., Metian, M., Beveridge, M., Tyedmers, P.H., Folke, C. et al. (2014) Does aquaculture add resilience to the global food system? *Proceedings of the National Academy of Sciences of the United States of America*, 111, 13257–13263.

Turchini, G.M., Trushenski, J.T. & Glencross, B.D. (2019) Thoughts for the future of aquaculture nutrition: realigning perspectives to reflect contemporary issues related to judicious use of marine resources in aquafeeds. *North American Journal of Aquaculture*, 81, 13–39.

Tzeng, T.-D., Pao, Y.Y., Chen, P.C., Weng, F.C., Jean, W.D. & Wang, D. (2015) Effects of host phylogeny and habitats on gut microbiomes of oriental river prawn (*Macrobrachium nipponense*). *PLoS ONE*, 10, e0132860.

Ventura, M., Turroni, F, Canchaya, C., Vaughan, E.E., O'Toole, P.W. & van Sinderen, D. (2009) Microbial diversity in the human intestine and novel insights from metagenomics. *Frontiers in Bioscience*, 14:3214–3221.

Walter, J. (2008) Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. *Applied and Environmental Microbiology*, 74, 4985–4996.

Wang, A.R., Ran, C., Ringø, E. & Zhou, Z.G. (2018) Progress in fish gastrointestinal microbiota research. *Rev Aquac*, 10, 626–640.

Wong, S., Waldrop, T., Summerfelt, S., Davidson, J., Barrows, F., Kenney, P.B et al. (2013) Aquacultured rainbow trout (*Oncorhynchus mykiss*) possess a large core intestinal microbiota that is resistant to variation in diet and rearing density. *Applied and Environmental Microbiology*, 79, 4974–4984.

WüSt, P.K., Horn, M.A. & Drake, H.L. (2009) *In situ* hydrogen and nitrous oxide as indicators of concomitant fermentation and denitrification in the alimentary canal of the earthworm *Lumbricus terrestris*. *Applied and Environmental Microbiology*, 75, 1852–1859.

Xu, Z., Parra, D., Gómez, D., Salinas, I., Zhang, Y.-A., von Gersdorff Jørgensen, L. et al. (2013) Teleost skin, an ancient mucosal surface that elicits gut-like immune responses. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 13097–13102.

Ye, Y. & Gutierrez, N.L. (2017) Ending fishery overexploitation by expanding from local successes to globalized solutions. *Nature Ecology & Evolution*, 1, 0179.

Yildirimer, C.C. & Brown, K.H. (2018) Intestinal microbiota lipid metabolism varies across rainbow trout (*Oncorhynchus mykiss*) phylogeographic divide. *Journal of Applied Microbiology*, 125, 1614–1625.

Zabidi, A., Yusoff, F.M., Amin, N., Yaminudin, N.J.M., Puvanasundram, P. & Karim, M.M.A. (2021) Effects of probiotics on growth, survival, water quality and disease resistance of red hybrid tilapia (*Oreochromis* spp.) fingerlings in a biofloc system. *Animals*, 11, 3514.

Zhang, M., Sun, Y., Liu, Y., Qiao, F., Chen, L., Liu, W.-T. et al. (2016) Response of gut microbiota to salinity change in two euryhaline aquatic animals with reverse salinity preference. *Aquaculture*, 454, 72–80.

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