Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

REVIEW ARTICLE

Fish intestinal microbiome: diversity and symbiosis unravelled by metagenomics

A.M. Tarnecki[1], F.A. Burgos[2], C.L. Ray[3] and C.R. Arias[2]

1 Mote Marine Laboratory, Sarasota, FL, USA
2 School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, AL, USA
3 United States Department of Agriculture, Agricultural Research Service, Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, AR, USA

Keywords
diversity, fish (live), intestinal microbiology, metagenomics, symbiosis.

Correspondence
Andrea M. Tarnecki, Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, USA.
E-mail: atarnecki@mote.org

2016/2275: received 21 October 2016, revised 11 January 2017 and accepted 2 February 2017

doi:10.1111/jam.13415

Summary

The gut microbiome of vertebrates plays an integral role in host health by stimulating development of the immune system, aiding in nutrient acquisition and outcompeting opportunistic pathogens. Development of next-generation sequencing technologies allows researchers to survey complex communities of microorganisms within the microbiome at great depth with minimal costs, resulting in a surge of studies investigating bacterial diversity of fishes. Many of these studies have focused on the microbial structure of economically significant aquaculture species with the goal of manipulating the microbes to increase feed efficiency and decrease disease susceptibility. The unravelling of intricate host–microbe symbioses and identification of core microbiome functions is essential to our ability to use the benefits of a healthy microbiome to our advantage in fish culture, as well as gain deeper understanding of bacterial roles in vertebrate health. This review aims to summarize the available knowledge on fish gastrointestinal communities obtained from metagenomics, including biases from sample processing, factors influencing assemblage structure, intestinal microbiology of important aquaculture species and description of the teleostean core microbiome.

Introduction

Nowadays, it is well accepted that the community of microbes occupying the gastrointestinal (GI) tract of vertebrates (gut microbiome) plays a critical role in host development, physiology and health (Mueller *et al.* 2012; Llewellyn *et al.* 2014). Most of our knowledge on microbiome structure and function derives from studies on mammals which comprise <10% of total vertebrate diversity (Sullam *et al.* 2012). Despite encompassing nearly half of vertebrate species (Nelson 2006), few studies have examined the gut microbiome of fishes (Llewellyn *et al.* 2014; Ringø *et al.* 2016). The first attempts to explore fish intestinal microbiology used culture-based methods that vastly underestimate the diversity of these communities, as <10% of bacteria can be isolated and cultured under laboratory conditions (Amann *et al.* 1995). More recently, in line with other efforts aimed at exploring microbial diversity in different ecosystems, molecular-based culture-independent methods have been applied to the study of microbial communities that colonize the GI tract of fishes.

There are three main strategies to characterize microbial communities using culture-independent methods. Fluorescence *in situ* hybridization (FISH) uses fluorescent-label probes to directly observe microbes, with minimal processing, using fluorescence or confocal microscopy (Amann *et al.* 1995). The probes target specific regions of the ribosomal RNA (rRNA). This method provides a 3D view of the community and allows observation of the intricate spatial relationships between microbes. Although not conducive for high-throughput sample processing, FISH has been used to track specific probiotics in the gut of fish (Del'Duca *et al.* 2013).

DNA fingerprinting methods have been extensively used to compare bacterial diversity of communities

colonizing the GI tract of fish. These methods include terminal restriction fragment length polymorphism (Fjellheim *et al.* 2012; Green *et al.* 2013), denaturing gradient gel electrophoresis (DGGE) (Le Nguyen *et al.* 2008; Zhou *et al.* 2009) and (automated) ribosomal intergenic spacer analysis (RISA) (Larsen *et al.* 2014b). These techniques are based on targeted PCR amplification of variable regions within the ribosomal operons that are unique to bacterial species or strains. Overall, these techniques are fairly quick to perform, relatively inexpensive and allow for medium to high-throughput analysis. However, results obtained with these methods are more qualitative than quantitative and, for the most part, inform on the complexity of the communities but not on the specific operational taxonomic units (OTUs) that constitute each community (DGGE allows for further analysis to identify specific OTUs, but this approach is cumbersome and has a low throughput).

A more comprehensive culture-independent approach to obtain a full inventory of the diversity present in a sample is to sequence the pool of bacterial 16S rRNA genes. Bacterial taxonomy heavily relies on 16S rRNA gene sequencing which has become an accurate method for routine bacterial identification (typically, a conservative cut-off point of 97% sequence similarity or higher is used to ascribe an unknown isolate to a known species) (Rosello-Mora 2005). Early efforts involved the generation of 16S clone libraries followed by sequencing of individual clones using Sanger sequencing (Clements *et al.* 2007; Kim *et al.* 2007; Wu *et al.* 2010b). Although very accurate in terms of sequence quality, sequencing hundreds of individual clones is expensive and time-consuming, limiting the applicability of this method. In 2008, sequencing centres started to transition from Sanger-based sequencing to new (or next-generation) DNA technologies (NGS) and the cost per megabase of DNA sequence plummeted (Wetterstrand 2016). These cost-effective technologies soon triggered an explosion of studies aimed at characterizing, with a level of detail hard to imagine only a few years ago, the microbial communities present in a myriad of environments including the GI tract of fishes. This review aims at providing an overview on the knowledge generated on the fish gut microbiome using NGS technology. Studies that have investigated the gut bacterial communities of fishes are described in Table 1.

Laboratory procedures

Numerous reviews address NGS sequencing technologies, including sample preparation, sequencing chemistry and pros and cons (Metzker 2010; Mardis 2013; Chiu and Miller 2016). In this review, sample preparation protocols are briefly discussed as they affect results and data interpretation. During design of a microbiome study, decisions regarding sample type (gut contents *vs* intestinal mucus), sample storage, DNA extraction method and sequencing protocols must be made, each of which may bias results. However, few studies have investigated these effects in fishes, specifically using NGS, despite the potential for these biases to differ based on environmental community analysed (Tremblay *et al.* 2015). This section will include insight from other methods and organisms to describe potential biases related to sample processing.

Sample type

The first choice in fish gut microbiome investigations is whether to analyse digesta (gut content) or intestinal mucus (emptied intestinal tract). It is generally accepted that rinsed intestinal tissue or mucus is used to characterize adherent (autochthonous) bacteria, whereas digesta is used to characterize non-adherent (allochthonous) communities (Ringø *et al.* 2016). Microbiome differences between these sample types (Wu *et al.* 2012; Li *et al.* 2015; Gajardo *et al.* 2016) are detectable despite variations in sample processing (Carda-Diéguez *et al.* 2014; Larsen *et al.* 2014b) which impact these communities independently. Larsen *et al.* (2014b) demonstrated that the influence of storage conditions and DNA extraction method was greater in digesta than in intestinal tissue samples. Similarly, Carda-Diéguez *et al.* (2014) determined that digesta samples were more sensitive to sampling procedures and environmental conditions than were intestinal samples. Thus, variation in sample processing may be more impactful when investigating non-adherent bacteria than when comparing adherent microbiomes. Due to the still limited number of articles on the topic, determining sample size (i.e. number of individuals analysed) for future studies is hard to predict. Statistical analysis using human microbiome data showed that power is a function of number of sequence reads and sample size; the more reads generated, the fewer individual replicates are needed to achieve significant levels (La Rosa *et al.* 2012). For most fish species, specific replicate numbers should still be determined empirically.

Sample storage

Often, field sampling prevents immediate processing of samples and sometimes even storage at −80°C, which are generally recognized as ideal storage conditions prior to DNA extraction and analysis. Choice of storage temperature can significantly impact gut microbiome results. Using RISA, Larsen *et al.* (2014b) demonstrated that freezing samples at −20°C for 15 days distorted the bacterial community fingerprint by decreasing apparent diversity (band number). Storage in RNA*later*® buffer

Table 1 Studies investigating the fish gastrointestinal microbiome using next-generation sequencing

Species group	Platform	Microbiome	Environment	Notable findings	Source
Bass, Bluegill, Catfish	PS	Non-adherent	FW; Wild	Communities differ by species, perhaps due to host selection or diet	Larsen *et al.* (2014a)
Bass, Bluegill, Gar	IMS	Non-adherent	FW; Wild	Gut microbiota differ significantly between sampling date (season) and species	Ray (2016)
Bream, Carps, Culter, Perch	IMS	Non-adherent	FW; Wild	Gut microbiota influenced by trophic level Gut microbiota related to metabolism	Liu *et al.* (2016)
Bream, Carps, Catfish, Goldfish, Perch, Snakehead	IMS	Both	FW; Wild; Captive	Microbiota differ based on developmental stage and trophic category Gut physiological changes elicit large influence on gut microbiota structure	Yan *et al.* (2016)
Carp	PS	Non-adherent	FW; Wild	Communities differ by fish species, gut segment, location and sampling time	Ye *et al.* (2016)
Carps, Drum, Goldfish	IHS	Non-adherent	FW; Wild; Captive	Captive and wild fish harbour different microbiota Environmental and host influences apparent in predicted microbial function	Eichmiller *et al.* (2016)
Carps	PS	Each	FW; Captive	Communities differ by species, sample type, trophic group and different from those of water	Li *et al.* (2015)
Carp	PS	Both	FW; Captive	Gut microbiota is correlated with growth rate	Li *et al.* (2013)
Carp	PS	Non-adherent	FW; Captive	Many taxa related to digestion of food, production of vitamins and nitrogen cycling	Van Kessel *et al.* (2011)
Carp	PS	Non-adherent	FW; Captive	High individual variation with differences from environment (feed, water, sediment)	Wu *et al.* (2013)
Carp	PS	Each	FW; Captive	Mucosa and digesta harbour unique microbiomes that differ from environmental communities	Wu *et al.* (2012)
Carp	IHS	Adherent	FW; Wild; Captive	Gut microbiota influenced by amount of food in the gut, diet and environment	Ni *et al.* (2014)
Carp	IMS	Each	FW; Wild	Communities share similarities with live food, water and sediment	Kashinskaya *et al.* (2015)
Catfish	PS	Non-adherent	FW; Captive	Diet influences community structure	Di Maiuta *et al.* (2013)
Cichlids	IMS	Both	FW; Wild; Captive	Communities are species- and ecology-specific, but only in earlier-diverging species	Franchini *et al.* (2014)
Cichlids	PS	Both	FW; Wild; Captive	Captive microbiota differed from wild individuals, but much of the core microbiota remained	Baldo *et al.* (2015)
Cod	PS	Both	SW; Captive	Assembly deterministic, differs by age and from water, with little influence of diet	Bakke *et al.* (2015)
Cod	PS	Non-adherent	SW; Wild	High variability between individuals	Star *et al.* (2013)
Grouper	IMS	Non-adherent	SW; Wild; Captive	Microbiota and functions distinct between environments with higher diversity in wild fish	Hennersdorf *et al.* (2016b)
Groupers, Scad	IMS	Non-adherent	SW; Wild; Captive	Differ between environments, microbiota cluster primarily by location Pathogens more common in or near cages	Hennersdorf *et al.* (2016a)
Grudgeon	IMS	Both	FW; Captive	Microbiota were distinct between diseased and healthy fish	Li *et al.* (2016)

(Continued)

Table 1 (*Continued*)

Species group	Platform	Microbiome	Environment	Notable findings	Source
Minnow	IMS	Both	FW; Captive	Gut microbiota changes over time and with exposure to triclosan	Narrowe *et al.* (2015)
Molly	IHS	Both	FW & SW; Captive	Microbiota is deterministically assembled, differs by salinity, and correlated with water quality parameters	Schmidt *et al.* (2015)
Mosquitofish	IHS	Both	FW; Captive	Rifampicin altered gut microbiota, increased susceptibility to disease and osmotic stress	Carlson *et al.* (2015)
Oilfish	PS	NS	FW; Wild	High diversity (301 OTUs) dominated by Proteobacteria (β subclass)	Bel'Kova *et al.* (2015)
Paddlefish, Carp	IMS	Non-adherent	FW; Captive	Communities are species-specific and differ from those of water	Li *et al.* (2014)
Parrotfishes, Rabbitfish, Surgeonfishes	PS	Non-adherent	SW; Wild	Communities mostly species-specific Correlation between gut microbiota composition and host phylogeny Gut microbiota also impacted by diet category	Miyake *et al.* (2015)
Perch, Stickleback	IHS	Both	FW; Wild; Captive	Diet impacts microbiota and it is deterministically assembled	Bolnick *et al.* (2014b)
Salmon	IMS	Non-adherent	FW; Captive	Variation between individuals with microbiota impacted by rearing environment Core microbiota indicate degree of host selection	Dehler *et al.* (2017)
Salmon	IHS	Both	FW; Captive	Intestine communities significantly different from those of gills, water and biofilter Diet primarily impacted members of the dominant order (Lactobacillales)	Schmidt *et al.* (2016)
Salmon	PS	Non-adherent	SW; Captive	Communities impacted by sampling time (season) and diet during certain months	Zarkasi *et al.* (2014)
Salmon	PS	Non-adherent	SW; Captive	Communities influenced by sampling time and diet	Zarkasi *et al.* (2016)
Salmon	IT	Each	SW; Captive	Mucosa and digesta communities significantly different	Gajardo *et al.* (2016)
Sea Bream	PS	Non-adherent	SW; Captive	Microbiota differs by gut segment and diet, and is different from the surrounding seawater	Estruch *et al.* (2015)
Sea Bream	PS	Adherent	SW; Captive	Gut microbiota differs by diet and by environment (wild *vs* captive)	Kormas *et al.* (2014)
Seabass	IHS	Adherent	SW; Captive	Starvation alters gut microbiota and enriches bacteria with antibiotic-producing abilities	Xia *et al.* (2014)
Seabass	PS	Each	SW; Captive	Digesta more influenced by environment and sampling procedures than diet Mucosa influenced by diet and time	Carda-Diéguez *et al.* (2014)
Seabass	IMS	Non-adherent	SW; Captive	No significant difference in gut microbiota based on diet	Wang *et al.* (2016)
Snook	IT	Both	SW; Captive	Community differed by age, probiotic treatment, was distinct from that of live food Larvae exhibiting high mortality dominated by potential fish pathogens	Tarnecki, A.M., Rhody, N.R., unpublished data

(Continued)

Table 1 (*Continued*)

Species group	Platform	Microbiome	Environment	Notable findings	Source
Stickleback	IMS	Both	FW & SW; Wild	Gut microbiota is selected by host genotype but no influence of sex Differences in populations attributable to geographic differences in prey microbiota	Smith *et al.* (2015)
Stickleback	IHS	Both	FW; Wild	Major histocompatibility class II genotype contributes to variation in microbiota	Bolnick *et al.* (2014a)
Sturgeon	PS	Non-adherent	FW; Captive	Diet (prebiotic and/or probiotics) significantly influenced gut microbiota	Geraylou *et al.* (2013a)
Sturgeon	PS	Non-adherent	FW; Captive	Diet (prebiotic arabinoxylan oligosaccharides) significantly alter gut microbiota	Geraylou *et al.* (2013b)
Tambaqui	IMS	Non-adherent	FW; Captive	pH and sample type significantly alters microbiome which is different from water	Sylvain *et al.* (2016)
Tilapia	PS	Non-adherent	FW; Captive	Essential oils act through host mechanisms, overshadow bacteria-mediated effects	Ran *et al.* (2016)
Tilapia	IMS	Both	FW; Captive	Microbiota altered by immune function, mucus production, pH, chemical changes, intestine size and varying abilities of microbes to survive in low-nutrient conditions	Kohl *et al.* (2014)
Tilapia	PS	Non-adherent	FW; Captive	Gut microbiota more similar to sediment that water samples	Fan *et al.* (2017)
Tilapia	IHS	Each	FW; Captive	Basal diet formulations influenced gut microbiota composition	Ran *et al.* (2015)
Tilapia	IT	Each	FW; Captive	Diet (probiotic supplementation) significantly impacts gut microbiota structure	Standen *et al.* (2015)
Tilapia	PS	Both	FW; Captive	Microbiota influenced by system type and diet, correlated to but different from water	Giatsis *et al.* (2015)
Tilapia	PS	Both	FW; Captive	Microbiota influenced by system type and changes over time, differs from water	Giatsis *et al.* (2014)
Tilapia	I (NS)	Non-adherent	SW & FW; Captive	Communities differed by salinity and species, more opportunists during osmotic stress	Zhang *et al.* (2016)
Trout	PS	Adherent	FW; Captive	*Acinetobacter*, *Cetobacterium*, *Pseudomonas*, *Psychrobacter* are primary genera	Etyemez and Balcazar (2015)
Trout	IMS	Each	FW; Captive	Gut microbiota significantly different based on sample type (contents *vs* mucosa)	Lyons *et al.* (2015)
Trout	IMS	Both	FW; Captive	Diet exerts no influence and gut microbiome differs from feed and tank biofilm	Lyons *et al.* (2016)
Trout	PS	Both	FW; Captive	Diet and rearing density affected specific taxa and non-dominant members	Wong *et al.* (2013)
Trout	PS	Non-adherent	FW; Captive	Plant-based diets altered microbiota and may play a role in negative health observations	Desai *et al.* (2012)

(Continued)

Table 1 (*Continued*)

Species group	Platform	Microbiome	Environment	Notable findings	Source
Trout	IHS	Adherent	FW; Captive	Diet and pathogen challenge significantly influences gut microbiota compositionEffect of bacterial challenge on microbiota impacted by diet	Ingerslev *et al.* (2014a)
Trout	IHS	Adherent	FW; Captive	Microbiota is influenced by ontogeny and diet	Ingerslev *et al.* (2014b)
Various (13 bony fish, three sharks)	PS	Both	SW; Wild; Captive	High individual variation, species-specificity; diet, age and environmental influences	Givens *et al.* (2015)
Zebrafish	IHS	Both	FW; Captive	No influence of sex but microbiota different from that of water, tank surfaces and food Shifts with age occurred with and without changes in diet and environmental conditions	Stephens *et al.* (2015)
Zebrafish	IMS	Adherent	FW; Captive	Triclosan significantly impacted microbiota structure altered microbial interactions	Gaulke *et al.* (2016)
Zebrafish	PS	Both	FW; Captive	Microbiota shifts with age and diet	Rurangwa *et al.* (2015)
Zebrafish	IMS	Both	FW; Captive	Microbiota influences anxiety-related behaviour and stress response	Davis *et al.* (2016)
Zebrafish	IT	Both	FW; Captive	Probiotic influenced behaviour, brain-derived gene expression and microbiota structure	Borrelli (2015)
Zebrafish	IHS	Both	FW; Captive	Microbiota shifts with age and was different from water, influenced by diet Some taxa are better evolved to live in the gut than the surrounding environment	Wong *et al.* (2015)
Zebrafish	PS	Variable	FW; Captive	Influenced by environment but with a large core microbiota Microbiota selected by host physiology, immunity, gut histology and salinity	Roeselers *et al.* (2011)

Platforms: PS, pyrosequencing; IMS, Illumina MiSeq; HIS, Illumina HiSeq; I (NS), Illumina instrument not specified; IT, Ion Torrent™ PGM.
Microbiome: Both, adherent and non-adherent were combined for analysis; each, adherent and non-adherent were analysed separately; NS, not specified.
FW, freshwater; SW, seawater.

maintained banding patterns similar to those obtained from fresh samples, but only when used with a particular commercial DNA extraction kit. The use with another kit decreased performance (replicate similarity, diversity) in some cases, suggesting the impacts of storage conditions may be exacerbated by further downstream processing. Carda-Diéguez *et al.* (2014) described a significant effect of freezing at −80°C on fish gut microbiome structure, in that dominant bacterial genera were shifted when compared to fresh samples. As a result, it is important to note storage conditions during between-study comparisons on the fish gut microbiome, particularly when studying larval fish, as the effects of storage on microbiome structure are enhanced in communities with lower diversity (Hill *et al.* 2016).

DNA extraction

DNA extraction protocols significantly impact results of studies on GI microbiomes (Kennedy *et al.* 2014; Walker *et al.* 2015). Bead beating influences DNA extraction, particularly as it pertains to Gram-positive bacteria (Walker *et al.* 2015) although its lysis efficiency appears to be bacterial species-specific (Sergeant *et al.* 2012). MacKenzie *et al.* (2015) reported that only methods that included heat during cell lysis detected Fusobacteria in human digesta samples. This may prove vital for accurate description of the fish gut microbiome, as Fusobacteria make up a large proportion of these communities in freshwater fishes (Di Maiuta *et al.* 2013; Geraylou *et al.* 2013b; Larsen *et al.* 2014a; Li *et al.* 2015). Commercial

extraction kits optimized for inhibitor removal perform better with fish digesta and intestinal mucus samples, likely due to the presence of PCR inhibitors in the fish GI tract (Larsen *et al.* 2014b). Despite these influences, baises due to extraction technique are often overshadowed by inter-individual variation (Wu *et al.* 2010a; MacKenzie *et al.* 2015).

PCR conditions

There are nine regions identified as variable within the bacterial 16S rRNA gene (Yarza *et al.* 2014), and the V4 region, spanning from positions 751 to 1050 of the 16S rRNA of *Escherichia coli*, is most commonly sequenced in studies on fishes. However, other regions may be investigated, resulting in bias due to primer choice. Mismatches between 'universal' bacterial primers and particular taxonomic groups can decrease the detection of those taxa compared to those with fewer mismatches (Walker *et al.* 2015). Within the 454 pyrosequencing platform, Engelbrektson *et al.* (2010) detected greater OTU richness using V1-2 primers as compared to those targeting the V8 region due to higher sequence variability in the V1-2 region. This variability also resulted in lower detected species evenness, as fewer sequences met the 97% identity threshold used to identify OTUs. Furthermore, sequences <400 bp resulted in increased species richness estimates and inconsistent detection of minor community members between replicates. Even different primers designed to amplify the same region can impact bacterial community structure (Hongoh *et al.* 2003; Walker *et al.* 2015).

PCR can result in other potential biases resulting from varying initial template concentration, annealing temperature and number of cycles. At higher DNA concentrations or more PCR cycles, PCR products are more likely to reanneal, resulting in reduced amplification of these products. These effects are most apparent in detecting abundances of dominant bacterial taxa (Hongoh *et al.* 2003). As a result, although the use of more PCR cycles may lead to increased diversity, it is likely less representative of the true structure of the community. Similarly, decreasing the annealing temperature may detect greater diversity due to decreased stringency allowing for more nucleotide mismatches (Hongoh *et al.* 2003).

Sequencing platform

Studies using the same primers with multiple sequencing platforms suggest that there is a bias based on technology used; however, these biases are overshadowed by those demonstrated with the use of different primers. Tremblay *et al.* (2015) demonstrated that many of the differences between pyrosequencing and Illumina MiSeq results were seen in poorly classified lineages and noted that these variations may have been artefacts of classification. Wu *et al.* (2010a) investigated the differences in faecal communities using two different pyrosequencing platforms, 454 GS FLX and 454 Titanium, the primary difference being resulting read lengths of 260 and 450 nucleotides, respectively. Few differences were identified, and those detected may have been due to slight variations in the primers used for the two instruments. Fouhy *et al.* (2016) compared sequences obtained from a mock community using Ion Torrent™ PGM and Illumina MiSeq. The PGM platform detected a higher proportion of community members than MiSeq when using V4-5 primers, but produced a profile most similar to that expected from the mock community using V1-2 primers. Interestingly, the differences between platforms were also dependent on primer selection, with V4-5 results being less variable between platforms than other primer combinations. Thus, despite the potential for biases resulting from choice of sequencing platform, those associated with primer choice seem to be more influential.

The methods associated with fish gut microbiome studies can greatly influence their results. Many NGS technologies require PCR for target amplification prior to sequencing, and the biases associated with this step should be considered and standardized in fish microbiome research to allow for accurate comparison studies. However, further technological advancements, such as single molecule real-time sequencing (Pacific BioSciences of California, Inc., Menlo Park, CA, USA) that do not rely on PCR amplification may alleviate some of these concerns.

The core gut microbiome of fishes

One of the main reasons for studying the gut microbiome of fishes is the idea that those communities can be modified to improve host health. A prerequisite to this approach is the characterization of the gut microbiome of the species of interest. Many factors contribute to the composition of the gut microbiome in vertebrates including host genetics, environment and nutrition among others. Discovering a core microbiome, that is, members of the microbial community present in all individuals of a species, has been a primary goal for many researchers interested in understanding gut microbial communities (Turnbaugh *et al.* 2007). However, defining a core microbiome has proven to be an elusive task in many species, including humans, as ecological relationships within each community are complex and several parameters need to be taken into consideration such as composition, phylogeny, persistence and connectivity (Shade and Handelsman 2012). The first study to investigate the presence of

a core gut microbiome in teleosts was conducted by Roeselers *et al.* (2011). In that study, the authors compared the gut microbiome of wild and domesticated zebrafish and, although they found significant differences among those populations, all fish shared 21 OTUs that were considered the core community. In a more recent study, the core gut microbiome of invasive carp species was compared between laboratory-reared and wild fish (Eichmiller *et al.* 2016). The authors only identified five shared OTUs, but they comprised up to 40% of the total OTU abundance in the samples, thus suggesting a key role for those members. While some studies point towards the environment as the primary driver for population structure assembly (Eichmiller *et al.* 2016), others point towards nutrition or trophic level as the main modifier for microbiome species composition (Liu *et al.* 2016). A meta-analysis on 25 16S rDNA libraries from previously published studies indicated that host trophic level, habitat and possibly host phylogeny are determinant factors for the core gut microbiome of fishes (Sullam *et al.* 2012). Not surprisingly, the study statistically demonstrated how culture-based approaches distort the actual composition of gut microbial communities. An interesting result from the study was the effect that salinity has over the gut microbiome structure. While some authors have suggested that host phylogeny was the determinant factor in shaping those microbial communities (Roeselers *et al.* 2011), Sullam *et al.* (2012) noted that fish-associated microbiomes were more similar between freshwater fishes, regardless of phylogeny, than to those of fishes inhabiting marine environments. Anecdotal evidence obtained by our group agrees with the latter statement, as we have observed a dominance of *Aeromonas* sp. in the gut of freshwater fishes, whereas *Vibrio* sp. tend to dominate the gut of marine fish species. It is likely that the phylogenetic origin of the bacteria is not as relevant as the role they play in the gut.

Overall, the fish gut microbiome seems to be dominated by the phylum Proteobacteria, followed by Fusobacteria and Firmicutes and in a lesser percentage Bacteroidetes, Actinobacteria and Verrucomicrobia (see Llewellyn *et al.* (2014) for a summary of main phyla identified in recent studies). Proteobacteria, Fusobacteria and Firmicutes reportedly dominate the gut microbiome of most fish species studied to date including marine (Hennersdorf *et al.* 2016a) and freshwater species (Larsen *et al.* 2014a; Eichmiller *et al.* 2016; Liu *et al.* 2016), and they can represent up to 90% of the communities. All these phyla are found in both allochthonous (transient) and autochthonous (adherent) microbial communities.

One of the most interesting species found in the gut of fishes, primarily in freshwater species, is *Cetobacterium somerae* of the phylum Fusobacteria. This species was first described from children with late-set autism (Finegold *et al.* 2003), and since then has been identified in a variety of freshwater fishes representing as much as 94% of total OTUs (Larsen *et al.* 2014a; Etyemez and Balcazar 2015; Lyons *et al.* 2015; Gaulke *et al.* 2016). This species is known to produce high amounts of vitamin B12 and can inhibit the growth of potential pathogens (Sugita *et al.* 1996). Unfortunately, *C. somerae* is a microaerophilic, fastidious bacterium that is hard to culture under laboratory conditions but warrants further investigations on its function in the fish gut.

The theory of a core gut microbiome has primarily been explored in humans and mammalian models (Tap *et al.* 2009; Turnbaugh and Gordon 2009), but many authors believe that the same concept applies to bony fish. It is likely that finding the common core microbiome of fishes will be harder than in mammals, due to the large phylogenetic diversity of teleosts including those of aquaculture importance. To date, it is unclear whether a core microbiome exists in all fish species or, if it does, at which phylogenetic level. A core microbiome must be present within the fish species across environments and some studies have pointed out that environment exerts a stronger effect on the core communities than host genetics (Wilson *et al.* 2008). However, several studies suggest a core microbiome exists at least at the fish species level (Roeselers *et al.* 2011; Hennersdorf *et al.* 2016a). Exploring the hypothesis that the gut microbiome is shaped by evolutionary forces dictated by host genetics and gut physiology, as well as by its bacterial symbionts, is definitely worth exploring. If this hypothesis is correct and a core microbiome exists, we will be one step closer to making informed decisions on how to manipulate the bacterial communities in order to promote host health and well-being in fishes.

Gut microbiome of economically significant species

Aquaculture is one of the largest and fastest growing industries worldwide. The increasing trend to develop large-scale production systems has led to intensive marine and freshwater aquaculture practices that are vulnerable to severe disease outbreaks. Traditional disease control methods include the use of vaccines and antibiotics to prevent and control diseases. In recent years, public pressure against the use of antibiotics in farm animals shifted the focus from 'killing pathogens' to 'promoting beneficial microbes'. As a result, many studies on the effects of prebiotics and probiotics on farm-reared fish and their associated microbiomes have been published in the last decade. Recently, the use of NGS has made possible the thorough description and characterization of the gut microbiomes of main aquaculture species and the

changes those communities exhibit when prebiotics, probiotics or other feed additives are incorporated into the diet. Nevertheless, we are still in the early stages of understanding the complex interactions between the gut microbiome and its host and how the bacterial communities can be most effectively manipulated to improve fish health and aquaculture production (Montalban-Arques *et al.* 2015).

Not surprisingly, as the top aquaculture fish group worldwide in terms of both value and tonnes produced (FAO 2016), carps have been the subject of numerous studies describing the fish GI microbiome. In general, the gut microbiome of carps is dominated by Proteobacteria, Firmicutes and Fusobacteria (Wu *et al.* 2013; Ni *et al.* 2014; Eichmiller *et al.* 2016; Liu *et al.* 2016; Yan *et al.* 2016), but their abundances often differ. Some species (Asian carp, common carp, grass carp, Prussian carp) have higher abundances of Bacteroidetes (Li *et al.* 2013, 2015; Ye *et al.* 2014; Kashinskaya *et al.* 2015) and Cyanobacteria (Wu *et al.* 2012; Ye *et al.* 2016) which is typically considered transient as ingested with food (Givens *et al.* 2015). The genus *Cetobacterium* has been recognized as a common member of the microbiome of grass, Asian, bighead, common and Crucian carps (Van Kessel *et al.* 2011; Ye *et al.* 2014; Li *et al.* 2015; Eichmiller *et al.* 2016; Yan *et al.* 2016) and may be considered a core genus among carps. In grass carp, the genera *Cetobacterium* and *Aeromonas* are present in large abundances throughout much of development, whereas many others such as *Pseudomonas* (1–4 days post-hatch, dph), *Bacillariophyta* (5–30 dph) and *Bacteroides* (juveniles) vary significantly by age (Yan *et al.* 2016). *Aeromonas* was also detected in relatively high abundances in Crucian and grass carp (Li *et al.* 2015). Other shared taxa include *Clostridium* (Wu *et al.* 2012; Ni *et al.* 2014; Li *et al.* 2015), *Veillonella*, *Rothia*, and Methylocystaceae (Wu *et al.* 2012, 2013). From these studies, it is clear that carps are a diverse group of fishes that harbour complex communities of bacteria; yet, there are many members of the gut microbiome that are shared across species despite studies being conducted in different environments.

Another major aquaculture species, Atlantic salmon (*Salmo salar*), has also been the subject of numerous sequencing studies. Proteobacteria and Firmicutes largely dominate the gut microbiome of this species, whether the fish are reared in freshwater or saltwater (Zarkasi *et al.* 2014, 2016; Gajardo *et al.* 2016; Schmidt *et al.* 2016; Dehler *et al.* 2017). Total OTUs range from the 100s in intestinal mucosa (Gajardo *et al.* 2016) to over 500 in digesta (Gajardo *et al.* 2016; Dehler *et al.* 2017). Dehler *et al.* (2017) described the gut microbiome of juvenile salmon in freshwater environments to be dominated by Ruminococcaceae, Mycoplasmataceae, and *Pseudomonas* sp., which were core members of the microbiome across captive and wild environments. Other studies have investigated the composition of the gut bacterial communities in adult (post-smolt) salmon in freshwater (Schmidt *et al.* 2016) and saltwater (Zarkasi *et al.* 2014; Gajardo *et al.* 2016) where the fish seem to harbour different communities. Freshwater salmon harboured a gut microbiome significantly different from that of the surrounding water, tank biofilms and gill of the fish, and were dominated by Aeromonadales and Lactobacillales (Schmidt *et al.* 2016). Dominant genera within the Lactobacillales differed by diet but included *Streptococcus* and *Lactobacillus*. Both Gajardo *et al.* (2016) and Zarkasi *et al.* (2014) identified *Leuconostoc* and *Weissella* as dominant members of marine adult salmon during colder temperatures (approximately 10–12°C), whereas that community shifted to one dominated by Vibrionaceae (*Vibrio*, *Aliivibrio* and *Photobacterium*) when waters were warmer (14–17°C; Zarkasi *et al.* 2014). Thus, gut communities in Atlantic salmon are dynamic and change according to season and fish growth phase, but shared taxa in different rearing environments (captive pens *vs* captive recirculating aquaculture systems) suggest host selection may also influence these assemblages.

Another salmonid of great economic interest, rainbow trout (*Oncorhynchus mykiss*), shares a number of community members with marine Atlantic salmon, despite being a freshwater species. For example, Proteobacteria and Firmicutes are the dominant phyla in rainbow trout. Interestingly, *Weissella* is identified as a major member of the gut microbiome (Desai *et al.* 2012; Ingerslev *et al.* 2014a, b), as were *Streptococcus*, *Leuconostoc* (Ingerslev *et al.* 2014a,b) and *Lactobacillus* (Ingerslev *et al.* 2014b). Similar to studies that identified these genera in Atlantic salmon, studies reported *Streptococcus*, *Leuconostoc* and *Lactobacillus* in rainbow trout in colder temperatures (10–13°C). Desai *et al.* (2012) performed their studies at 15°C and identified a more varied community from Atlantic salmon. These similarities suggest salmonids (rainbow trout, Atlantic salmon) share dominant members of the Firmicutes as a core microbiome, despite differences in environment (freshwater *vs* marine) and diet, providing further support for a host-related selection that may be tied to phylogeny (Sullam *et al.* 2012).

The dominant phyla in the gut microbiome of Nile tilapia (*Oreochromis niloticus*) are generally Proteobacteria and Fusobacteria although the relative abundances of these phyla vary between studies (Kohl *et al.* 2014; Giatsis *et al.* 2015; Ran *et al.* 2016; Zhang *et al.* 2016). However, Standen *et al.* (2015) examined the allochthonous microbiome of Nile tilapia and identified Firmicutes as the primary phylum, and Zhang *et al.* (2016) identified the most frequent phyla to be Actinobacteria, Bacteroidetes

and Proteobacteria in tilapia reared in saline water, suggesting these proportions are different depending on rearing conditions. Between-study comparisons suggest that aspects of study design largely influence lower level taxa as there are few similarities in dominant microbiome members among studies. For example, Ran *et al.* (2015) identified *Plesiomonas* as the dominant OTU; Standen *et al.* (2015) identified *Enterococcus*, *Bacillus* and *Streptophyta* as common members of the microbiome; and Giatsis *et al.* (2015) characterized members of the families Isophaeraceae, Peptostreptococcaceae and Bradyrhizobiaceace, the genera *Arthrobacter* and *Rhodococcus*, and the species *Mycobacterium llatzerense* as dominating these communities. These differences may be due to the herbivorous/omnivorous nature of this species, as these trophic positions generally harbour greater diversity than those of carnivores (Larsen *et al.* 2014a; Givens *et al.* 2015; Yan *et al.* 2016).

Factors influencing fish gut microbiome structure

Bacteria are everywhere, present across all habitats that other organisms call home, but the establishment of the communities formed by these ubiquitous, unicellular organisms is far from random (Sullam *et al.* 2012; Bakke *et al.* 2015; Yan *et al.* 2016). Studies indicate that the microbiomes of fishes, including that of the gut, are largely influenced by the environment surrounding the host and diet, but overall, the gut microbiome is distinct from that of the external environment (Wu *et al.* 2012, 2013; Zhang *et al.* 2016; Dehler *et al.* 2017), suggesting an influence of genetic factors as well.

Environmental factors

Environmental factors such as salinity, season and geographic location can heavily influence the composition of free-living and symbiotic bacterial communities (Sullam *et al.* 2012; Ye *et al.* 2014; Zarkasi *et al.* 2014; Ray 2016; Zhang *et al.* 2016).

Variations in salinity result in substantial differences in the composition of taxa in free-living microbial communities (Schmidt *et al.* 2015). Similarly, studies have reported significant differences in the gut microbiome of freshwater and marine fishes (Roeselers *et al.* 2011; Zhang *et al.* 2016). However, understanding the influence of variables such as salinity in the wild is hampered by covariation of other environmental parameters and host taxonomy (Sullam *et al.* 2012; Schmidt *et al.* 2015). Fishes live in dynamic environments where conditions such as temperature, hydrostatic pressure and salinity are highly variable (Zhang *et al.* 2016). The intestine, a major osmoregulatory organ in fishes, adapts to osmolality in seawater and is involved in gene expression for salinity acclimation (Wong *et al.* 2014; Zhang *et al.* 2016), but how these processes impact the gut microbiome is still poorly understood. Sullam *et al.* (2012) showed that the majority of fish gut communities clustered with free-living and non-fish-associated microbial assemblages inhabiting similar salinities as their fish host (freshwater *vs* marine). However, an exception to the rule were marine herbivorous fishes that harboured gut communities more closely resembling those found in the gut of mammalian species. Zhang *et al.* (2016) also identified differences in the GI microbiome of Nile tilapia acclimated to freshwater and water at 24 psu (practical salinity units), with those reared in freshwater harbouring greater abundances of Actinobacteria *vs* seawater-reared fish which harboured more Fusobacteria. These findings indicate that the bacterial communities of the gut of fishes must be adapted to tolerate the same conditions as the host organism.

Seasonal parameters also influence the gut microbiome structure in fishes. Ray (2016) identified a shift in the microbiome of bluegill (*Lepomis macrochirus*) where *Clostridium* dominated in the late summer and fall, but co-dominated with *Cetobacterium* in spring. Differences due to season were also apparent in largemouth bass (*Micropterus salmoides*) and spotted gar (*Lepisosteus oculatus*). However, seasonal changes were greater on the skin microbiome, suggesting that the gut is able to harbour a relatively stable community composition despite seasonal influences. Zarkasi *et al.* (2014) also reported seasonal impacts on the gut microbiome of Atlantic salmon (*S. salar*). Specifically, in winter, communities were dominated by Gram-positive fermentative bacteria (i.e. *Lactococcus*, *Weissella*, *Leuconostoc*), whereas in summer, Gram-negative Vibrionaceae became the most abundant members of the community. Interestingly, come the next winter, lactic acid bacteria did not repopulate, suggesting that colonization is not only affected by seasonal factors (temperature), but also influenced by aspects of fish physiology and diet (Zarkasi *et al.* 2014).

Factors related to geographical location can also alter the GI microbiome composition in fishes. Microbiomes of gizzard shad (*Dorosoma cepedianum*) collected from Illinois, Louisiana, Indiana and Missouri clustered together from each location but did not cluster by sampling month (season) (Ye *et al.* 2014). Interestingly, silver carp (*Hypophthalmichthys molitrix*) were the opposite, clustering by season but not by location, indicating that factors may influence fish microbiomes in different ways. However, the authors state that these differences may reflect dietary behaviours unique to each fish species. This observation was also put forth by Smith *et al.* (2015) who observed significant differences between geographic locations in the bacterial communities of threespine stickleback (*Gasterosteus aculeatus*) but were able to

relate those differences to prey microbiomes. These studies further emphasize the difficulties in exploring these influences in wild populations.

Dietary factors

Changes in the environment such as fluctuations in oxygen concentration, temperature and salinity can lead to periods of starvation for fishes in the wild. Physiological changes in the host that occur during starvation force its associated gut microbiome to adapt to the new conditions, but few studies have characterized the changes in the fish gut microbiome in response to starvation. A recent analysis of the gut microbiome of cultured Asian seabass (*Lates calcarifer*) under starvation revealed bacterial communities shift, resulting in greater abundances of Bacteroidetes and fewer Betaproteobacteria in starved fish (Xia *et al.* 2014). Bacteroidetes, which are often dominant in the gut, produce digestive enzymes (Crawford *et al.* 2009), and some genera (i.e. *Bacteroides*) aid in digestion of polysaccharides (Xia *et al.* 2014). Therefore, during periods of starvation, members of the Bacteroidetes are capable of harvesting additional energy from food, providing them with a competitive advantage over other phyla and allowing for their proliferation. Likewise, Nile tilapia (*O. niloticus*) exhibited changes in the microbial communities of two gut regions: the colon and the caecum, under starvation (Kohl *et al.* 2014). Microbial phylogenetic diversity increased as a result of fasting in the colon, whereas this diversity decreased in the caecum. Shifts due to reduced nutrition may negatively impact fish health as members of the microbiome compete with opportunistic pathogens.

Diet is recognized as a primary factor influencing diversity and community structure of fish gut microbiomes. The majority of studies relating diet and microbiome composition alter diet treatments in culture or controlled settings (Desai *et al.* 2012; Ingerslev *et al.* 2014a,b; Estruch *et al.* 2015; Schmidt *et al.* 2016), as concern over the use of fishmeal forces aquaculturists to test new, plant protein-based formulations. As microbial communities play an important role in nutrient acquisition and provide a protective barrier against opportunistic pathogens (Llewellyn *et al.* 2014), gut microbiome manipulation is often tested as a mechanism to increase feed efficiency, growth and disease resistance of fish in aquaculture (Dimitroglou *et al.* 2011). Research supporting dietary influences on the fish gut microbiome is vast and has recently been reviewed elsewhere (see Llewellyn *et al.* 2014; Ringø *et al.* 2016).

Although diet seems to clearly impact GI microbiome composition, studies on larvae have identified shifts in bacterial community structure that do not coincide with dietary shifts, but instead with changes in larval gut physiology (Stephens *et al.* 2015). These studies provide evidence for another set of influential parameters on microbiome structure that include host-related genetic factors.

Evolutionary factors

Bacteria constituting the gut microbiome play a critical role in metabolic processes and are thus key components of host evolution and fitness (Llewellyn *et al.* 2014; Sylvain *et al.* 2016). In a world dominated by microorganisms, metazoans have had to evolve and form tactical associations with their unicellular neighbours in order to inhabit a wide array of niches and habitats (Rawls *et al.* 2006). The essential partnership between organisms and their microbes suggests the host and its associated microbiome is best described as a single meta-organism or holobiont (Guerrero *et al.* 2013).

Genetic diversity of hosts, both within and among populations, is likely to impact bacterial community structure. Schmidt *et al.* (2015) hypothesized that fish habitat regions harbour functionally redundant bacterial taxa, and the environmental parameters associated with these habitats, including salinity, alter host physiology in a way that forms a habitat filter which allows only a subset of those functionally redundant taxa to colonize the host. These bacteria then compete with one another for the niche. Other studies investigating migratory fishes provide evidence for this hypothesis. Smith *et al.* (2015) found that population level differences in the composition of the gut microbiome of wild-caught three-spine sticklebacks (*G. aculeatus*), despite being influenced by habitat type, may be more related to internal sorting and selective pressures exerted by the host genotype than to environmentally linked colonization processes. Similarly, Llewellyn *et al.* (2016) determined that the gut microbiome structure of Atlantic salmon (*S. salar*) was influenced by life cycle stage and not by geography. Despite migration between freshwater and marine habitats, adult salmon returning to rivers maintained many of the bacterial taxa associated with marine adults, and members of the Mycoplasmataceae were present throughout life stages. Similarly, the presence of a core gut microbiome between laboratory- and cage-reared Atlantic salmon (*S. salar*) suggests selection of specific microorganisms resulting from host physiology (Dehler *et al.* 2017). Additional evidence for genetics-based selection of the microbiome is demonstrated in culture environments, as gut bacterial communities of different fish species fed similar diets exhibit species-specificity, despite being reared in the same environment (Li *et al.* 2014, 2015). Genetic differences between fish

species and individuals can lead to alterations in immune function, metabolism and behaviour (Li *et al.* 2015), all of which likely influence the structure of the fish gut microbiome. These studies indicate that the composition, diversity and structure of fish microbiomes are not simply a reflection of the environment and ecology, but are a result of pressure exerted by evolutionary relationships.

Traditionally, studies concerning the fish gut microbiome focus on cultured fish species, particularly related to the promotion of growth characteristics, non-pathogenic bacteria and disease control (Uchii *et al.* 2006). Although a symbiotic relationship exists involving the host metabolism and bacterial communities of the gut, much is still unknown about host–microbiome interactions at a functional level, especially for wild fish populations. A majority of research concerning host–microbiome interactions and assembly processes derive from laboratory-based studies, often using model species that have been domesticated in laboratory settings for generations (Roeselers *et al.* 2011). However, the interactions of the gut microbiome with the host organism are complex, and these domestic studies may not adequately represent these relationships in the wild; therefore, better understanding of the natural bacterial communities of healthy individuals and how they interact with the host and other environmental factors is of critical importance (Sullam *et al.* 2012; Eichmiller *et al.* 2016).

Conclusion and future directions

Gut microbiomes of fishes are complex, dynamic communities influenced by a wide variety of environmental, physiological and genetic factors. Although fish species generally harbour unique microbiomes, the exact relationship of these differences to phylogeny is not yet understood. Furthermore, a large number of shared taxa persist across fish species, but it is not known whether it is physical characteristics of the taxon itself or biological functions performed by specific members that lead to this preservation. The key to unravelling the mysteries of the microbiome lies in deciphering host–microbe interactions. It is important to characterize the bacterial communities present in fish and understand what factors influence that composition, but until we understand how those microbes influence digestion, immune function, behaviour and overall fish health, we will be unable to use them to their full potential in aquaculture. It is important for future studies to delve deeper into functional aspects of the microbiome in order to advance our knowledge on fish health and microbial manipulation for disease prevention in culture systems.

Conflict of Interest

The authors have no conflict of interest to declare.

References

Amann, R.I., Ludwig, W. and Schleifer, K.H. (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* **59**, 143–169.

Bakke, I., Coward, E., Andersen, T. and Vastein, O. (2015) Selection in the host structures the microbiota associated with developing cod larvae (*Gadus morhua*). *Environ Microbiol* **17**, 3914–3924.

Baldo, L., Riera, J.L., Tooming-Klunderud, A., Albà, M.M. and Salzburger, W. (2015) Gut microbiota dynamics during dietary shift in Eastern African cichlid fishes. *PLoS ONE* **10**, e0127462.

Bel'Kova, N.L., Denikina, N.N. and Dzyuba, E.V. (2015) Study of the microbiome of the intestine of the *Comephorus dybowski* Korotneff, 1904. *Biol Bull* **42**, 463–469.

Bolnick, D.I., Snowberg, L.K., Caporaso, J.G., Lauber, C., Knight, R. and Stutz, W.E. (2014a) Major histocompatibility complex class IIb polymorphism influences gut microbiota composition and diversity. *Mol Ecol* **23**, 4831–4845.

Bolnick, D.I., Snowberg, L.K., Hirsch, P.E., Lauber, C.L., Knight, R., Caporaso, J.G. and Svanbäck, R. (2014b) Individuals' diet diversity influences gut microbial diversity in two freshwater fish (threespine stickleback and Eurasian perch). *Ecol Lett* **17**, 979–987.

Borrelli, L. (2015) *The microbiota-gut brain axis. A study in zebrafish (Danio rerio)*. Dissertation, Università degli Studi di Napoli Federico II, Italy.

Carda-Diéguez, M., Mira, A. and Fouz, B. (2014) Pyrosequencing survey of intestinal microbiome diversity in cultured sea bass (*Dicentrarchus labrax*) fed functional diets. *FEMS Microbiol Ecol* **87**, 451–459.

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

Chiu, C. and Miller, S. (2016) Next-generation sequencing. In *Molecular Microbiology: Diagnostic Principles and Practice*, 3rd edn ed. Persing, D.H., Tenover, F.C., Hayden, R.T., Ieven, G., Miller, M.B. and Nolte, F.S. pp. 68–79. Washington DC: ASM Press.

Clements, K.D., Pasch, I.B.Y., Moran, D. and Turner, S.J. (2007) Clostridia dominate 16S rRNA gene libraries prepared from the hindgut of temperate marine herbivorous fishes. *Mar Biol* **150**, 1431–1440.

Crawford, P.A., Crowley, J.R., Sambandam, N., Muegge, B.D., Costello, E.K., Hamady, M., Knight, R. and Gordon, J.I. (2009) Regulation of myocardial ketone body metabolism

by the gut microbiota during nutrient deprivation. *Proc Natl Acad Sci U S A* **106**, 11276–11281.

Davis, D.J., Bryda, E.C., Gillespie, C.H. and Ericsson, A.C. (2016) Microbial modulation of behavior and stress responses in zebrafish larvae. *Behav Brain Res* **311**, 219–227.

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

Del'Duca, A., Cesar, D.E., Diniz, C.G. and Abreu, P.C. (2013) Evaluation of the presence and efficiency of potential probiotic bacteria in the gut of tilapia (*Oreochromis niloticus*) using the fluorescent in situ hybridization technique. *Aquaculture* **388–391**, 115–121.

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

Di Maiuta, N.D., Schwarzentruber, P., Schenker, M. and Schoelkopf, J. (2013) Microbial population dynamics in the faeces of wood-eating loricariid catfishes. *Lett Appl Microbiol* **56**, 401–407.

Dimitroglou, A., Merrifield, D.L., Carnevali, O., Picchietti, S., Avella, M., Daniels, C., Güroy, D. and Davies, S.J. (2011) Microbial manipulations to improve fish health and production – a Mediterranean perspective. *Fish Shellfish Immunol* **30**, 1–16.

Eichmiller, J.J., Hamilton, M.J., Staley, C., Sadowsky, M.J. and Sorensen, P.W. (2016) Environment shapes the fecal microbiome of invasive carp species. *Microbiome* **4**, 44.

Engelbrektson, A., Kunin, V., Wrighton, K.C., Zvenigorodsky, N., Chen, F., Ochman, H. and Hugenholtz, P. (2010) Experimental factors affecting PCR-based estimates of microbial species richness and evenness. *ISME J* **4**, 642–647.

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

Etyemez, M. and Balcázar, J.L. (2015) Bacterial community structure in the intestinal ecosystem of rainbow trout (*Oncorhynchus mykiss*) as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Res Vet Sci* **100**, 8–11.

Fan, L., Chen, J., Meng, S., Song, C., Qiu, L., Hu, G. and Xu, P. (2017) Characterization of microbial communities in intensive GIFT tilapia (*Oreochromis niloticus*) pond systems during the peak period of breeding. *Aquaculture Res.* **48**, 459–472.

FAO (Food and Agricultural Organization) (2016) World aquaculture production by species groups. In *FAO Yearbook, Fishery and Aquaculture Statistics* 2014. pp. 52–53. Rome: FAO of the United Nations.

Finegold, S.M., Vaisanen, M.-L., Molitoris, D.R., Tomzynski, T.J., Song, Y.L., Liu, C., Collins, M.D. and Lawson, P.A. (2003) *Cetobacterium somerae* sp. nov. from human feces and emended description of the genus *Cetobacterium*. *Syst Appl Microbiol* **26**, 177–181.

Fjellheim, A.J., Playfoot, K.J., Skjermo, J. and Vadstein, O. (2012) Inter-individual variation in the dominant intestinal microbiome of reared Atlantic cod (*Gadus morhua* L.) larvae. *Aquaculture Res* **43**, 1499–1508.

Fouhy, F., Clooney, A.G., Stanton, C., Claesson, M.J. and Cotter, P.D. (2016) 16S rRNA gene sequencing of mock microbial populations – impact of DNA extraction method, primer choice and sequencing platform. *BMC Microbiol* **16**, 123.

Franchini, P., Fruciano, C., Frickey, T., Jones, J.C. and Meyer, A. (2014) The gut microbial community of Midas cichlid fish in repeatedly evolved limnetic-benthic species pairs. *PLoS ONE* **9**, e95027.

Gajardo, K., Rodiles, A., Kortner, T.M., Krogdahl, A., Bakke, A.M., Merrifield, D.L. and Sørum, H. (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.

Gaulke, C.A., Barton, C.L., Proffitt, S., Tanguay, R.T. and Sharpton, T.J. (2016) Triclosan exposure is associated with rapid restructuring of the microbiome in adult zebrafish. *PLoS ONE* **11**, e0154632.

Geraylou, Z., Souffreau, C., Rurangwa, E., De Meester, L., Courtin, M., Delcour, J.A., Buyse, J. and Ollevier, F. (2013a) Effects of dietary arabinoxylan-oligosaccharides (AXOS) and endogenous probiotics on the growth performance, non-specific immunity and gut microbiota of juvenile Siberian sturgeon (*Acipenser baerii*). *Fish Shellfish Immunol* **35**, 766–775.

Geraylou, Z., Souffreau, C., Rurangwa, E., Maes, G.E., Spanier, K.I., Courtin, C.M., Delcour, J.A., Buyse, J. *et al.* (2013b) Prebiotic effects of arabinoxylan oligosaccharides on juvenile Siberian sturgeon (*Acipenser baerii*) with emphasis on the modulation of the gut microbiome using 454 pyrosequencing. *FEMS Microbiol Ecol* **86**, 357–371.

Giatsis, C., Sipkema, D., Smidt, H., Verreth, J. and Verdegem, M. (2014) The colonization dynamics of the gut microbiota in tilapia larvae. *PLoS ONE* **9**, e103641.

Giatsis, C., Sipkema, D., Smidt, H., Heilig, H., Benvenuti, G., Verreth, J. and Verdegem, M. (2015) The impact of rearing environment on the development of gut microbiome in tilapia larvae. *Sci Rep* **5**, 18206.

Givens, C.E., Ransom, B., Bano, N. and Hollibaugh, J.T. (2015) Comparison of the gut microbiomes of 12 bony fish and 3 shark species. *Mar Ecol Prog Ser* **518**, 209–223.

Green, T.J., Smullen, R. and Barnes, A.C. (2013) Dietary soybean protein concentrate-induced intestinal disorder in marine farmed Atlantic salmon, *Salmo salar* is associated with alterations in gut microbiota. *Vet Microbiol* **166**, 286–292.

Guerrero, R., Margulis, L. and Berlanga, M. (2013) Symbiogenesis: the holobiont as a unit of evolution. *Int Microbiol* **16**, 133–143.

Hennersdorf, P., Kleinertz, S., Theisen, S., Abdul-Aziz, M., Mrotzek, G., Palm, H.W. and Saluz, H.P. (2016a) Microbial diversity and parasitic load in tropical fish of different environmental conditions. *PLoS ONE* **11**, e0151594.

Hennersdorf, P., Mrotzek, G., Abdul-Aziz, M.A. and Saluz, H.P. (2016b) Metagenomic analysis between free-living and cultured *Epinephelus fuscoguttatus* under different environmental conditions in Indonesian waters. *Mar Poll Bull* **110**, 726–734.

Hill, C.J., Brown, J.R.M., Lynch, D.B., Jeffery, I.B., Ryan, C.A., Ross, R.P., Stanton, C. and O'Toole, P.W. (2016) Effect of room temperature transport vials on DNA quality and phylogenetic composition of faecal microbiota of elderly adults and infants. *Microbiome* **4**, 19.

Hongoh, Y., Yuzawa, H., Ohkuma, M. and Kudo, T. (2003) Evaluation of primers and PCR conditions for the analysis of 16S rRNA genes from a natural environment. *FEMS Microbiol Lett* **221**, 299–304.

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

Ingerslev, H.C., Jørgensen, L.v., Strube, M., Larsen, N., Dalsgaard, I., Boye, M. and Madsen, L. (2014b) The development of the gut microbiota in rainbow trout (*Oncorhynchus mykiss*) is affected by first feeding and diet type. *Aquaculture* **424–425**, 24–34.

Kashinskaya, E.N., Belkova, N.L., Izvekova, G.I., Simonov, E.P., Andree, K.B., Glupov, V.V., Baturina, O.A., Kabilov, M.R. *et al.* (2015) A comparative study on microbiota from the intestine of Prussian carp (*Carassius gibelio*) and their aquatic environmental compartments, using different molecular methods. *J Appl Microbiol* **119**, 948–961.

Kennedy, N.A., Walker, A.W., Berry, S.H., Duncon, S.H., Farquarson, F.M., Louis, P., Thomson, J.M., UK IBD Genetics Consortium, Satsangi, J., Flint, H.J., Parkhill, J., Lees, C.W. and Hold, G.L. (2014). The impact of different DNA extraction kits and laboratories upon the assessment of human gut microbiota composition by 16S rRNA gene sequencing. *PLoS ONE* **9**, e88982.

Kim, D.H., Brunt, J. and Austin, B. (2007) Microbial diversity of intestinal contents and mucus in rainbow trout (*Oncorhynchus mykiss*). *J Appl Microbiol* **102**, 1654–1664.

Kohl, K.D., Amaya, J., Passement, C.A., Dearing, M.D. and McCue, M.D. (2014) Unique and shared responses of the gut microbiota to prolonged fasting: a comparative study across five classes of vertebrate hosts. *FEMS Microbiol Ecol* **90**, 883–894.

Kormas, K.A., Meziti, A., Mente, E. and Frentzos, A. (2014) Dietary differences are reflected on the gut prokaryotic community structure of wild and commercially reared sea bream (*Sparus aurata*). *MicrobiologyOpen* **3**, 718–728.

La Rosa, P.S., Brooks, J.P., Deych, E., Boone, E.L., Edwards, D.J., Wang, Q., Sodergren, E., Weinstock, G. *et al.* (2012) Hypothesis testing and power calculations for taxonomic-based human microbiota data. *PLoS ONE* **7**, e52078.

Larsen, A.M., Mohammed, H.H. and Arias, C.R. (2014a) Characterization of the gut microbiota of three commercially valuable warmwater fish species. *J Appl Microbiol* **116**, 1396–1404.

Larsen, A.M., Mohammed, H.H. and Arias, C.R. (2014b) Comparison of DNA extraction protocols for the analysis of gut microbiota in fishes. *FEMS Microbiol Lett* **362**, fnu031.

Li, X., Yan, Q., Xie, S., Hu, W., Yu, Y. and Hu, Z. (2013) Gut microbiota contributes to the growth of fast-growing transgenic common carp (*Cyprinus carpio* L.). *PLoS ONE* **8**, e64577.

Li, X.M., Zhu, Y.J., Yan, Q.Y., Ringø, E. and Yang, D.G. (2014) Do the intestinal microbiotas differ between paddlefish (*Polyodon spathala*) and bighead carp (*Aristichthys nobilis*) reared in the same pond? *J Appl Microbiol* **117**, 1245–1252.

Li, T., Long, M., Gatesoupe, F.-J., Zhang, Q., Li, A. and Gong, X. (2015) Comparative analysis of the intestinal bacterial communities in different species of carp by pyrosequencing. *Microb Ecol* **69**, 25–36.

Li, T., Long, M., Ji, C., Shen, Z., Gatesoupe, F.-J., Zhang, X., Zhang, Q., Zhang, L. *et al.* (2016) Alterations of the gut microbiota of largemouth bronze grudgeon (*Coreius guichenoti*) suffering from furunculosis. *Sci Rep* **6**, 30606.

Liu, H., Guo, X., Gooneratne, R., Lai, R., Zeng, C., Zhan, F. and Wang, W. (2016) The gut microbiota and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. *Sci Rep* **6**, 24340.

Llewellyn, M.S., Boutin, S., Hoseinifar, S.H. and Derome, N. (2014) Teleost microbiotas: the state of the art in their characterization, manipulation, and importance in aquaculture and fisheries. *Front Microbiol* **5**, 207.

Llewellyn, M.S., McGinnity, P., Dionne, M., Letourneau, J., Thonier, F., Carvalho, G.R., Creer, S. and Derome, N. (2016) The biogeography of the atlantic salmon (*Salmo salar*) gut microbiota. *ISME J* **10**, 1280–1284.

Lyons, P.O., Turnbull, J.F., Dawson, K.A. and Crumlish, M. (2015) Exploring the microbial diversity of the distal intestinal lumen and mucosa of farmed rainbow trout *Oncorhynchus mykiss* (Walbaum) using next generation sequencing (NGS). *Aquaculture Res* **48**, 77–91.

Lyons, P.P., Turnbull, J.F., Dawson, K.A. and Crumlish, M. (2016) Effects of low-level dietary microalgae supplementation on the distal intestinal microbiota of farmed rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquaculture Res*. doi:10.1111/are.13080.

MacKenzie, B.W., Waite, D.W. and Taylor, M.W. (2015) Evaluating variation in human gut microbiota profiles due

to DNA extraction method and inter-subject differences. *Front Microbiol* **6**, 130.

Mardis, E.R. (2013) Next-generation sequencing platforms. *Annu Rev Anal Chem* **6**, 287–303.

Metzker, M.L. (2010) Sequencing technologies – the next generation. *Nature Rev Genet* **11**, 31–46.

Miyake, S., Ngugi, D.K. and Stingl, U. (2015) Diet strongly influences the gut microbiota of surgeonfishes. *Mol Ecol* **24**, 656–672.

Montalban-Arques, A., De Schryver, P., Bossier, P., Gorkiewicz, G., Mulero, V., Gatlin, D.M. and Galindo-Villegas, J. (2015) Selective manipulation of the gut microbiota improves immune status in vertebrates. *Front Immunol* **6**, 512.

Mueller, K., Ash, C., Pennisi, E. and Smith, O. (2012) The gut microbiota. *Science* **336**, 1245.

Narrowe, A.B., Albuthi-Lantz, M., Smith, E.P., Bower, K.J., Roane, T.M., Vajda, A.M. and Miller, C.S. (2015) Perturbation and restoration of the fathead minnow gut microbiota after low-level triclosan exposure. *Microbiome* **3**, 6.

Nelson, J.S. (2006) *Fishes of the World*, 4th edn. Hoboken, NJ: John Wiley & Sons, Inc.

Le Nguyen, D.D., Ngoc, H.H., Dijoux, D., Loiseau, G. and Montet, D. (2008) Determination of fish origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: an application on Pangasius fish from Viet Nam. *Food Control* **19**, 454–460.

Ni, J., Yan, Q., Yu, Y. and Zhang, T. (2014) Fish gut microecosystem: a model for detecting spatial pattern of microorganisms. *Chin J Oceanol Limnol* **32**, 54–57.

Ran, C., Huang, L., Liu, Z., Xu, L., Yang, Y., Tacon, P., Auclair, E. and Zhou, Z. (2015) A comparison of the beneficial effects of live and heat-inactivated baker's yeast on Nile tilapia: suggestions on the role and function of the secretory metabolites released from the yeast. *PLoS ONE* **10**, e0145448.

Ran, C., Hu, J., Liu, W., Liu, Z., He, S., Dan, B.C.T., Diem, N.N., Ooi, E.L. *et al.* (2016) Thymol and carvacrol affect hybrid tilapia through the combination of direct stimulation and an intestinal microbiota-mediated effect: insights from a germ-free zebrafish model. *J Nutr* **146**, 1132–1140.

Rawls, J.F., Mahowald, M.A., Ley, R.E. and Gordan, J.I. (2006) Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. *Cell* **127**, 423–433.

Ray, C.L. (2016) *Characterization of the gut and skin microbiotas of wild-caught fishes from Lake Guntersville, Alabama*. Master Thesis, Auburn University, Auburn, AL, USA.

Ringø, E., Zhou, Z., Vecino, J.L.G., Wadsworth, S., Romero, J., Kroghdal, Å., Olsen, R.E., Dimitoglou, A. *et al.* (2016) Effect of dietary components on the gut microbiota of aquatic animals: the never-ending story? *Aquaculture Nutr* **22**, 219–282.

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

Rosello-Mora, R. (2005) Updating prokaryotic taxonomy. *J Bacteriol* **187**, 6225–6257.

Rurangwa, E., Sipkema, D., Kals, J., ter Veld, M., Forlenza, M., Bacanu, G.M., Smidt, H. and Palstra, A.P. (2015) Impact of a novel protein meal on the gastrointestinal microbiota and the host transcriptome of larval zebrafish *Danio rerio*. *Front Physiol* **6**, 133.

Schmidt, V.T., Smith, K.F., Melvin, D.W. and Amaral-Zettler, L.A. (2015) Community assembly of euryhaline fish microbiota during salinity acclimation. *Mol Ecol* **24**, 2537–2550.

Schmidt, V., Amaral-Zettler, L., Davidson, J., Summerfelt, S. and Good, C. (2016) Influence of fishmeal-free diets on microbial communities in Atlantic Salmon (*Salmo salar*) recirculation aquaculture systems. *Appl Environ Microbiol* **82**, 4470–4481.

Sergeant, M.J., Constantinidou, C., Cogan, T., Penn, C.W. and Pallen, M.J. (2012) High-throughput sequencing of 16S rRNA gene amplicons: effects of extraction procedure, primer length, and annealing temperature. *PLoS ONE* **7**, e38094.

Shade, A. and Handelsman, J. (2012) Beyond the Venn diagram: the hunt for the core microbiota. *Environ Microbiol* **14**, 4–12.

Smith, C.C.R., Snowberg, L.K., Caporaso, J.G., Knight, R. and Bolnick, D.I. (2015) Dietary input of microbes and host genetic variation shape among-population differences in stickleback gut microbiota. *ISME J* **9**, 2515–2526.

Standen, B.T., Rodiles, A., Peggs, D.L., Davies, S.J., Santos, G.A. and Merrifield, D.L. (2015) Modulation of the intestinal microbiota and morphology of tilapia, *Oreochromis niloticus*, following the application of a multi-species probiotic. *Appl Microbiol Biotechnol* **99**, 8403–8417.

Star, B., Haverkamp, T., Jentoft, S. and Jakobsen, K. (2013) Next generation sequencing shows high variation of the intestinal microbial species composition in Atlantic cod caught at a single location. *BMC Microbiol* **13**, 248.

Stephens, W.Z., Burns, A., Stagaman, K., Wong, S., Rawls, J., Guillemin, K. and Bohannan, B. (2015) The composition of the zebrafish intestinal microbial community varies across development. *ISME J* **10**, 644–654.

Sugita, H., Shibuya, K., Shimooka, H. and Deguchi, Y. (1996) Antibacterial abilities of intestinal bacteria in freshwater cultured fish. *Aquaculture* **145**, 195–203.

Sullam, K.E., Essinger, S.D., Lozupone, C.A., O'Connor, M.P., Rosen, G.L., Knight, R., Kilham, S.S. and Russell, J.A. (2012) Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Mol Ecol* **21**, 3363–3378.

Sylvain, F.É., Cheaib, B., Llewellyn, M., Correia, T.G., Fagundes, D.B., Val, A.L. and Derome, N. (2016) pH drop

impacts differentially skin and gut microbiota of the Amazonian fish tambaqui (*Colossoma macropomum*). *Sci Rep* **6**, 32032.

Tap, J., Mondot, S., Levenez, F., Pelletier, E., Caron, C., Furet, J.-P., Ugarte, E., Muñoz-Tamayo, R. *et al.* (2009) Towards the human intestinal microbiota phylogenetic core. *Environ Microbiol* **11**, 2574–2584.

Tremblay, J., Singh, K., Fern, A., Kirton, E.S., He, S., Woyke, T., Lee, J., Chen, F. *et al.* (2015) Primer and platform effects on 16S rRNA tag sequencing. *Front Microbiol* **6**, 771.

Turnbaugh, P.J. and Gordon, J.I. (2009) The core microbiota, energy balance and obesity. *J Physiol* **587**, 4153–4158.

Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R. and Gordon, J.I. (2007) The human microbiota project. *Nature* **449**, 804–810.

Uchii, K., Matsui, K., Yonekura, R., Tani, K., Kenzaka, T., Nasu, M. and Kawabata, Z. (2006) Genetic and physiological characterization of the intestinal bacterial microbiota of bluegill (*Lepomis macrochirus*) with three different feeding habits. *Microb Ecol* **51**, 277–284.

Van Kessel, M.A.H.J., Dutilh, B.E., Neveling, K., Kwint, M.P., Veltman, J.A., Flik, G., Jetten, M.S.M., Klaren, P.H.M. *et al.* (2011) Pyrosequencing of 16S rRNA gene amplicons to study the microbiota in the gastrointestinal tract of carp (*Cyprinus carpio* L.). *AMB Express* **1**, 41.

Walker, A.W., Martin, J.C., Scott, P., Parkhill, J., Flint, H.J. and Scott, K.P. (2015) 16S rRNA gene-based profiling of the human infant gut microbiota is strongly influenced by sample processing and PCR primer choice. *Microbiome* **3**, 26.

Wang, J., Tao, Q., Wang, Z., Mai, K., Xu, W., Zhang, Y. and Ai, Q. (2016) Effects of fish meal replacement by soybean meal with supplementation of functional compound additives on intestinal morphology and microbiota of Japanese seabass (*Lateolabrax japonicus*). *Aquaculture Res*. doi:10.1111/are.13055.

Wetterstrand, K.A. (2016) DNA sequencing costs: data from the NHGRI genome sequencing program (GSP). Available at: http://www.genome.gov/sequencingcostsdata. Accessed 27 Sept 2016

Wilson, B., Danilowicz, B.S. and Meijer, W.G. (2008) The diversity of bacterial communities associated with Atlantic cod *Gadus morhua*. *Microb Ecol* **55**, 425–434.

Wong, S., Waldrop, T., Summerfelt, S., Davidson, J., Barrows, F., Kenney, B.B., Welch, T., Wiens, G.D. *et al.* (2013) Aquacultured rainbow trout (*Onchorhynchus mykiss*) possess a large core intestinal microbiota that is resistant to variation in diet and rearing density. *Appl Environ Microbiol* **79**, 4974–4984.

Wong, M.K., Ozaki, H., Suzuki, Y., Iwasaki, W. and Takei, Y. (2014) Discovery of osmotic sensitive transcription factors in fish intestine via a transcriptomic approach. *BMC Genom* **15**, 1134.

Wong, S., Stephens, W.Z., Burns, A.R., Stagaman, K., David, L.A., Bohannan, B.J.M., Guillemin, K. and Rawls, J.F. (2015) Ontogenetic differences in dietary fat influence microbiota assembly in the zebrafish gut. *mBio* **6**, e00687–15.

Wu, G.D., Lewis, J.D., Hoffman, C., Chen, Y.-Y., Knight, R., Bittinger, K., Hwang, J., Chen, J. *et al.* (2010a) Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16S sequence tags. *BMC Microbiol* **10**, 206.

Wu, S.-G., Gao, T.H., Zheng, Y.Z., Wang, W.W., Cheng, Y.Y. and Wang, G.T. (2010b) Microbial diversity of intestinal contents and mucus in yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture* **303**, 1–7.

Wu, S., Wang, G., Angert, E.R., Wang, W., Li, W. and Zou, H. (2012) Composition, diversity, and origin of the bacterial community of grass carp intestine. *PLoS ONE* **7**, e30440.

Wu, S.-G., Tian, J.-Y., Gatesoupe, F.-J., Li, W.-X., Zou, H., Yang, B.-J. and Wang, G.-T. (2013) Intestinal microbiota of gibel carp (*Carassius auratus gibelio*) and its origin as revealed by 454 pyrosequencing. *World J Microbiol Biotechnol* **29**, 1585–1595.

Xia, J.H., Lin, G., Fu, G.H., Wan, Z.Y., Lee, M., Wang, L., Liu, X.J. and Yue, G.H. (2014) The intestinal microbiota of fish under starvation. *BMC Genom* **15**, 266.

Yan, Q., Li, J., Yu, Y., Wang, J., He, Z., Van Nostrand, J.D., Kempher, M.L., Wu, L. *et al.* (2016) Environmental filtering decreases with fish development for the assembly of gut microbiota. *Environ Microbiol* **18**, 4739–4754.

Yarza, P., Yilmaz, P., Pruesse, E., Glöckner, F.O., Ludwig, W., Schleifer, K.-H., Whitman, W.B., Euzéby, J., Amann, R. *et al.* (2014) Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature Rev Microbiol* **12**, 635–645.

Ye, L., Amberg, J., Chapman, D., Gaikowski, M. and Liu, W.T. (2016) Fish gut microbiota analysis differentiates physiology and behavior of invasive Asian carp and indigenous American fish. *ISME J* **8**, 541–551.

Zarkasi, K.Z., Abell, G.C.J., Taylor, R.S., Neuman, C., Hatje, E., Tamplin, M.L., Katouli, M. and Bowman, J.P. (2014) Pyrosequencing-based characterization of gastrointestinal bacteria of Atlantic salmon (*Salmo salar* L.) within a commercial mariculture system. *J Appl Microbiol* **117**, 18–27.

Zarkasi, K.Z., Taylor, R.S., Abell, G.C.J., Tamplin, M.L., Glencross, B.D. and Bowman, J.P. (2016) Atlantic salmon (*Salmo salar* L.) gastrointestinal microbial community dynamics in relation to digesta properties and diet. *Microb Ecol* **71**, 589–603.

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

Zhou, Z.K., Shi, P., He, S., Liu, Y., Huang, G.H., Yao, B. and Ringø, E. (2009) Identification of adherent microbiota in the stomach and intestine of emperor red snapper (*Lutjanus sebae* Cuvier) using 16S rRNA-DGGE. *Aquaculture Res* **40**, 1213–1218.