



# Salinity significantly affects intestinal microbiota and gene expression in striped catfish juveniles

Dang Quang Hieu<sup>1</sup> · Bui Thi Bich Hang<sup>2</sup> · Jep Lokesh<sup>3</sup> · Mutien-Marie Garigliany<sup>4</sup> · Do Thi Thanh Huong<sup>2</sup> · Duong Thuy Yen<sup>2</sup> · Pham Thanh Liem<sup>2</sup> · Bui Minh Tam<sup>2</sup> · Dao Minh Hai<sup>2,5</sup> · Vo Nam Son<sup>2</sup> · Nguyen Thanh Phuong<sup>2</sup> · Frédéric Farnir<sup>5</sup> · Patrick Kestemont<sup>1</sup>

Received: 21 January 2022 / Revised: 8 March 2022 / Accepted: 19 March 2022 / Published online: 2 April 2022  
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

## Abstract

In the present study, juvenile striped catfish (*Pangasianodon hypophthalmus*), a freshwater fish species, have been chronically exposed to a salinity gradient from freshwater to 20 psu (practical salinity unit) and were sampled at the beginning (D20) and the end (D34) of exposure. The results revealed that the intestinal microbial profile of striped catfish reared in freshwater conditions were dominated by the phyla *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia*. Alpha diversity measures (observed OTUs (operational taxonomic units), Shannon and Faith's PD (phylogenetic diversity)) showed a decreasing pattern as the salinities increased, except for the phylogenetic diversity at D34, which was showing an opposite trend. Furthermore, the beta diversity between groups was significantly different. *Vibrio* and *Akkermansia* genera were affected differentially with increasing salinity, the former being increased while the latter was decreased. The genus *Sulfurospirillum* was found predominantly in fish submitted to salinity treatments. Regarding the host response, the fish intestine likely contributed to osmoregulation by modifying the expression of osmoregulatory genes such as *nka1a*, *nka1b*, *slc12a1*, *slc12a2*, *cfr*, and *aqp1*, especially in fish exposed to 15 and 20 psu. The expression of heat shock proteins (*hsp60*, *hsp70*, and *hsp90*) was significantly increased in fish reared in 15 and 20 psu. On the other hand, the expression of pattern recognition receptors (PRRs) were inhibited in fish exposed to 20 psu at D20. In conclusion, the fish intestinal microbiota was significantly disrupted in salinities higher than 10 psu and these effects were proportional to the exposure time. In addition, the modifications of intestinal gene expression related to ion exchange and stressful responses may help the fish to adapt hyperosmotic environment.

## Key points

- It is the first study to provide detailed information on the gut microbiota of fish using the amplicon sequencing method.
- Salinity environment significantly modified the intestinal microbiota of striped catfish.
- Intestinal responses may help the fish adapt to hyperosmotic environment.

**Keywords** Striped catfish · Intestinal microbiota · Gene expression · Salinity · Osmoregulation

✉ Dang Quang Hieu  
quanghieu.dang.87@gmail.com

✉ Patrick Kestemont  
patrick.kestemont@unamur.be

<sup>1</sup> Research Unit in Environmental and Evolutionary Biology, Institute of Life Earth & Environment (ILEE), University of Namur, Namur, Belgium

<sup>2</sup> College of Aquaculture and Fisheries, Can Tho University, Can Tho City, Vietnam

<sup>3</sup> Université de Pau Et Des Pays de L'Adour, Saint-Pee-sur-Nivelle, E2S UPPA INRAE, NuMéA, France

<sup>4</sup> Department of Pathology, Faculty of Veterinary Medicine, University of Liège, Liege, Belgium

<sup>5</sup> Department of Animal Production, Faculty of Veterinary Medicine, University of Liège, Liege, Belgium

## Introduction

Striped catfish *Pangasianodon hypophthalmus* industry has been an important inland aquaculture production in Southeast Asia, particularly in Mekong Delta in Vietnam. In 2019, striped catfish production in Vietnam reached 1.4 million of tons with estimated value of US\$2.3 billion (FAO 2020). Nevertheless, the industry is currently facing climatic challenges, especially extensive salinity intrusion caused by the climate change. The saline water was observed up to 40 km far from the river mouth in places and increased salinity level in striped catfish farms up to 9 psu (practical salinity units) (Schmitz et al. 2017a, b, c). Several studies have been conducted to investigate the effects of salinity on physiological striped catfish (Nguyen et al. 2014; Schmitz et al. 2016a,b). However, the intestinal response of fish is largely unknown.

The fish intestine is a complex multifunctional organ that plays crucial roles in nutrient absorption, the immune response, and osmoregulation (Martin et al. 2016; Dehler et al. 2017a). In addition to the gills and kidney, the gastro-intestinal tract contributes to internal osmoregulation through balancing the uptake and maintenance of electrolytes and water when fish migrate from fresh water to seawater (Ringø et al. 2003; Malakpour Kolbadinezhad et al. 2018). When adapting to hyperosmotic environments, fish continuously drink water to compensate water loss that may alter intestinal physiology resulting in changes in physiochemical factors in the intestine, including gastric acidity, digestive enzymes, and bile salts (Usher et al. 1990). These changes are known to affect the intestinal colonization by specific microbes in teleosts (Ringø et al. 2003). Therefore, the changes in internal pH and osmolality could significantly affect the resident and transient intestinal microbes (Ray and Ringø 2014). In fact, previous research on anadromous and euryhaline fish species has shown that salinity could decrease the intestinal microbial richness and evenness and can cause changes in its composition (Zhang et al. 2016; Dehler et al. 2017a). The salinity gradient is an important factor that can shape fish microbial communities, with marked differences in the community composition in freshwater versus seawater fish (Dehler et al. 2017a). Furthermore, the environmental water and diets can also influence the intestinal microbial composition in fish. Hence, it could be predicted that freshwater and seawater fish have different intestinal microbiota due to different compositions in the environment (Herlemann et al. 2011) and, therefore, the fish intestinal microbiota and the respective environmental microbiota should have a similar composition. However, it is known that several bacterial species are specifically abundant in the intestine but not in the water (Schmidt

et al. 2015; Dehler et al. 2017b). This suggests that there is a complex mechanism for bacterial colonization of the fish intestine that responds to both temporal environmental changes and host physiology (Schmidt et al. 2015; Dehler et al. 2017a).

Regarding fish responses to environmental salinity changes, fish living in hyperosmotic environments are required to retain water and secrete ions against the concentration gradient (Ronkin et al. 2015). The regulatory mechanisms involve many processes and most of them take place in the gill, kidney, and intestine (Marshall and Grosell 2005). In the euryhaline species Mozambique tilapia (*Oreochromis mossambicus*), the ability to tolerate hypersaline water may be related to its capacity to upregulate ion exchange in the posterior intestine with significantly increased expressions of  $\text{Na}^+\text{-K}^+\text{-Cl}^-$  2 cotransporter (*nkcc2*),  $\text{Na}^+\text{-K}^+\text{-Cl}^-$  1 cotransporter (*nkcc1*), cystic fibrosis transmembrane regulator (*cftr*) and  $\text{Na}^+/\text{K}^+\text{-ATPase}$  (*nka*), which are involved in exchanging sodium, potassium, and chloride ions (Li et al. 2014). Another euryhaline fish, silver sea bream (*Sparus sarba*) acclimated to 33 psu also exhibited an increase of aquaporin 1 (*aqp1*) transcripts, regulating water and solute transport, when exposed to hyposaline (12 psu) and hypersaline (70 psu) environments (Deane et al. 2011). In striped catfish, osmoregulation is known to increase through upregulation of gill  $\text{Na}^+/\text{K}^+\text{-ATPase}$  activity when the fish are transferred from fresh water (0 psu) to seawater (20 psu) (Schmitz et al. 2016a). In the case of insufficient ionic regulation, salinity fluctuations can lead to osmotic stress in aquatic organisms, eventually causing disruption of protein synthesis and protein damage (Deane et al. 2002). During osmotic stress, the synthesis of heat shock protein (hsp) families increases remarkably; therefore, these proteins are considered to be a biomarker of stress in fish (Hightower 1991; Deane et al. 2011). For instance, hsp70 (heat shock protein 70) expression in gills of tilapia (*Sarotherodon melanotheron*) significantly increased with hyperosmotic stress. Additionally, hepatic expression of *hsp* genes, including *hsp60* (heat shock protein 60), *hsp70*, and *hsp90* (heat shock protein 90), were lowest in fish reared in isosmotic salinity and highest in those adapted to hypoosmotic and hyperosmotic salinities (Deane et al. 2002). However, not much is known about the physiological changes in the intestine during such osmotic changes.

The interactions between the host and intestinal microbiota play an important role in many aspects of physiology, metabolism, and immune homeostasis in fish (Rawls et al. 2004). Microbial colonization of intestinal mucosal surfaces results in positive effects on gut immune responses, while microbiota imbalance disrupts immunoregulatory functions and may lead to disease development (Pérez et al. 2010). Intestinal epithelial cells are capable of sensing components of the expression of pattern recognition receptors (PRRs), among which toll-like receptors can recognize bacterial lipopolysaccharides and other microbial molecules such as pathogen-associated

molecular patterns (Tlaskalova-Hogenova et al. 2005; Hörmannspurger and Haller 2010). The interaction between the receptor and the ligand activates the signaling molecules, resulting in an increase of solute mediators that recruit and regulate the immune and inflammatory responses (Fasano and Shea-Donohue 2005). In sea bass (*Dicentrarchus labrax*), a downregulation of intestinal inflammatory genes such as interleukin 1 beta (*il1 $\beta$* ), interleukin 10 (*il10*), transforming growth factor  $\beta$  (*tgf $\beta$* ) in fish fed with probiotics has been previously shown (Picchiatti et al. 2009). In contrast, another probiotic mix upregulated toll-like receptor 1 (*tlr1*), toll-like receptor 3 (*tlr3*), *il1 $\beta$* , tumor necrosis factor alpha (*tnf $\alpha$* ), *il10*, and *tgf $\beta$*  in the gut of zebrafish (*Danio rerio*) (Gioacchini et al. 2017). Collectively, these studies indicate that there is an alteration of the microbe composition that may lead to locally regulated host inflammatory responses.

In the present study, we investigated alterations in the intestinal microbiota as well as host gene expression in the intestine of striped catfish under different conditions of salinity.

## Materials and methods

### Experimental conditions

Juvenile striped catfish weighing  $24.04 \pm 1.02$  g/fish were collected from Viet-Uc hatchery, An Giang province, and acclimated in fresh water for 2 weeks before starting the experiment. The juveniles were distributed (40 fish/tank) into 5 experimental recirculating systems with triplicate for each treatment. The final salinities in the different treatments were 0, 5, 10, 15, and 20 psu, and the salinities were gradually increased at a rate of 0, 0.25, 0.5, 0.75, and 1 psu per day, respectively. The increase was done twice daily to reduce the shock to juveniles. Fish were fed ad libitum twice daily with commercial dry pellets (Aquaexcel 7434, Cargill, Vietnam), and the residual feed was removed after 30 min of feeding. All treatments reached the target salinities after 20 experimental days (D20). Then, the fish were continuously reared in the corresponding salinities for 2 weeks (until D34). Water quality parameters, including pH ( $7.6 \pm 0.2$ ), temperature ( $29.5 \pm 0.6$  °C), dissolved oxygen ( $6.7 \pm 0.5$  mg/l), nitrites ( $0.2 \pm 0.1$  mg/l), and total ammonia nitrogen ( $0.6 \pm 0.1$  mg/l) were measured and maintained at optimal levels for striped catfish during the experiment.

### Sampling

Three individual juveniles/tank were sampled at D20 and D34 (Supplementary Fig. S1). There were 5 treatments in D20, and 4 treatments in D34 were sampled because all fish

exposed 20 psu were dead in the end of the experiment. The fish were first euthanized with 200 mg/L MS-222 (ethyl 3-aminobenzoate methane sulfonate; Sigma, St. Louis, MO, USA). Then, the fish abdominal cavity was opened aseptically. The intestinal samples were separated and transferred to a cryotube containing RNAlater (prepared following the protocol detailed at <https://www.protocols.io/view/RNAlater-Recipe-c56y9d>). The tubes were stored at  $-80$  °C until further analysis.

### DNA extraction, preparation of 16S rRNA gene amplicon libraries (V3-V4 region), library quantification, and sequencing

DNA was extracted from the intestinal samples using the NucleoMag VET kit (Macherey–Nagel, Düren, Germany) according to the manufacturer's protocol. The DNA quantities were measured using the Qubit dsDNA BR Assay Kit (Life Technologies, Carlsbad, CA, USA).

Library preparation followed the standard Illumina (San Diego, CA, USA) 16S rRNA metagenomics sequencing protocol (15,044,223 Rev. B). The V3-V4 region of 16S rRNA was amplified using the primers 341F-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 785R-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC. These primers were shown to have a broad phyletic coverage (Klindworth et al. 2013). The PCR comprised 50  $\mu$ l reactions, including 10  $\mu$ l of 5  $\times$  KAPA HiFi (Kapa Biosystems, Woburn, USA), 1.5  $\mu$ l of 10 mM dNTP, 1.5  $\mu$ l of each forward and reverse primer (1  $\mu$ M), 1  $\mu$ l of MgCl<sub>2</sub> (2.5 mM), 1  $\mu$ l Kapa HiFi Hstart (Kapa Biosystems, Woburn, USA), 5  $\mu$ l of genomic DNA (50 ng), and 28.5  $\mu$ l of PCR grade water. Thermocycling conditions included an initial denaturation step at 95 °C for 3 min, then 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. A final extension step was conducted at 72 °C for 5 min. After the PCR, the products were subsequently purified using AmpPure XP beads (Beckman Coulter, High Wycombe, UK) and the concentration was checked using Qubit dsDNA BR Assay Kit. Then, a second PCR amplification was performed to attach dual indices and Illumina sequencing adapters using the Nextera XT Index kit (Illumina, San Diego, CA, USA). The reaction contained 14  $\mu$ l of DNA elution and PCR grade water, 2.5  $\mu$ l each of Nextera XT Index 1 Primer (N7XX) and Nextera XT Index 2 Primer (S5XX) (200 nM), 5  $\mu$ l of 5  $\times$  KAPA HiFi, 0.75  $\mu$ l of 10 mM dNTP, and 0.25  $\mu$ l of 2  $\times$  KAPA HiFi Hstart (0.5 U). The process included an initial denaturation step at 95 °C for 3 min, then 8 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension step at 72 °C for 5 min. AMPure XP beads were used to purify PCR products. The 16S rRNA amplicon libraries for each sample were quantified by a fluorometric

method using dsDNA binding dyes. Then, 5  $\mu$ L of the 4 nM DNA library pool was prepared for sequencing on the Illumina MiSeq platform. The library pool was denatured with 0.2 M NaOH and diluted with Pre-chilled HT1 buffer (Illumina, San Diego, CA, USA) to a final concentration of 4 pM. Sequencing was performed with a MiSeq Reagent Kit v3–600 cycle (Illumina, San Diego, CA, USA) following the manufacturer's protocols. Paired-end sequencing was performed on an Illumina MiSeq platform with 5% PhiX spike-in quality control using PhiX Control Kit v3 (Illumina, San Diego, CA, USA). Raw sequencing data from all samples can be openly accessed on NCBI database with BioProject accession number PRJNA802379.

## Data analysis

The data analysis was performed according to the protocol of Lokesh et al. (2019). The FastQ files were used as the input for the UPARSE pipeline (usearch version 11.0.667) (Edgar 2013). First, the forward and reverse reads were merged, with maximum mismatches of 10 and minimum ID percentage of 80%. The primer binding sites were trimmed, and the merged reads were truncated at 400 bp. The resulting sequences were then quality filtered with maximum expected error rate set to 1. Next, the dereplication was performed, and the reads with < 10 sequences were discarded. OTUs (operational taxonomic units) were clustered at a threshold of 97% similarity level and chimeric sequences were discarded. After clustering, the raw reads were mapped to OTUs by searching the reads as queries against the OTU representative sequences. Taxonomic ranks were assigned to OTUs using the SINTAX algorithm using Ribosome Database Project database downloaded at the link of [https://drive5.com/syntax/rdp\\_16s\\_v18.fa.gz](https://drive5.com/syntax/rdp_16s_v18.fa.gz) (rdp\_16s sequences, version v18). Taxonomic assignments with scores < 0.5 were not considered in the downstream analyses.

Alpha and beta diversities were computed using QIIME2 (Hall and Beiko 2018). Then, one-way ANOVA followed by Tukey's post hoc tests were used to statistically compare the treatments on the same sampling day and an independent *t*-test applying a 95% confidence interval was used for the treatments at the same salinity levels from different sampling days in SPSS 20 software (IBM Corporation, Armond, NY, USA). Appropriate transformation was performed when the assumptions of normality and equal variance were not met. Beta diversity was assessed based on the Bray–Curtis distance. Pairwise comparisons between treatments were performed using ANOSIM in QIIME2. The results of Bray–Curtis distance and relative abundance were plotted using R package (version 4.0.5) (Team RC 2013) and phyloseq (version 1.34.0) (McMurdie and Holmes 2013). The significantly abundant OTUs were calculated using Linear Discriminant Analysis Effect Size (LEfSe) (Segata et al.

2011) on Galaxy (version 1.0) with *p*-value set at 0.05 and LDA log score threshold at 3.5. The LEfSe results were plotted using GraPhlAn (version 1.1.3) (Asnicar et al. 2015). To predict the metagenomes of each sample, the OTU table was used in PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States2) version 2.4.1 with the maximum value of Nearest Sequenced Taxon Index (NSTI) of 0.03 (Douglas et al. 2020). The generated tree was complemented with OTU resulting in hidden-state prediction (hsp) (Louca and Doebeli 2018) using the maximum-parsimony method, generating predicted Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology. The abundance profiles of KEGG orthologs were statistically analysed by LEfSe with *p*-value set at 0.05 and LDA log score threshold set at 2.5.

## RNA extraction and qPCR

Intestinal RNA was extracted using Tri-Reagent solution (Ambion, ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The RNA concentration and quality were determined by NanoDrop-2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) and after migration on agarose gel (1%), respectively. Genomic DNA contamination was removed by digestion using rDNase I (Thermo Fischer Scientific, Waltham, MA, USA). Then, 1  $\mu$ g of total RNA was reverse-transcribed using RevertAid RT kit (Thermo Fischer Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The cDNA samples were diluted 1:25 and used to determine gene expressions of *nka1a* (Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 1a); *nka1b* (Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 1b), *slc12a1* (solute carrier protein family protein 12 group A, member 1); *slc12a2* (solute carrier protein family protein 12 group A, member 2); *cfr*, *aqp1*, *hsp70*, *hsp90*, *hsp60*, *tlr1*, and *tlr2* (toll-like receptor 2); *il1 $\beta$* , *il10*, and *tnfa* (tumour necrosis factor alpha); and *tgfb* and *alpl* (alkaline phosphatase) by quantitative PCR (qPCR). The reactions were performed using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA) on QuantStudio 5 (Applied Biosystems, Waltham, MA, USA). The thermal conditions were set at 95 °C for 3 min in the initial denaturation step, followed by 40 cycles at 95 °C for 30 s and 60 °C for 30 s. The primers used in the present study were designed using Primer-BLAST (Ye et al. 2012) from the striped catfish sequence published on GenBank. The efficiency of each gene was tested before analysing. The details concerning the primers are given in Supplementary Table S1. For analysis, sample value and normalization were determined using the relative expression ratio mathematical model (Pfaffl 2001). Briefly, Ct values are transformed according to the equation: Ratio (fold change) =  $E_{\text{target}}^{\Delta C_t(\text{control}-\text{sample})} / E_{\text{reference}}^{\Delta C_t(\text{control}-\text{sample})}$ , where  $E_{\text{target}}$  is the qPCR efficiency of the target gene and

$E_{reference}$  is the qPCR efficiency of the reference gene (beta actin *actb*) (Pfaffl 2001). To determine the qPCR efficiency, the serial dilution of pooled cDNA from all samples was included in the qPCR reaction. Statistical comparisons were carried out by one-way ANOVA, followed by Tukey’s post hoc test between treatments on the same sampling day and an independent *t*-test applying a 95% confidence interval was used for the treatments at the same salinity levels in different sampling days in SPSS 20 (IBM Corporation, Armond, NY, USA). Appropriate transformation was performed when the assumptions of normality and equal variance were not met.

## Results

### Differences in the intestinal microbial diversity and composition

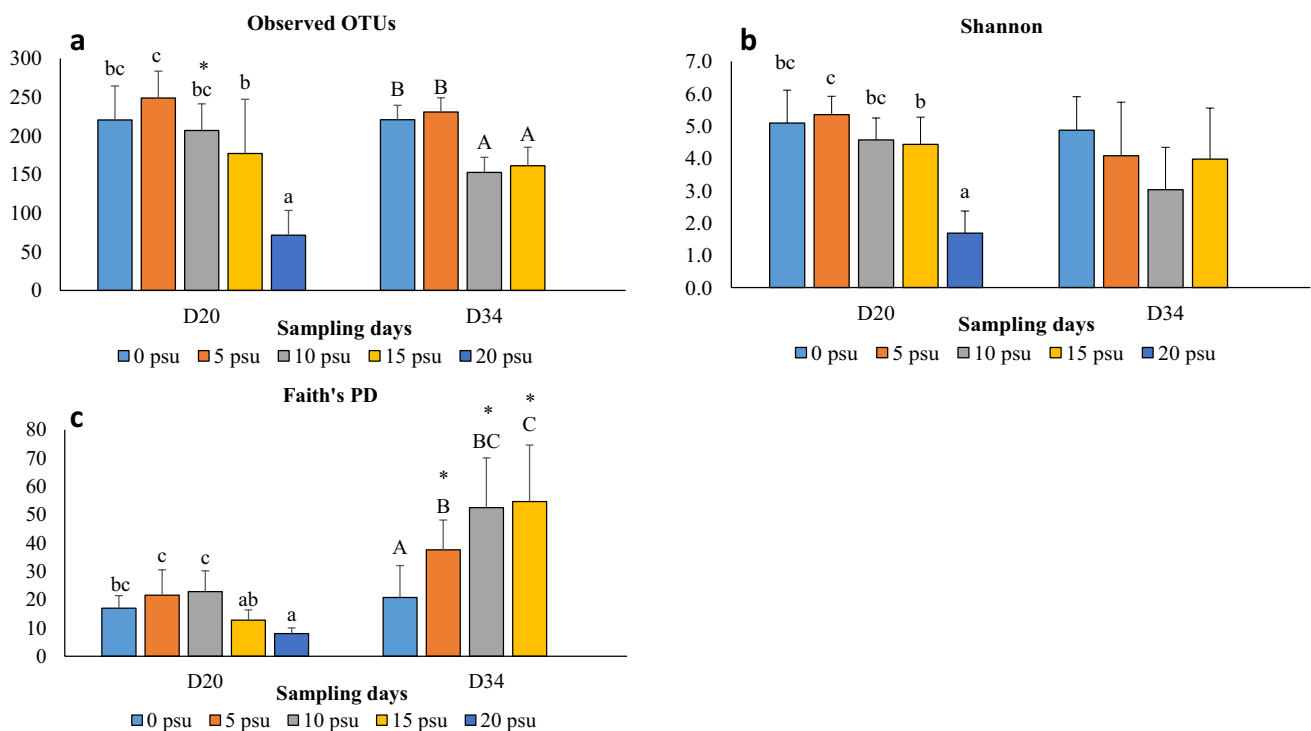
#### Alpha diversity

The intestinal microbiota was clustered in 939 OTUs. Alpha diversity, including observed OTUs, Shannon index, and Faith’s PD (phylogenetic tree) of the intestinal microbiota had a decreasing pattern with the increase in salinity at D20. Notably, the highest values were expressed in the 5 psu

(observed OTUs and Shannon index) and 10 psu (Faith’s PD) treatments, which were significantly different compared to the communities of the 15 psu and 20 psu treatments at D20 ( $p < 0.05$ ) (Fig. 1). The richness at D34 decreased significantly ( $p < 0.05$ ) in fish exposed to 10 psu compared to the same salinity at D20 (Fig. 1a). Although Shannon index decreased similarly at 5 psu and 10 psu, the changes were not statistically different (Fig. 1b). Interestingly, there was a significant increase in Faith’s PD in saline treatments at D34 compared to the corresponding treatments at D20 ( $p < 0.05$ ) (Fig. 1c).

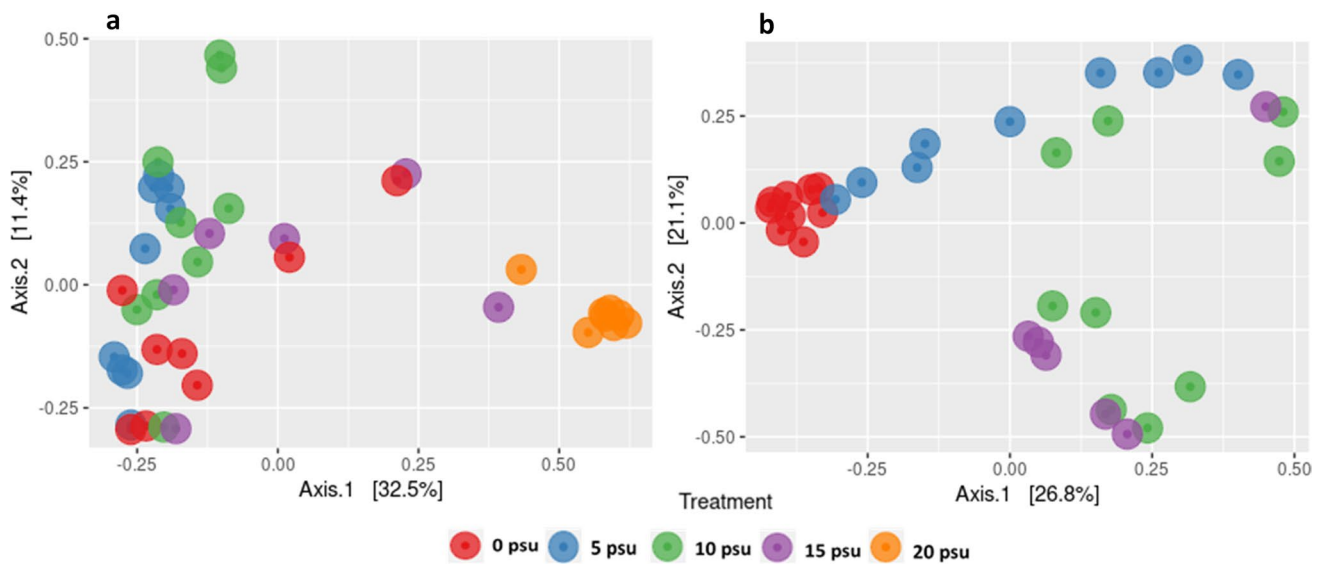
#### Beta diversity

To visualize the intestinal microbiota impaired under different salinity conditions, a Bray–Curtis similarity-based ordination analysis (PCoA) was performed. The ordination at D20 showed that the samples in 20 psu were completely separated from those in the other treatments (Fig. 2a). This was confirmed with the  $p$ -value  $< 0.01$  and  $R$ -value  $> 0.7$  (ANOSIM) (Table 1). Furthermore, the pairwise comparison between 15 psu and the other treatments showed similar patterns, with the most significant difference found between 5 and 15 psu with a  $p$ -value of 0.001 and an  $R$ -value of 0.655. The remaining pairwise comparisons varied between



**Fig. 1** Alpha diversity in the intestinal microbiota of striped catfish exposed to different levels of salinity. **a** Observed operational taxonomic units (OTUs), **b** Shannon index, and **c** Faith’s PD (phylogenetic diversity). Results are given as mean  $\pm$  SD. Different lower-case and upper-case let-

ters denote statistically significant differences between treatments at D20 and D34, respectively ( $p < 0.05$ ). Asterisks denote significantly higher values in the treatment compared to the corresponding values on the other sampling days ( $p < 0.05$ )



**Fig. 2** Principal coordinate analysis (PCoA) plot based on Bray–Curtis distance similarities in the intestinal microbiota of striped catfish exposed to different salinity levels. **a** Treatments plotted at D20. **b** Plot of treatments at D34

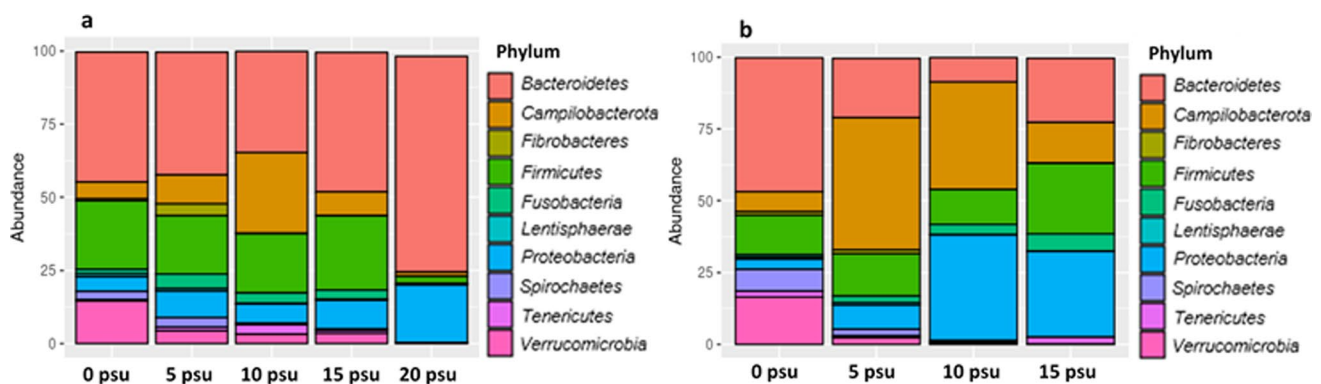
**Table 1** Pairwise ANOSIM test based on Bray–Curtis dissimilarity in intestinal microbiota of striped catfish juveniles at D20 and D34

Pairwise comparison	D20		D34	
	<i>p</i> value	<i>R</i> value	<i>p</i> value	<i>R</i> value
0 ppt vs 5ppt	0.003	0.338	0.001	0.632
0 ppt vs 10 ppt	0.002	0.428	0.001	0.856
0 ppt vs 15 ppt	0.008	0.337	0.001	0.938
0 ppt vs 20 ppt	0.001	0.924		
5 ppt vs 10 ppt	0.001	0.462	0.004	0.313
5 ppt vs 15 ppt	0.001	0.655	0.001	0.723
5 ppt vs 20 ppt	0.001	1		
10 ppt vs 15 ppt	0.009	0.323	0.157	0.098
10 ppt vs 20 ppt	0.001	1		
15 ppt vs 20 ppt	0.001	0.786		

0.001 and 0.009 for the *p*-value, and 0.323 and 0.462 for the *R*-value. At D34, there were significant differences in all pairwise comparisons ( $p < 0.01$  and  $R > 0.6$ ), except between the 5 and 10 psu treatments and 10 and 15 psu (Fig. 2b). The differences in beta diversities under each salinity treatment between two different sampling points (D20 and D34) were significant in all groups, except for the groups with 0 psu (Supplementary Fig. S2 and Supplementary Table S2).

### Relative abundance of the prominent taxa in the intestinal microbiota of striped catfish

It is clear that *Bacteroidetes* was the dominant phylum in all treatment groups at D20 (Fig. 3) with the highest abundance (73.7%) in the 20 psu treatment. This was followed



**Fig. 3** Mean relative abundance of the top 10 bacterial phyla in striped catfish intestinal microbiota at D20 (**a**) and D34 (**b**)

by *Firmicutes* (23.3%) and then *Verrucomicrobia* (14.7%) in the freshwater treatment. *Firmicutes* was also the predominant phylum in fish exposed to 5, 10, and 15 psu with 20.0, 20.4 and 25.4%, respectively. *Campilobacterota* increased significantly up to 27.5% in the 10 psu group. In addition, a gradual decrease in *Verrucomicrobia* was observed with increasing salinity, from 14.7% in fresh water (0 psu) to 1% at 20 psu. In the 20 psu treatment group, *Proteobacteria* (20%) were the most abundant after *Bacteroidetes* (73.7%) (Fig. 3a). At D34, there was a significant increase in the *Campilobacterota* phylum in the 5 and 10 psu groups (46.0% and 37.4%, respectively), which became one of the most abundant phyla in the intestinal microbiota. In addition, the proportion of *Proteobacteria* as the predominant bacteria in the gut microbiota changed in fish treated with 10 and 15 psu, at 38.8% and 30%, respectively (Fig. 3b).

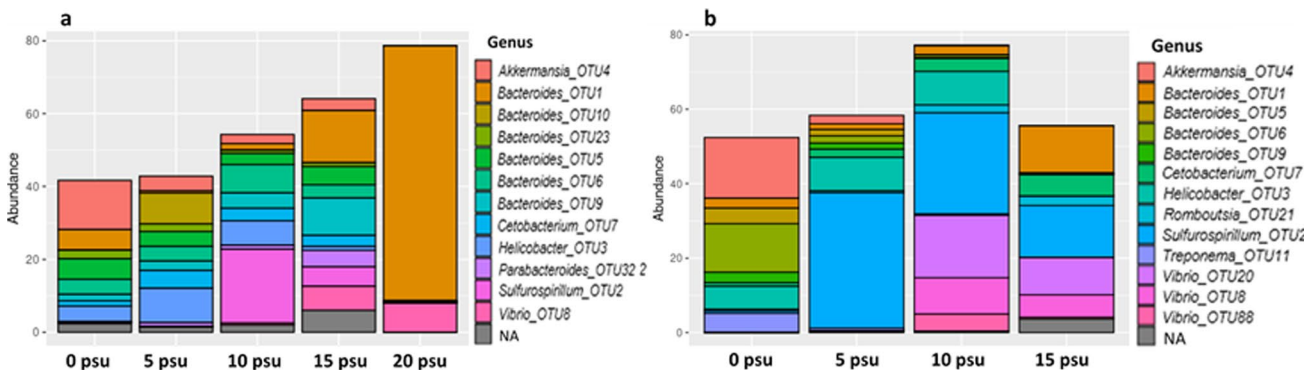
At D20, *Akkermansia* was the most dominant genus in the gut microbiota of fish kept in freshwater conditions (13.4%) (Fig. 4a), followed by several different *Bacteroides* species, *Helicobacter* (4.2%), and *Cetobacterium* (1.5%). In fish exposed to 5 psu, *Helicobacter* was the most abundant genus (9.5%), followed by several *Bacteroides* species and *Cetobacterium* (4.9%). Under 10 psu, *Sulfurospirillum* accounted for 20.4% and was the predominant genus in the intestinal microbiota. When treated with 15 psu, the *Bacteroides* species were still predominant in the microbiota, but a significant increase in *Vibrio* and *Parabacteroides* was observed up to 6.7 and 5.4%, respectively. The most notable change in the 20 psu treatment was that the *Bacteroides\_OTU1* accounted for the greatest proportion of the microbiota at 70%, followed by the *Vibrio* genus (8%). The fish at D34 had a similar diversity of gut microbiota as those at D20, except for the increase in the genus *Treponema* (5.3%). *Sulfurospirillum* was the prominent genus in fish in 5 psu, accounting for over 36% of the community, followed by *Helicobacter* at 9.1%. In the 10 and 15 psu treatments, the genera *Sulfurospirillum* and *Vibrio* dominated (27.1 and 14.0%, respectively). In the

15 psu group, *Bacteroides\_OTU1* was dominant (12.7%). In addition, the proportion of the genera *Cetobacterium* (3.5 and 5.7% at 10 and 15 psu, respectively) and *Romboutsia* (2.1 and 2.5% at 10 and 15 psu, respectively) increased with salinity (Fig. 4b).

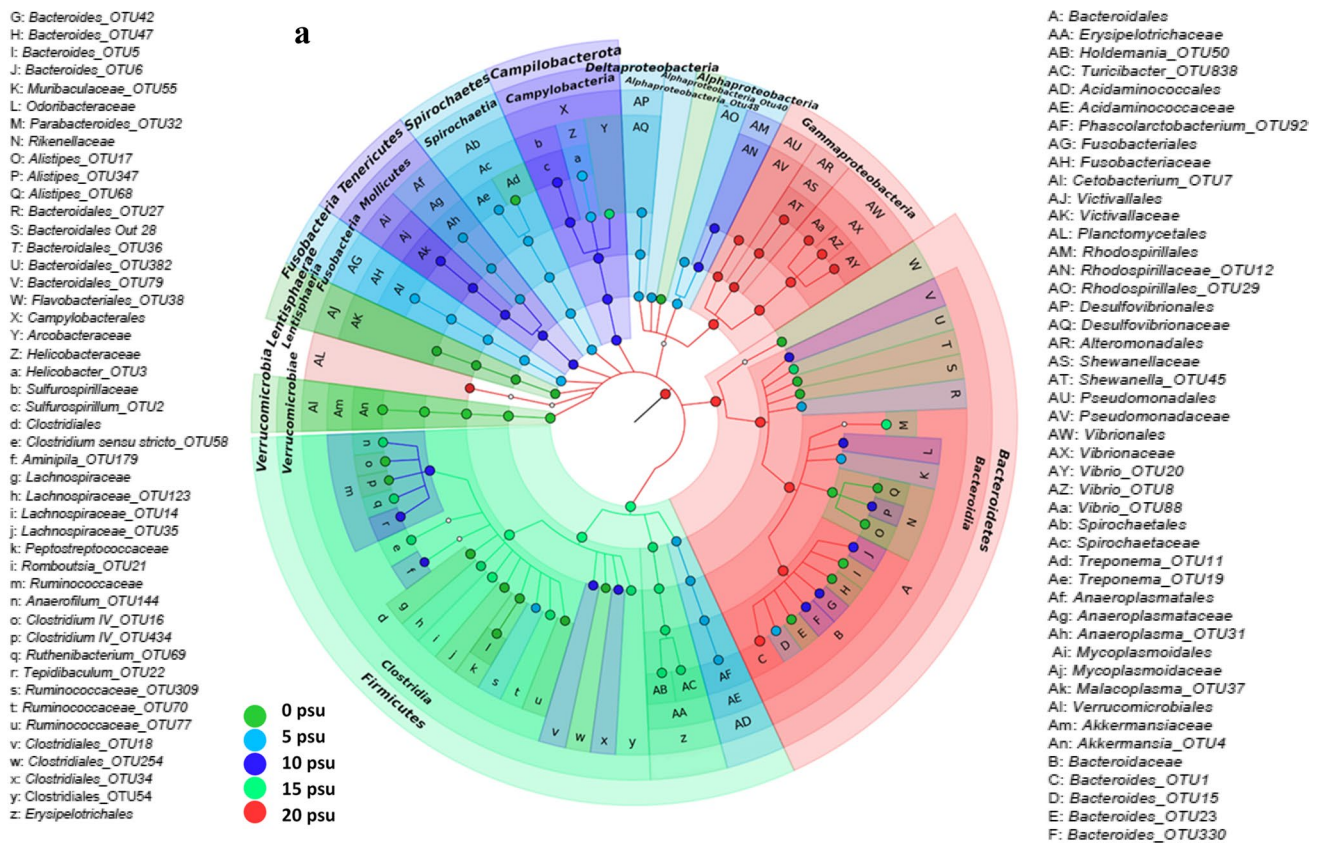
### Differential abundance of the OTUs in intestinal microbiota of striped catfish

LEfSe was used to determine the intestinal biomarkers (OTUs) in striped catfish exposed to different levels of salinity (Fig. 5a). At the phylum level, *Verrucomicrobia*, mainly reflecting the abundance of OTUs of the genera *Akkermansia* and *Lentisphaerae*, were biomarkers in the 0 psu treatment. In addition, *Fusobacter*, mainly represented by the genus *Cetobacterium*, were differentially abundant in the 5 psu treatment, while *Tenericutes* and *Campilobacterota* were biomarkers in 10 psu. The latter was mainly comprised of the genera *Sulfurospirillum* and *Helicobacter*. The abundance of *Treponema* genus, belonging to the *Spirochaetes* phylum, was significantly higher in the 0 and 5 psu treatments than for the other treatments. The 15 psu treatment was significantly enriched in the *Firmicutes* phylum, reflecting the abundance of the *Clostridia* class, although the lower taxonomic levels shared significant abundance in all treatments except 20 psu. The *Bacteroidia* clade, which belongs to the *Bacteroidetes* phylum, was significantly abundant in the 20 psu treatment, but the *Bacteroides* genus shared high abundances in all the different salinities. Moreover, the most significant abundance of *Vibrio* genus belonging to the class *Gammaproteobacteria* were found in the 20 psu treatment, indicating that the genus is a biomarker in fish subject to a saline environment.

At D34, the phyla *Bacteroidetes*, *Verrucomicrobia*, and *Spirochaetes*, reflecting the abundance of the genera *Bacteroides*, *Akkermansia*, and *Treponema*, respectively,



**Fig. 4** The mean relative abundance of the intestinal microbiota of striped catfish at the genus level at D20 (a) and D34 (b). Only the OTUs present at an abundance greater than 1% were retained



**Fig. 5** Cladogram showing differentially abundant taxa in the intestinal microbiota of striped catfish at D20 (a) and D34 (b) for the various salinity conditions. Linear discriminant analysis effect size

(LEfSe) was used to validate the statistical significance and the effect size of the different abundances of taxa in the treatments compared to the control

were significantly more abundant in the freshwater group (Fig. 5b). In the 5 psu treatment, the phyla *Letisphaerae* and *Campylobacterota* had the highest abundances, the latter being reflected in the highly abundant genera *Sulfurospirillum* and *Helicobacter*. Although the phylum *Proteobacteria* was a biomarker in the 10 psu treatment, it reflected only the *Gammaproteobacteria* class with the representation of the *Vibrio* genus. The point of interest in the 15 psu treatment was the high abundance of *Bacteroides\_OTU 1*.

### Presumptive metabolic potential of the intestinal microbiota of striped catfish

The OTU table was used as the input file for PICRUSt2, and accuracy was calculated using the NSTI value. The results according to LEfSe showed that there were 20 significantly different abundances of KEGG pathways at level 2 in which the treatment of 20 psu had the highest number (9) of significantly enriched pathways, such as carbohydrate metabolism, lipid metabolism, cell motility, and membrane transport (Table 2). This was followed by 6, 4, and 1 enriched pathways at 0, 5 and 15 psu, respectively, at D20. At D34, the

0 psu treatments had the highest number of enriched pathways with 9 of 18 total pathways, while the 5, 10, and 15 psu treatments were significantly abundant with 5, 3, and 1 pathways, respectively.

### Gene expression

#### Expression of genes related to osmoregulation

The expression of *nk1a* and *nk1b* showed a significant increase with salinity at D20 and D34. In addition, a remarkable increase in mRNA levels of *nk1b* was observed after fish were maintained at the targeted salinities of 5, 10, and 15 psu for two weeks (Fig. 6a, b). A similar pattern was observed for the solute carrier family genes *slc12a1* and *slc12a2*, which encode *nkcc2* and *nkcc1*, respectively (Fig. 6c, d). Notably, the mRNA level of *slc12a2* was the lowest at 5 and 10 psu, whereas it was the highest at 20 psu at D20 (Fig. 6d). Moreover, *slc12a1* showed a gradual increase with salinity at D20 (Fig. 6c). At D34, the expression of both genes was significantly higher in the 15 psu group than in the other groups. Fish



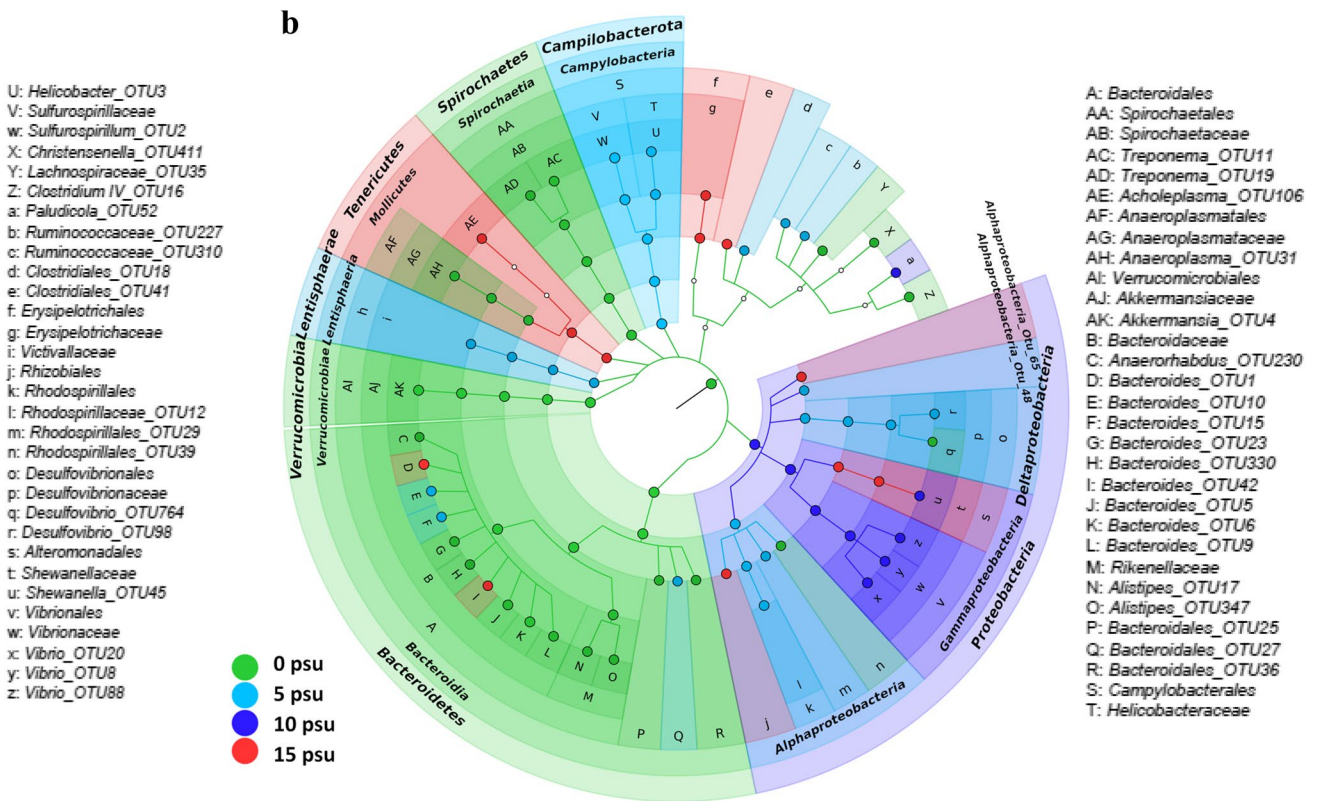


Fig. 5 (continued)

exposed to salinity of 5, 10, and 15 psu at D34 had significantly higher mRNA levels for these genes than the corresponding fish at D20. The transcripts of *nka1a*, *nka1b*, *slc12a1*, and *slc12a2* were lowest on D20 at 5 psu compared to the other conditions. With respect to cystic fibrosis transmembrane conductance regulator (*cftr*) and aquaporin 1 (*aqp1*), the highest levels of the genes were observed in the 0 psu treatment and the lowest in the 20 psu treatment at D20. Similarly, the mRNA levels of *cftr* and *aqp1* were significantly higher in fish exposed to fresh water than in fish exposed to 10 and 15 psu (Fig. 6e, f). Notably, both gene expressions significantly decreased on D34 compared to D20 when treated with 10 psu.

**Heat shock protein family**

The highest expression of heat shock protein 70 (*hsp70*) was found in the 15 and 20 psu groups, and was significantly different from that of the freshwater group at D20. However, the changes recovered after 2 weeks of exposure to salinity (Fig. 7a). A similar pattern was observed in the mRNA levels of heat shock protein 90 and 60 at D20, where there was a significant increase with salinity. Moreover, the increased responses were prolonged until D34 when treated with

15 psu (Fig. 7b, c). In general, salinity greater than 10 psu had a significant effect on the expression of *hsp* genes in the initial period of exposure and these responses decreased with exposure time.

**Intestinal pattern recognition receptor and its associated pathways (TLRs)**

In general, the expression of genes involved in PRRs, such as *tlr1*, *tlr2*, *il1β*, *tnfa*, *il10*, and *tgfβ*, showed the lowest expression in fish exposed to 20 psu at D20 (Fig. 8). Notably, the expression of *tlr1* was significantly lower in fish exposed to 20 psu than that in the lower salinity levels, while the statistical significance of *tlr2* was found only between the treatments of 5 psu and 20 psu ( $p < 0.05$ ) (Fig. 8a, b). Pro-inflammatory genes such as *il1β* and *tnfa* were remarkably downregulated in the 20 psu treatment compared to the others ( $p < 0.05$ ) (Fig. 8c, d). Moreover, *il1β* expression was significantly lower in fish exposed to 15 psu compared to the others at D34. For anti-inflammatory responses, *tgfβ* transcripts were the lowest at 20 psu and significantly different from 0, 5, and 10 psu treatments. For *il10* expression, there was no statistical

**Table 2** Differentially abundant presumptive functions in level 2 of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of the intestinal microbiota of striped catfish exposed to different salinities. PICRUSt2 was used to predict the metagenome and the significantly abundant pathways were identified with the help of LEfSe

No	KEGG pathway in level 2	D20			D34		
		Group	LDA	<i>p</i> value	Group	LDA	<i>p</i> value
1	Transcription	0 psu	4.027	0.000	0 psu	4.062	0.000
2	Folding, sorting and degradation	0 psu	3.843	0.002	0 psu	3.801	0.001
3	Infectious disease bacterial	0 psu	3.743	0.030			
4	Amino acid metabolism	0 psu	3.759	0.034	0 psu	3.852	0.014
5	Translation	0 psu	3.949	0.000	0 psu	4.165	0.000
6	Glycan biosynthesis and metabolism	0 psu	4.176	0.000	0 psu	4.154	0.001
7	Immune system				0 psu	3.419	0.006
8	Digestive system				0 psu	3.672	0.001
9	Endocrine system				0 psu	3.753	0.001
10	Nucleotide metabolism	5 psu	4.084	0.000	0 psu	3.988	0.001
11	Xenobiotics biodegradation and metabolism	5 psu	3.962	0.029	5 psu	3.922	0.044
12	Metabolism of cofactors and vitamins	5 psu	4.071	0.000	5 psu	3.942	0.014
13	Metabolism of terpenoids and polyketides	5 psu	4.883	0.000	5 psu	4.639	0.001
14	Biosynthesis of other secondary metabolites	15 psu	3.413	0.015			
15	Cellular community prokaryotes	20 psu	4.400	0.000	10 psu	4.413	0.000
16	Environmental adaptation	20 psu	3.781	0.000	10 psu	3.805	0.000
17	Carbohydrate metabolism	20 psu	4.039	0.003			
18	Cell motility	20 psu	4.724	0.000	10 psu	4.598	0.000
19	Lipid metabolism	20 psu	3.964	0.001	10 psu	3.862	0.001
20	Replication and repair	20 psu	3.767	0.001			
21	Transport and catabolism	20 psu	3.721	0.005			
21	Membrane transport	20 psu	4.130	0.000	10 psu	3.979	0.001
23	Metabolism of other amino acids	20 psu	4.128	0.000	15 psu	3.995	0.002

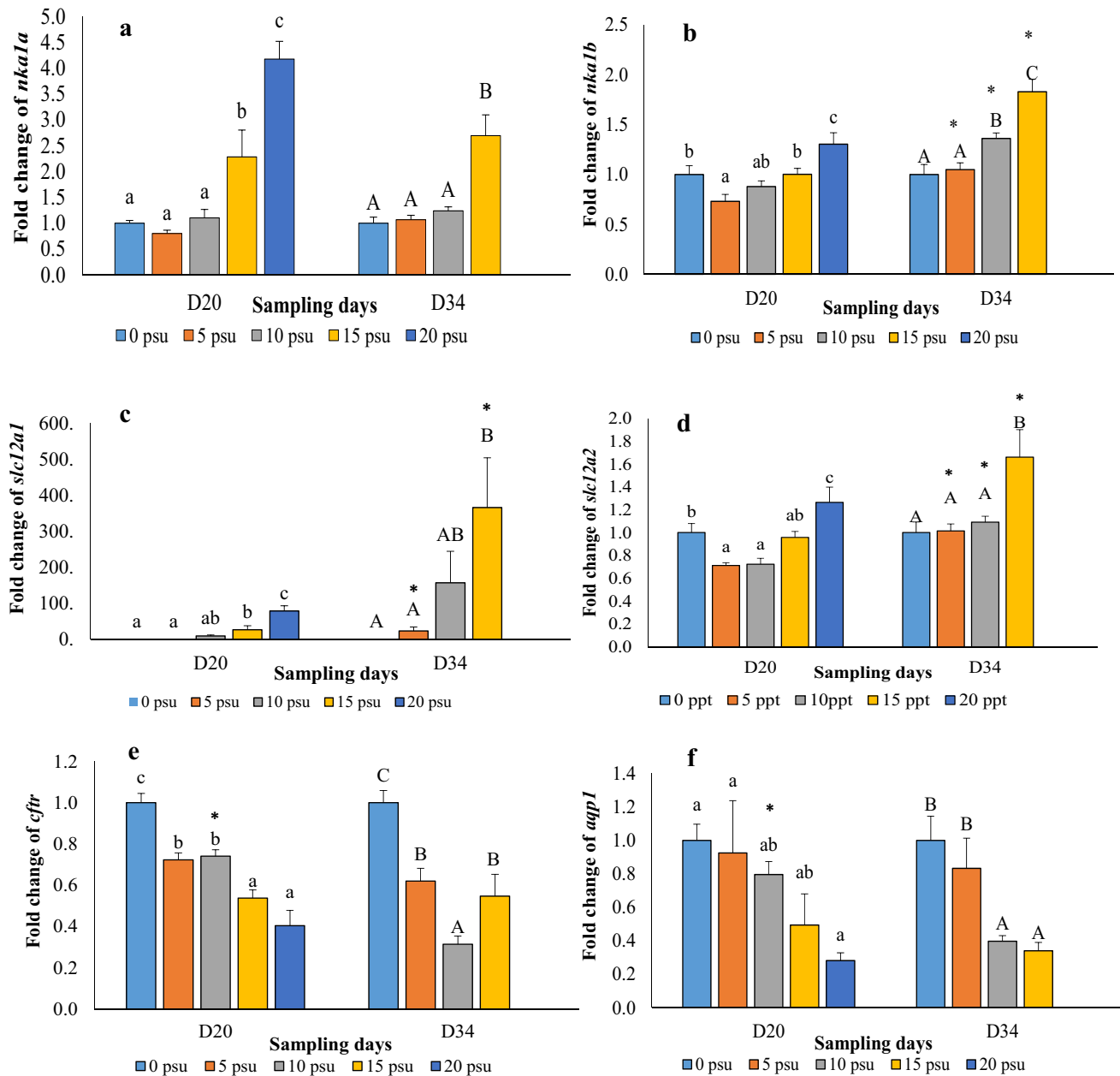
difference between treatments at D20, although it was the lowest in the 20 psu treatment, whereas at D34, it was highest in the freshwater treatment and significantly different compared to that of 10 and 15 psu (Fig. 8e, f). Moreover, *il10* gene expression was significantly decreased in the 10 psu treatment after 2 weeks of exposure to salinity. The expression of a gene related to lipopolysaccharide detoxification, alkaline phosphatase, was examined (Fig. 8g). It showed a gradual increase with the salinity gradient, and was statistically higher in the 20 psu treatment than in the 0, 5, and 10 psu treatments. There were no remarkable differences in the expression of this gene after two weeks of exposure to salinity.

## Discussion

### Fish intestine responds to changes in the osmotic conditions

In teleost fishes, the gut plays an important role in water balance in marine environments but its role in osmoregulation is less important in freshwater fish, which are hyperosmotic to the environment (Takei and Hwang 2016). Entry into a

saline environment results in a 10- to 50-fold increase in drinking volume to compensate for water losses in fish (Grosell 2006). The oesophageal-gastrointestinal tract absorbs more than 95% of the NaCl in seawater during ingestion and this absorption facilitates water absorption (Grosell, 2010). The major components of the absorption are basolateral Na<sup>+</sup>/K<sup>+</sup>-ATPase and apical Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> (Grosell 2006). In the present study, the genes involved in ion transporters, especially *nka1a* and *slc12a1* were generally upregulated at salt levels of 15 and 20 psu for short-term exposure (D20) and even at 10 psu for long-term exposure (D34). The genes *nka1a* and *nka1b* are two similar paralogues of *nka1* that encode the  $\alpha$ -subunit of Na<sup>+</sup>/K<sup>+</sup>-ATPase (Lind et al. 2013). Na<sup>+</sup>/K<sup>+</sup>-ATPase, known to be an active pump, represents the primary driving force for activation of many ion transport systems in osmoregulatory organs, including the intestinal tract in teleost fish (Yang et al. 2018), and its upregulated expression indicates a higher need for ion uptake or excretion (Lin et al. 2003; Yang et al. 2009; Whitamore 2012). Also, *slc12a1* coding *nkcc2* plays a dominant role in intestinal NaCl absorption in euryhaline fish (Takei 2021). The upregulation of *nka1* and *slc12a1* was also found in a study of the euryhaline fish Mozambique tilapia and sea bream when transferred from hypo- to hyperosmotic environments (Gregório et al. 2013; Li et al. 2014). Salinities

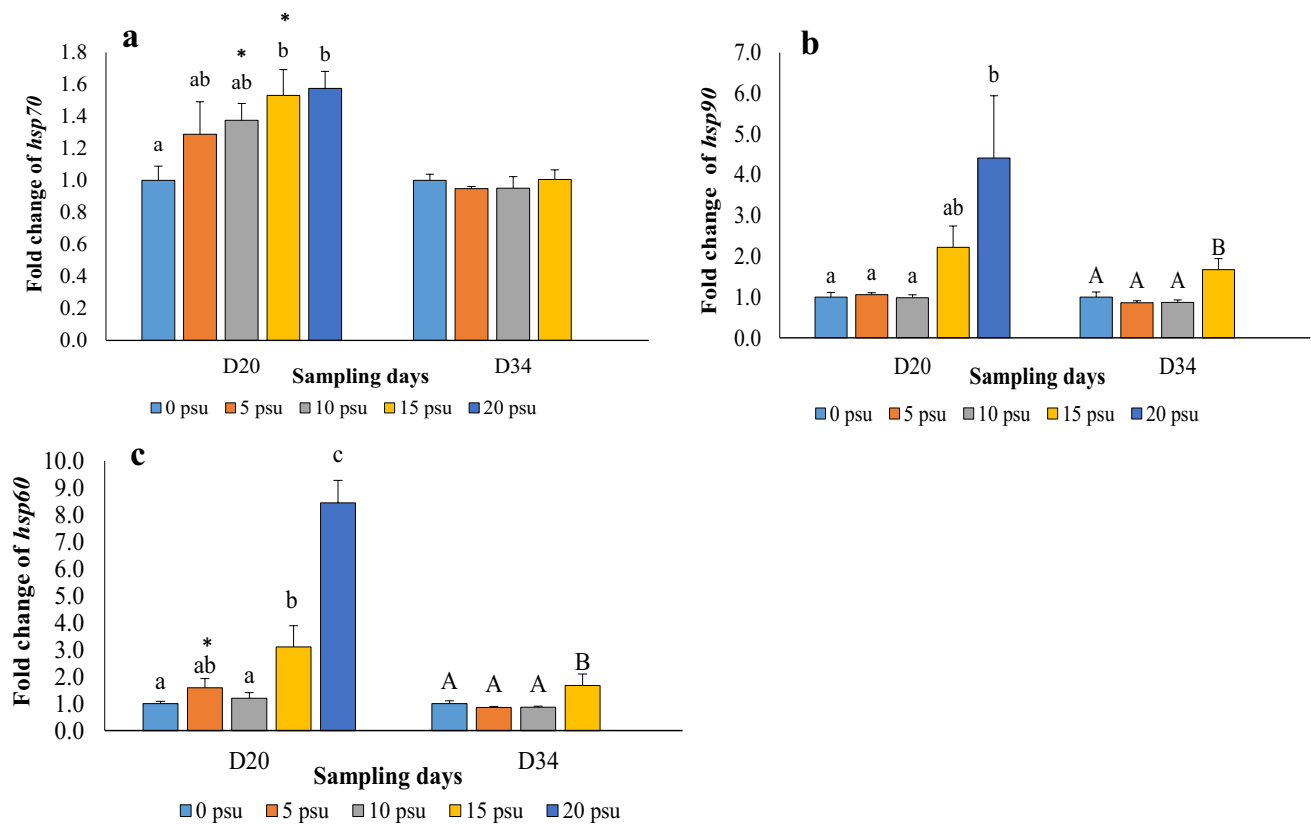


**Fig. 6** Fold changes in the expression of genes related to osmoregulation in the intestine of striped catfish exposed to different salinity levels. **a** *nka1a*, **b** *nka1b*, **c** *slc12a1*, **d** *slc12a2*, **e** *cftr*, and **f** *aqp1*. Results are expressed as mean ± SEM. Different lower-case and

upper-case letters denote statistically significant differences between treatments at D20 and D34, respectively ( $p < 0.05$ ). Asterisks denote significantly higher values in the treatment compared to the corresponding values on the other sampling day ( $p < 0.05$ )

between freshwater and 10 psu generally did not upregulate the expressions of these osmoregulatory genes, even fish subjected to 5 and 10 psu showed a significant decrease in *slc12a2* expressions compared to that in freshwater in the early exposure. The regulations indicate that fish may have a tendency to reduce ion exchange salinities close to their isosmotic point which is estimated to be 9 psu in striped catfish (Nguyen et al. 2014). In mammals,  $Cl^-$  secretion is regulated by *cftr* on the apical membrane and by *nkcc1*

on the basolateral membrane (Takei 2021). In the present study, the upregulated expression of *slc12a2* in the highest salinity treatment, i.e., 20 psu at D20 and 15 psu at D34, may demonstrate their effective activities in  $Cl^-$  regulation; however, *cftr* expression showed downregulation. A decline in *cftr* transcript expression during seawater adaption was also found in the intestine of killifish (*Fundulus heteroclitus*) and European eel (*Anguilla anguilla*) (Marshall and Singer 2002; Takei 2021), while the opposite pattern was



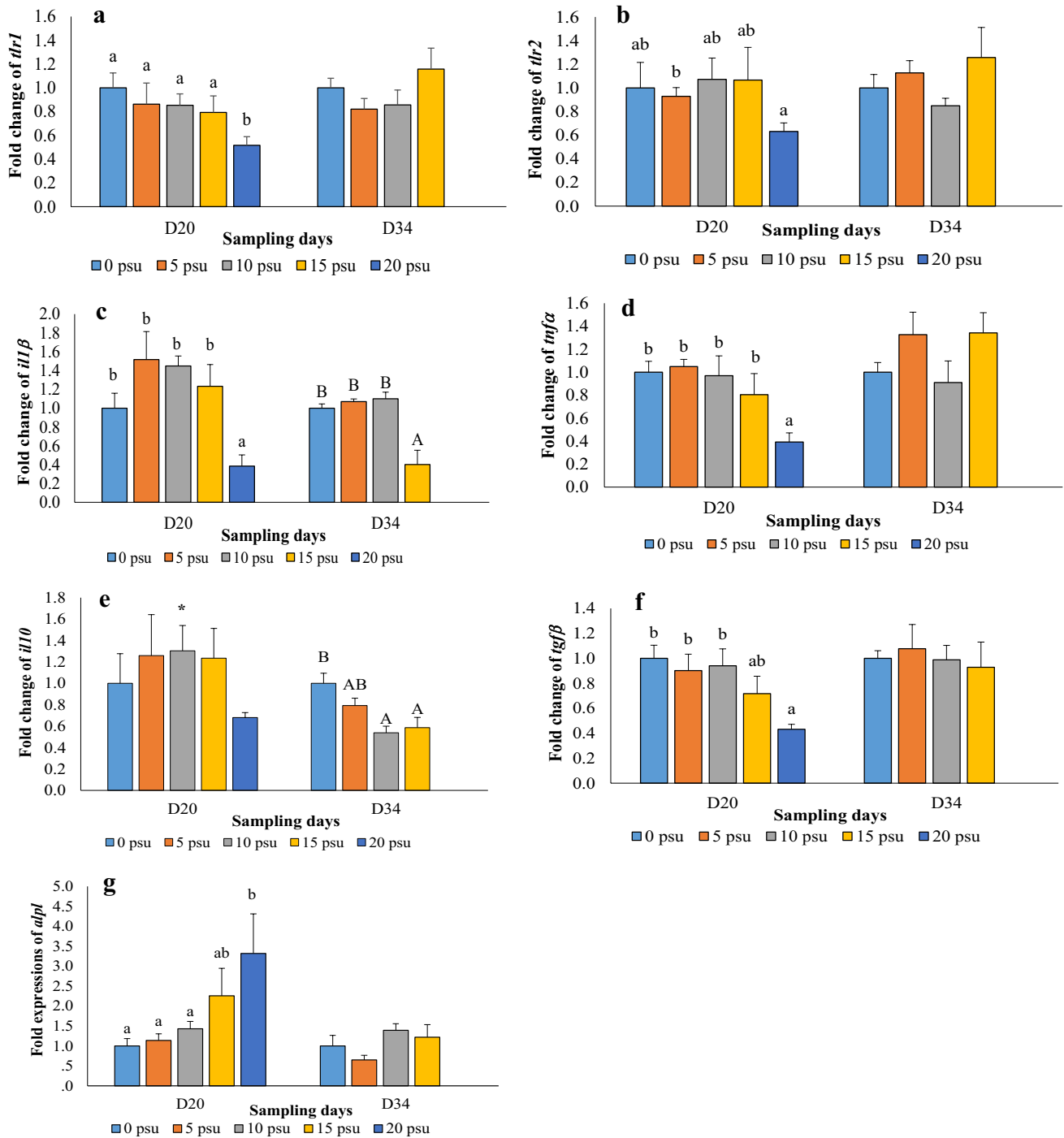
**Fig. 7** Fold change expression of genes in the heat shock protein family in the intestine of striped catfish exposed to different salinities. **a** *hsp70*, **b** *hsp90*, and **c** *hsp60*. Results are given as mean  $\pm$  SEM. Different lower-case and upper-case letters denote statistically sig-

nificant differences between treatments on D20 and D34, respectively ( $p < 0.05$ ). Asterisks denote significantly higher values in the treatment compared to the corresponding values on the other sampling days ( $p < 0.05$ )

found in different euryhaline fish species (Li et al. 2014). The downregulated expression is possibly explained by the fact that the CFTR of striped catfish intestine is localized in the basolateral membrane for  $\text{Cl}^-$  absorption, as suggested in previous research (Marshall and Singer 2002; Takei 2021). The transport of NaCl parallels that of water, moving from the intestinal lumen into the body fluid through the epithelium when luminal fluid is almost isotonic to the body fluid (Li et al. 2005). Water absorption by transcellular passage is greatly facilitated by aquaporins (AQPs) which showed an upregulated expression in European and Japanese eels (*Anguilla japonica*) acclimated to seawater (Aoki et al. 2003; Martinez et al. 2005). However, the expression of *aqp1* decreased when striped catfish were exposed to salinity in the current study. A similar result was found in medaka (*Oryzias latipes*), which may be explained by the movement of AQP1a from the apical membrane to the subapical area and the possible transport of water via paracellular routes after fish enter seawater (Madsen et al. 2014). Additionally, the duration of exposure to salinity also affected the osmoregulation process in the gut. In general, these genes were upregulated in fish exposed to increased salinity in

a manner proportional to the exposure time. Similar findings were reported in Sacramento splittail (*Pogonichthys macrolepid*) and killifish, showing an increase of  $\text{Na}^+/\text{K}^+$ -ATPase activity during seawater acclimation periods (Scott et al. 2008; Mundy et al. 2020). These results confirm the osmoregulatory ability of the intestinal tract in striped catfish during saline exposure through differential regulation of  $\text{Na}^+/\text{K}^+$ -ATPase activity. However, the exact mechanism of changes in  $\text{Cl}^-$  secretion and water absorption in striped catfish under the influence of salinity requires further research.

Heat shock proteins are known as stress proteins and extrinsic chaperones and are produced in all cellular organisms exposed to stressors (Roberts et al. 2010). HSPs are a useful biomarker indicating the stress status of teleost fish (Roberts et al. 2010). The increase in HSPs has been found in freshwater and euryhaline fishes such as common carp (*Cyprinus carpio*) and salmon (*Salmo salar*) under salinity stress, although it is not systematic in all organs (De Wachter et al. 1998; Pan et al. 2000). In the current study, the increase in all tested *hsp* transcripts were exhibited in the highly osmotic environments, such as 15 and 20 psu, although the levels decreased slightly with the duration of exposure to



**Fig. 8** Fold change expression of intestinal pattern recognition receptors (PRRs) in the intestine of striped catfish exposed to different salinity levels. **a** *tlr1*, **b** *tlr2*, **c** *il1β*, **d** *tnfa*, **e** *il10*, **f** *tgf*, and **g** *apl*. Results are given as mean ± SEM. Different lower-case and upper-

case letters indicate statistically significant differences between treatments on D20 and D34, respectively ( $p < 0.05$ ). Asterisks indicate that the values for each treatment are significantly higher compared to the corresponding values on the other sampling days ( $p < 0.05$ )

salinity. The upregulation of HSPs plays an important role in preventing protein misfolding under stress conditions (Winkelhofer et al. 2008). Interestingly, there was a highly similar pattern between HSP gene expression and the major reactive genes of the osmoregulation process, i.e., *nka1a* and

*slc12a1*, especially on D20. This observation is supported by results in other species showing a positive correlation between the expression of *hsp70* and *nka* under hyperosmotic stress (Deane and Woo 2004; Larsen et al. 2008; Tine et al. 2010). However, Schmitz et al. (2016a) found that

HSP70 expression in the kidney of juvenile striped catfish did not vary significantly with the salinity gradient from fresh water to 20 psu. Since a significantly lower survival is observed at 20 psu, it may be hypothesized that juvenile striped catfish have mechanisms to respond to hyperosmotic conditions, through *hsp*, *nka*, and *slc12a* expression changes, but these are not sufficient for adaptation to 20 psu.

### Osmotic stress alters the intestinal microbial diversity

The effect of salinity on striped catfish has been investigated in previous studies; however, most of them were focused on physiological and immunological responses (Nguyen et al., 2014; Schmitz et al. 2016a,b). To date, no attention has been given to how the intestinal microbiota changes under high salinity conditions in this species. Additionally, the intestinal microbiota in striped catfish has not been investigated by amplicon sequencing-based methods, except a study with general phylum information by Sutriana et al (2018).

We obtained 4.5 million raw sequence reads from the Miseq runs, distributed across 74 samples and 4,066,509 quality filtered sequences were clustered into 939 OTUs ( $63,486 \pm 23,401$ , mean  $\pm$  SD with the min and max values of 2,849 and 90,739 sequences, respectively). This allowed for rarefaction and even sampling of 6000 sequences per sample. After rarefaction, 73 of 81 samples were retained for further steps. The present study revealed that the alpha diversity indicators of the intestinal microbiota were significantly decreased in salinities higher than 10 psu at D20, which is in accordance with the previous studies on euryhaline species (Schmidt et al. 2015; Dehler et al. 2017a). The salinity gradient is an important barrier for bacterial colonization, and the significant decrease in microbial species richness and diversity in saline treatments might reflect this evolutionary barrier (Dehler et al. 2017a). Furthermore, the increase of water uptake and salt excretion in order to regulate ions in the intestinal tract may lead to changes in the niche and contribute to alterations in the microbiota structure and composition (Whittamore 2012; Dehler et al. 2017a). In fact, changes in ionic regulation with increasing salinity were confirmed through the modified expression of genes related to osmoregulatory processes, such as *nka1a*, *slc12a1*, *aqp1*, and *cftr*, which could reflect the physiological changes observed in the current study. These genes generally showed significant differences in 15 and 20 psu treatment groups, in which higher *nka1a* and *slc12a1* and lower *aqp1* and *cftr* compared to the lower salinity treatments were observed. The physiological changes (possibly including pH, osmolality, passage rate and residence time, which are different in fresh water and seawater) significantly affect microbiota establishment (Ray and Ringø 2014). Additionally, phylogenetic diversity estimated by Faith's PD significantly

increased with salinity after two weeks of exposure to highly saline conditions. Faith's PD is calculated based on the sum of branch lengths of a phylogenetic tree connecting all species in the community (Faith 1992); therefore, there is a strong positive correlation between species richness and phylogenetic diversity. However, our results showed an opposite trend between phylogenetic diversity and richness at D34. This finding might be explained by the fact that although the number of OTUs was reduced, the phylogenetic differences of remaining OTUs in salinity treatments were extremely significant and this reflected the destabilized microbiota under saline conditions. Furthermore, the results of the Bray–Curtis index, followed by the ANOSIM test, showed significant differences in the intestinal microbiota structure between treatments. Notably, differences in diversity increased with duration of saline exposure, with medium salinity treatments (i.e., 5 psu and 10 psu) showing the greatest changes. Lokesh et al. (2019) reported that the diversity of intestinal microbiota in Atlantic salmon decreased significantly after 3 weeks of exposure to saline conditions. Striped catfish exposed to 10 psu showed the greatest difference in diversity according to exposure duration in the current study. Taken together, these results suggest that the salinity gradient and exposure time can disturb the intestinal community in striped catfish.

### Osmotic stress alters the intestinal microbiota taxonomic composition

In the present study, we found that *Bacteroidetes*, *Firmicutes*, *Verrucomicrobia*, and *Proteobacteria* phyla were predominant in the intestinal microbiota of striped catfish in freshwater. This is consistent with previous studies on striped catfish (Sutriana et al. 2018) and channel catfish *Ictalurus punctatus* (Bledsoe et al. 2016). Fish exposed to 20 psu had a significantly lower diversity, in which *Bacteroides\_OTU1* and *Vibrio* accounted for around 80%. The low diversity combined with the loss of some *Bacteroides* species and the increase in the *Proteobacteria* phylum may reflect a dysbiosis status of the intestinal tract (Humphreys 2020). The *Bacteroides* genus was dominant in the intestinal microbiota in all treatment groups at the beginning of exposure to salinity, however its abundance decreased in all groups by the end of the experiment, except for *Bacteroides\_OTU1*. Although its significantly higher relative abundance at 20 psu possibly reflected low diversity in the treatment, this OTU was also abundant at 15 psu, suggesting a high salinity tolerance. Although *Bacteroides* is considered the dominant genus in the endogenous microbiota of freshwater fish (Gómez and Balcázar 2008), some strains prefer estuarine and riverine environments (Langenheder et al. 2003). *Bacteroides* species are anaerobic, bile-resistant, non-spore-forming, Gram-negative rods, and the genus accounts for approximately 25% of

anaerobes in the healthy human colon (Salyers 1984; Wexler 2007). They perform a variety of functions in the human gut that may be related to carbohydrate metabolism, secretion of toxins, and interaction with the host immune system by controlling pathogens (Wexler 2007). The common presence of *Bacteroides* species under different osmotic conditions indicates their functional importance in the striped catfish gut. However, further studies are needed to determine their functional role, especially *Bacteroides\_OTU1*, which has a high tolerance to the high salinity environment. Similarly, *Verrucomicrobia*, mostly represented by the *Akkermansia* genus, were significantly affected by the salinity gradient and exposure time. Previous studies have shown its abundance in the intestine of freshwater common carp, cichlid fish, and marine rabbit fish (*Siganus guttatus*) (Baldo et al. 2019; Chang et al. 2020; Le et al. 2020). In humans, salinity in faeces was associated with reduced *Akkermansia* abundance, independent of geography, age, and obesity status (Seck et al. 2019). Although the study of this bacterial genus in aquatic animals is limited, it is known as a mucin degrader in human and mice intestine and plays a key role in reducing systemic inflammation, improving glucose metabolism and the barrier function of the gut (Everard et al. 2013; Xu et al. 2020). Therefore, *Akkermansia* has been considered as a promising probiotic due to its ability to ameliorate host immune responses and metabolic functions (Gómez-Gallego et al. 2016; Zhang et al. 2019). In the present study, the relative abundance of *Akkermansia* decreased in fish intestines exposed to salinity, suggesting that the functional barrier of the intestine might be disrupted by osmotic conditions. It is clear that the increase of *Proteobacteria* with increasing osmotic conditions was mainly represented in the abundance of *Vibrio* spp. The observation is not surprising because *Vibrio* spp. abundance in the gut microbiota has been shown to increase in Nile tilapia (*Oreochromis niloticus*), Chinook salmon (*Oncorhynchus tshawytscha*), and Atlantic salmon after transfer to saline environments (Zhang et al. 2016; Lokesh et al. 2019; Zhao et al. 2020). Although *Vibrio* could be found in the intestinal tract of freshwater fish (Mishra et al. 2010; Givens et al. 2014), it was almost absent in freshwater conditions in our study, representing less than 0.002% of abundance. It may be assumed that *Vibrio* is not an inherent bacterium in striped catfish gut and that they initially entered the intestinal tract with the increased drinking of water in the saline environment. In one of the *Vibrio* species, *Vibrio vulnificus*, the optimal salinity for survival was shown to vary between 5 and 10 psu and this strain develops well up to 25 psu (Randa et al. 2004), which is consistent with the abundance of the bacteria in high salinity environments. The abundance of *Campilobacterota*, mainly represented by the genera *Helicobacter* and *Sulfurospirillum*, was significantly affected by the salinity gradient, the former being inhibited in saline conditions higher than 10 psu and

the latter being stimulated in all salinities after two weeks of exposure. *Helicobacter pylori* was observed in faecal samples of farmed and wild tilapia from different water types and locations, while this bacterium was absent in all other tested fish species, even when collected from the same sources (Abdel-Moein et al. 2015). It is likely that the presence of this bacterium is more related to fish species-specific genetic traits than to environmental factors. Our results are in accordance with the findings of Gancz et al. (2008) who showed that a strain of *H. pylori*, known as a human pathogen, could not grow at a concentration of 15 g/L of sodium chloride. *Sulfurospirillum*, known as a free-living organism, produces hydrogen, which is an important electron supplier in an anoxic environment (Kruse et al. 2018). This genus also plays a key role in the metabolic cycle of sulphur and nitrogen (Ross et al. 2016). It is positively correlated with dissolved oxygen and negatively correlated with biological and chemical oxygen demands (Tyagi et al. 2021). Fish gut microbiota contain relatively few free-living environmental bacteria (Wong and Rawls 2012). *Sulfurospirillum* spp. was present in the freshwater treatment during our experiment with a relative abundance around 0.5%, indicating a relationship between the surrounding environment and the intestinal microbiota. Its increase in the intestine may be explained by the increased drinking of water by fish when transferred to a highly osmotic environment. However, the functions of *Sulfurospirillum* in the intestine of aquatic animals are still largely unknown.

### Presumptive metabolic pathways changed under an osmotic environment

Global changes in putative functions in intestinal microbiota were examined using PICRUSt2. In the first phase of salinity exposure, beside carbohydrate and lipid metabolism, the 20 psu treatment was enriched in pathways related to the metabolism of a number of amino acids, such as arginine, proline, tryptophane, tyrosine, and glutathione (Supplementary Table S3). Furthermore, the energy production from nitrogen, sulphur, and oxidative phosphorylation process were enhanced in fish subject to 20 psu (Supplementary Table S3). A study in intestinal Atlantic salmon supports these findings that the metabolism of carbohydrates, lipids, and amino acids is enriched during seawater adaptation (Dehler et al. 2017a). Bacteria can use amino acids and fatty acids as an energy source for their growth (Kazakov et al. 2009). They also restore glutathione, which bacteria use to protect their cells from external stressors such as low pH, cold, oxidation, and osmoregulation (Masip et al. 2006). These changes in metabolic pathways may be evidence that bacteria in the striped catfish gut tend to obtain their energy from different sources, such as carbohydrates, lipids, amino acids,

and inorganic compounds, to maintain their metabolism during salinity adaptation. Interestingly, a proteomic study of striped catfish kidney also showed that amino acid metabolism pathways, including amino acid synthesis, branched amino acid degradation, and metabolism of 13 amino acids, had higher abundance in fish exposed to salt stress (Schmitz et al. 2017a, b, c). This could explain the interaction between host and gut microbiota in response to salt stress through changes in energy metabolism. Interestingly, the signaling pathways mainly related to stress responses, such as membrane transport, cell motility, and signal transduction (two component system) were significantly higher in abundance in fish exposed to 20 psu (D20) and 10 psu (D34) compared to the others (Table 2 and Supplementary Table S3). The two-component system is the main way microorganisms perceive and regulate their expression profile under environmental changes (Teschler et al. 2017; Xu et al. 2017). External signals are detected and transmitted by the sensor histidine kinase component of the system. This protein senses the signals and then autophosphorylates a conserved histidine residue of the phosphotransferase domain. Subsequently, the phosphoryl group is transferred to the response regulator, which may activate gene transcription in response to the specific signals from environmental stimuli (Fabret et al. 1999; Liu et al. 2019). The process may also upregulate gene expression encoding a bacterial secretion pathway (Gallique et al. 2017). Bacterial motility is an important ability to explore suitable conditions via regulation of energy demand and plays a key role in halotolerance (Mitchell and Kogure 2006; Chen et al. 2017). Bacterial motility and chemotaxis are influenced by changes in the osmotic environment in species such as *Vibrio anguillarum*, *Aeromonas hydrophila*, and *Vibrio salmonicida*, which showed differential motility with salinity gradient (Larsen et al. 2004; Karlsen et al. 2008; Jahid et al. 2015). Based on these results, we hypothesize that intestinal bacteria might respond to the saline environment through a two-component pathway and upregulated expression of genes involved in their motility to adapt to osmotic stress during long-term exposure. The most intensive responses were seen in the 20 psu condition in the first phase of exposure to salinity, but 10 psu in the longer period.

### Host-microbiota interactions under osmotic stress

The interaction between host and microbiota plays an important role in many aspects of fish health, such as metabolic activity and immune status (Pérez et al. 2010). In the present study, the interaction was investigated via PRR changes and the presence of dominant bacteria in the intestine. The mucosal immune system discriminates between pathogenic and commensal bacteria via the PRR pathway,

which recognizes bacterial antigens and activates signalling cascades that regulate the immune response (Fasano and Shea-Donohue 2005). In the present study, a highly saline environment of 20 psu resulted in a significant decrease in the expression of *tlr1* and *tlr2*, which followed the down-regulation of inflammatory genes such as *il1 $\beta$* , *tnfa*, *il10*, and *tgfb* at D20. Schmitz et al. (2017a) found similar results in striped catfish, in which the expression of toll-like receptors was decreased in the kidneys under conditions of 20 psu. Interestingly, a previous study found that *Vibrio* infection increased the expression of inflammatory genes (Wu et al. 2015). In addition, the presence of *Akkermansia* in freshwater has been shown to suppress inflammatory responses through increasing the anti-inflammatory cytokine *il10* to prevent the activation of pro-inflammatory cytokines like *il12* (Demirci et al. 2019). However, the saline treatments in the current study showed a high abundance of the genus *Vibrio* and low abundance of the genus *Akkermansia*, but their inflammatory responses were unchanged and even decreased at 20 psu. A plausible explanation could be the increase in the expression of alkaline phosphatase in the intestine (Bates et al. 2007). This plays a crucial role in the detoxification of lipopolysaccharide and prevention of intestinal inflammation, and enhances mucosal tolerance to the resident microbiota (Bates et al. 2007). However, the effect of hyperosmotic stress diminished over time, and responses tended to recover at D34, except for *il1 $\beta$*  and *il10*. These results suggest that striped catfish may regulate excessive inflammation due to invasive bacteria by responding to high lipopolysaccharide stress. However, due to the complex intestinal microbiota, the exact mechanism remains unknown.

In conclusion, this study revealed microbiota and gene expression alterations in striped catfish intestine under different saline conditions. A detailed profile of the intestinal microbiota in striped catfish was elucidated using the 16S rRNA sequencing method. The results were strongly concordant with those of other freshwater fish, with a predominance of the phyla *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia*. Regarding host responses to salinity, the intestine contributed to the regulation of the osmoregulatory and adaptive stress processes; however, this may be insufficient at 20 psu resulting in intestinal dysbiosis as well as high mortality. The osmoregulation was more intensive while the stress indicators returned to normal after a 2-week exposure to salinity. These osmotic changes in the intestine significantly reduced the diversity of the microbiota. However, extended salt exposure destabilized the intestinal microbiota, leading to an increase in phylogenetic diversity with the salt gradient. Despite the predominance of *Bacteroides* OTUs in all treatments, their abundances were mostly significantly decreased after extended exposure to salinity, except *Bacteroides\_OTU1*.



The roles of this OTU need to be clarified due to its high tolerance to salinity. *Akkermansia*, known to be a probiotic, had a lower abundance at the higher salinities and longer exposures. *Sulfurospirillum* and *Vibrio* were the most abundant in high salinity treatments at the end of the experiment, indicating a strong correlation between environmental conditions and fish intestinal bacterial communities. Regarding predicted metabolic pathways, when treated with 20 psu the fish obtained higher energy from various sources that might be needed to cope with osmotic stress. Additionally, the intestinal microbiota at 20 psu was most sensitive to environmental changes in the first phase of exposure to salinity, while this was 10 psu for the extended salt exposure. The salinity gradient of up to 10 psu did not negatively affect local immune responses. However, the exact mechanism needs further research, especially the interaction with the microbiota.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00253-022-11895-1>.

**Acknowledgements** The authors thank to the Académie de Recherche et d'Enseignement Supérieur (ARES) and the General Directorate and Development for Cooperation (DGD) in Belgium through the Research for Development Project (RDP) "Towards a sustainable pangasius breeding, a selection approach (PANGAGEN)."

**Author contribution** PK, FF, and NTP designed and coordinated the project. PK, DQH, and BTBH conceived of the experiment. DQH carried out the experiment and analysed the data. BTBH, DTT, DTY, PTL, BMT, DMH, and VNS supported to carry out and sample the experiment. LJ and MMG guided and advised for bioinformatics analysis. DQH, BTBH, JL, MMG, and PK prepared the manuscript. All the authors contributed to the article and approved the final manuscript.

**Funding** The research was supported by the Académie de Recherche et d'Enseignement Supérieur (ARES) and the General Directorate and Development for Cooperation (DGD) in Belgium.

**Data availability** The datasets supporting the conclusions of the article are included within the article and its supplementary materials. The sequencing raw data was deposited in the NCBI Sequence Read Archive database (accession: PRJNA759359).

## Declarations

**Ethics approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Competing interests** The authors declare no competing interests.

## References

- Abdel-Moein KA, Saeed H, Samir A (2015) Novel detection of *Helicobacter pylori* in fish: a possible public health concern. *Acta Trop* 152:141–144. <https://doi.org/10.1016/j.actatropica.2015.09.005>
- Aoki M, Kaneko T, Katoh F, Hasegawa S, Tsutsui N, Aida K (2003) Intestinal water absorption through aquaporin 1 expressed in the apical membrane of mucosal epithelial cells in seawater-adapted Japanese eel. *J Exp Biol* 206:3495–3505. <https://doi.org/10.1242/jeb.00579>
- Asnicar F, Weingart G, Tickle TL, Huttenhower C, Segata N (2015) Compact graphical representation of phylogenetic data and metadata with GraPhlAn. *PeerJ* 3:e1029
- Baldo L, Riera JL, Salzburger W, Barluenga M (2019) Phylogeography and ecological niche shape the cichlid fish gut microbiota in Central American and African Lakes. *Front Microbiol* 10:2372
- Bates JM, Akerlund J, Mittge E, Guillemin K (2007) Intestinal Alkaline Phosphatase Detoxifies Lipopolysaccharide and Prevents Inflammation in Zebrafish in Response to the Gut Microbiota. *Cell Host Microbe* 2:371–382. <https://doi.org/10.1016/j.chom.2007.10.010>
- Bledsoe JW, Peterson BC, Swanson KS, Small BC (2016) Ontogenetic characterization of the intestinal microbiota of channel catfish through 16S rRNA gene sequencing reveals insights on temporal shifts and the influence of environmental microbes. *PLoS ONE* 11:e0166379–e0166379. <https://doi.org/10.1371/journal.pone.0166379>
- Chang X, Wang X, Feng J, Su X, Liang J, Li H, Zhang J (2020) Impact of chronic exposure to trichlorfon on intestinal barrier, oxidative stress, inflammatory response and intestinal microbiome in common carp (*Cyprinus carpio* L.). *Environ Pollut* 259:113846. <https://doi.org/10.1016/j.envpol.2019.113846>
- Chen Y-H, Lu C-W, Shyu Y-T, Lin S-S (2017) Revealing the saline adaptation strategies of the halophilic bacterium *Halomonas beimenensis* through high-throughput omics and transposon mutagenesis approaches. *Sci Rep* 7:13037. <https://doi.org/10.1038/s41598-017-13450-9>
- De Wachter B, Scholliers A, Blust R (1998) Semiquantitative immunoblot detection of 70 kDa stress proteins in the carp *Cyprinus carpio*. *Bull Environ Contam Toxicol* 60:37–44. <https://doi.org/10.1007/s001289900588>
- Deane EE, Woo NYS (2004) Differential gene expression associated with euryhalinity in sea bream (*Sparus sarba*). *Am J Physiol Integr Comp Physiol* 287:R1054–R1063
- Deane EE, Kelly SP, Luk JCY, Woo NYS (2002) Chronic salinity adaptation modulates hepatic heat shock protein and insulin-like growth factor I expression in black sea bream. *Mar Biotechnol* 4:193–205
- Deane E, Luk J, Woo NYS (2011) Aquaporin 1a expression in gill, intestine, and kidney of the euryhaline silver sea bream. *Front Physiol* 2:39
- Dehler CE, Secombes CJ, Martin SAM (2017) Seawater transfer alters the intestinal microbiota profiles of Atlantic salmon (*Salmo salar* L.). *Sci Rep* 7:13877. <https://doi.org/10.1038/s41598-017-13249-8>
- Dehler CE, Secombes CJ, Martin SAM (2017) Environmental and physiological factors shape the gut microbiota of Atlantic salmon parr (*Salmo salar* L.). *Aquaculture* 467:149–157. <https://doi.org/10.1016/j.aquaculture.2016.07.017>
- Demirci M, Tokman HB, Uysal HK, Demiryas S, Karakullukcu A, Saribas S, Cokugras H, Kocazeybek BS (2019) Reduced *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* levels in the gut microbiota of children with allergic asthma. *Allergol Immunopathol (madr)* 47:365–371. <https://doi.org/10.1016/j.aller.2018.12.009>
- Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI (2020) PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* 38:685–688. <https://doi.org/10.1038/s41587-020-0548-6>
- Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 10:996–998. <https://doi.org/10.1038/nmeth.2604>

- Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, de Vos WM, Cani PD (2013) Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci* 110:9066 LP – 9071. <https://doi.org/10.1073/pnas.1219451110>
- Fabret C, Feher VA, Hoch JA (1999) Two-component signal transduction in *Bacillus subtilis*: how one organism sees its world. *J Bacteriol* 181:1975–1983. <https://doi.org/10.1128/JB.181.7.1975-1983.1999>
- Faith DP (1992) Conservation evaluation and phylogenetic diversity. *Biol Conserv* 61:1–10. [https://doi.org/10.1016/0006-3207\(92\)91201-3](https://doi.org/10.1016/0006-3207(92)91201-3)
- FAO (2020) GLOBEFISH Highlights January 2020 issue, with Jan. – Sep. 2019 Statistics – A quarterly update on world seafood markets. Globefish Highlights no. 1–2020. Rome. <https://doi.org/10.4060/ca7968en>
- Fasano A, Shea-Donohue T (2005) Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. *Nat Clin Pract Gastroenterol Hepatol* 2:416–422. <https://doi.org/10.1038/ncpgasthep0259>
- Gallique M, Bouteiller M, Merieau A (2017) The type VI secretion system: a dynamic system for bacterial communication? *Front Microbiol* 8:1454
- Gancz H, Jones KR, Merrell DS (2008) Sodium chloride affects *Helicobacter pylori* growth and gene expression. *J Bacteriol* 190:4100–4105. <https://doi.org/10.1128/JB.01728-07>
- Gioacchini G, Rossi G, Carnevali O (2017) Host-probiotic interaction: new insight into the role of the endocannabinoid system by in vivo and ex vivo approaches. *Sci Rep* 7:1261. <https://doi.org/10.1038/s41598-017-01322-1>
- Givens CE, Bowers JC, DePaola A, Hollibaugh JT, Jones JL (2014) Occurrence and distribution of *Vibrio vulnificus* and *Vibrio parahaemolyticus* – potential roles for fish, oyster, sediment and water. *Lett Appl Microbiol* 58:503–510. <https://doi.org/10.1111/lam.12226>
- Gómez GD, Balcázar JL (2008) A review on the interactions between gut microbiota and innate immunity of fish. *FEMS Immunol Med Microbiol* 52:145–154. <https://doi.org/10.1111/j.1574-695X.2007.00343.x>
- Gómez-Gallego C, Pohl S, Salminen S, De Vos WM, Kneifel W (2016) Akkermansia muciniphila: a novel functional microbe with probiotic properties. *Benef Microbes* 7:571–584
- Gregório SF, Carvalho ESM, Encarnação S, Wilson JM, Power DM, Canário AVM, Fuentes J (2013) Adaptation to different salinities exposes functional specialization in the intestine of the sea bream (*Sparus aurata* L.). *J Exp Biol* 216:470–479. <https://doi.org/10.1242/jeb.073742>
- Grosell M (2006) Intestinal anion exchange in marine fish osmoregulation. *J Exp Biol* 209:2813–2827. <https://doi.org/10.1242/jeb.02345>
- Grosell M (2010) The role of the gastrointestinal tract in salt and water balance. In: Grosell M (ed) *Fish physiology*. Elsevier, pp 135–164
- Hall M, Beiko RG (2018) 16S rRNA gene analysis with QIIME2. In: Beiko RG, Hsiao W, Parkinson J (eds) *Microbiome analysis*. Springer New York, pp 113–129. [https://doi.org/10.1007/978-1-4939-8728-3\\_8](https://doi.org/10.1007/978-1-4939-8728-3_8)
- Herlemann DPR, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J* 5:1571–1579. <https://doi.org/10.1038/ismej.2011.41>
- Hightower LE (1991) Heat shock, stress proteins, chaperones, and proteotoxicity. *Cell* 66:191–197
- Hörmannspurger G, Haller D (2010) Molecular crosstalk of probiotic bacteria with the intestinal immune system: clinical relevance in the context of inflammatory bowel disease. *Int J Med Microbiol* 300:63–73. <https://doi.org/10.1016/j.ijmm.2009.08.006>
- Humphreys C (2020) Intestinal Permeability. In: Pizzorno JE, Murray MT (eds) *Textbook of Natural Medicine*, 5th edn. Churchill Livingstone, St. Louis (MO), pp 166–177
- Jahid IK, Mizan MFR, Ha AJ, Ha S-D (2015) Effect of salinity and incubation time of planktonic cells on biofilm formation, motility, exoprotease production, and quorum sensing of *Aeromonas hydrophila*. *Food Microbiol* 49:142–151. <https://doi.org/10.1016/j.fm.2015.01.016>
- Karlsen C, Paulsen SM, Tunsjø HS, Krinner S, Sørum H, Haugen P, Willassen N-P (2008) Motility and flagellin gene expression in the fish pathogen *Vibrio salmonicida*: Effects of salinity and temperature. *Microb Pathog* 45:258–264. <https://doi.org/10.1016/j.micpath.2008.06.002>
- Kazakov AE, Rodionov DA, Alm E, Arkin AP, Dubchak I, Gelfand MS (2009) Comparative genomics of regulation of fatty acid and branched-chain amino acid utilization in proteobacteria. *J Bacteriol* 191:52–64. <https://doi.org/10.1128/JB.01175-08>
- Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 41:e1–e1. <https://doi.org/10.1093/nar/gks808>
- Kruse S, Goris T, Westermann M, Adrian L, Diekert G (2018) Hydrogen production by *Sulfurospirillum* species enables syntrophic interactions of *Epsilonproteobacteria*. *Nat Commun* 9:4872. <https://doi.org/10.1038/s41467-018-07342-3>
- Langenheder S, Kisand V, Wikner J, Tranvik LJ (2003) Salinity as a structuring factor for the composition and performance of bacterioplankton degrading riverine DOC. *FEMS Microbiol Ecol* 45:189–202. [https://doi.org/10.1016/S0168-6496\(03\)00149-1](https://doi.org/10.1016/S0168-6496(03)00149-1)
- Larsen MH, Blackburn N, Larsen JL, Olsen JE (2004) Influences of temperature, salinity and starvation on the motility and chemotactic response of *Vibrio anguillarum*. *Microbiology* 150:1283–1290. <https://doi.org/10.1099/mic.0.26379-0>
- Larsen PF, Nielsen EE, Koed A, Thomsen DS, Olsvik PA, Loeschcke V (2008) Interpopulation differences in expression of candidate genes for salinity tolerance in winter migrating anadromous brown trout (*Salmo trutta* L.). *BMC Genet* 9:1–9
- Laverty G, Skadhauge E (2012) Adaptation of teleosts to very high salinity. *Comp Biochem Physiol Part A Mol Integr Physiol* 163:1–6. <https://doi.org/10.1016/j.cbpa.2012.05.203>
- Le D, Nguyen P, Nguyen D, Dierckens K, Boon N, Lacoere T, Kerckhof F-M, De Vrieze J, Vadstein O, Bossier P (2020) Gut microbiota of migrating wild rabbit fish (*Siganus guttatus*) larvae have low spatial and temporal variability. *Microb Ecol* 79:539–551. <https://doi.org/10.1007/s00248-019-01436-1>
- Li J, Dai Z, Jana D, Callaway DJE, Bu Z (2005) Ezrin controls the macromolecular complexes formed between an adapter protein Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor and the cystic fibrosis transmembrane conductance regulator\*. *J Biol Chem* 280:37634–37643. <https://doi.org/10.1074/jbc.M502305200>
- Li Z, Lui EY, Wilson JM, Ip YK, Lin Q, Lam TJ, Lam SH (2014) Expression of key ion transporters in the gill and esophageal-gastrointestinal tract of euryhaline Mozambique tilapia *Oreochromis mossambicus* acclimated to fresh water, seawater and hypersaline water. *PLoS One* 9:e87591
- Lin YM, Chen CN, Lee TH (2003) The expression of gill Na, K-ATPase in milkfish, *Chanos chanos*, acclimated to seawater, brackish water and fresh water. *Comp Biochem Physiol Part A Mol Integr Physiol* 135:489–497

- Lind U, Alm Rosenblad M, Wrangé A-L, Sundell KS, Jonsson PR, André C, Havenhand J, Blomberg A (2013) Molecular characterization of the  $\alpha$ -subunit of  $\text{Na}^+/\text{K}^+$  ATPase from the euryhaline barnacle *Balanus improvisus* reveals multiple genes and differential expression of alternative splice variants. *PLoS ONE* 8:e77069–e77069. <https://doi.org/10.1371/journal.pone.0077069>
- Liu C, Sun D, Zhu J, Liu W (2019) Two-Component Signal Transduction Systems: A Major Strategy for Connecting Input Stimuli to Biofilm Formation. *Front Microbiol* 9:3279
- Lokesh J, Kiron V, Sipkema D, Fernandes JMO, Moum T (2019) Succession of embryonic and the intestinal bacterial communities of Atlantic salmon (*Salmo salar*) reveals stage-specific microbial signatures. *Microbiologyopen* 8:e00672. <https://doi.org/10.1002/mbo3.672>
- Louca S, Doebeli M (2018) Efficient comparative phylogenetics on large trees. *Bioinformatics* 34:1053–1055
- Madsen SS, Bujak J, Tipsmark CK (2014) Aquaporin expression in the Japanese medaka (*Oryzias latipes*) in freshwater and seawater: challenging the paradigm of intestinal water transport? *J Exp Biol* 217:3108–3121. <https://doi.org/10.1242/jeb.105098>
- Malakpour Kolbadinezhad S, Coimbra J, Wilson JM (2018) Osmoregulation in the Plotosidae catfish: Role of the salt secreting dendritic organ. *Front Physiol* 9:761
- Marshall WS, Grosell M (2005) Ion transport, osmoregulation, and acid-base balance. In: Evans DH, Clairborne JB (eds) *The physiology of fishes*. CRC Press, Boca Raton, pp 177–230
- Marshall WS, Singer TD (2002) Cystic fibrosis transmembrane conductance regulator in teleost fish. *Biochim Biophys Acta - Biomembr* 1566:16–27. [https://doi.org/10.1016/S0005-2736\(02\)00584-9](https://doi.org/10.1016/S0005-2736(02)00584-9)
- Martin SAM, Dehler CE, Król E (2016) Transcriptomic responses in the fish intestine. *Dev Comp Immunol* 64:103–117. <https://doi.org/10.1016/j.dci.2016.03.014>
- Martinez A-S, Cutler CP, Wilson GD, Phillips C, Hazon N, Cramb G (2005) Regulation of expression of two aquaporin homologs in the intestine of the European eel: effects of seawater acclimation and cortisol treatment. *Am J Physiol Integr Comp Physiol* 288:R1733–R1743. <https://doi.org/10.1152/ajpregu.00747.2004>
- Masip L, Veeravalli K, Georgiou G (2006) The many faces of glutathione in bacteria. *Antioxid Redox Signal* 8:753–762. <https://doi.org/10.1089/ars.2006.8.753>
- McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217
- Mishra P, Samanta M, Mohanty S, Maiti NK (2010) Characterization of *Vibrio* species isolated from freshwater fishes by ribotyping. *Indian J Microbiol* 50:101–103. <https://doi.org/10.1007/s12088-010-0010-4>
- Mitchell JG, Kogure K (2006) Bacterial motility: links to the environment and a driving force for microbial physics. *FEMS Microbiol Ecol* 55:3–16
- Mundy PC, Jeffries KM, Fangué NA, Connon RE (2020) Differential regulation of select osmoregulatory genes and  $\text{Na}^+/\text{K}^+$ -ATPase paralogs may contribute to population differences in salinity tolerance in a semi-anadromous fish. *Comp Biochem Physiol Part A Mol Integr Physiol* 240:110584. <https://doi.org/10.1016/j.cbpa.2019.110584>
- Nguyen PTH, Do HTT, Mather PB, Hurwood DA (2014) Experimental assessment of the effects of sublethal salinities on growth performance and stress in cultured tra catfish (*Pangasianodon hypophthalmus*). *Fish Physiol Biochem* 40:1839–1848. <https://doi.org/10.1007/s10695-014-9972-1>
- Pan F, Zarate JM, Tremblay GC, Bradley TM (2000) Cloning and characterization of salmon hsp90 cDNA: Upregulation by thermal and hyperosmotic stress. *J Exp Zool* 287:199–212. [https://doi.org/10.1002/1097-010X\(20000801\)287:3%3c199::AID-JEZ2%3e3.0.CO;2-3](https://doi.org/10.1002/1097-010X(20000801)287:3%3c199::AID-JEZ2%3e3.0.CO;2-3)
- Pérez T, Balcázar JL, Ruiz-Zarzuela I, Halaihel N, Vendrell D, de Blas I, Múzquiz JL (2010) Host–microbiota interactions within the fish intestinal ecosystem. *Mucosal Immunol* 3:355
- Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:e45. <https://doi.org/10.1093/nar/29.9.e45>
- Picchiatti S, Fausto AM, Randelli E, Carnevali O, Taddei AR, Buonocore F, Scapigliati G, Abelli L (2009) Early treatment with *Lactobacillus delbrueckii* strain induces an increase in intestinal T-cells and granulocytes and modulates immune-related genes of larval *Dicentrarchus labrax* (L.). *Fish Shellfish Immunol* 26:368–376. <https://doi.org/10.1016/j.fsi.2008.10.008>
- Randa MA, Polz MF, Lim E (2004) Effects of temperature and salinity on *Vibrio vulnificus* population dynamics as assessed by quantitative PCR. *Appl Environ Microbiol* 70:5469–5476. <https://doi.org/10.1128/AEM.70.9.5469-5476.2004>
- Rawls JF, Samuel BS, Gordon JI (2004) Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proc Natl Acad Sci U S A* 101:4596–4601. <https://doi.org/10.1073/pnas.0400706101>
- Ray AK, Ringø E (2014) The gastrointestinal tract of fish. In: Merrifield D, Ringø E (eds) *Aquaculture nutrition: gut health, probiotics and prebiotics*. Wiley-Blackwell Publishing, Oxford, UK, pp 1–13
- Ringø E, Olsen RE, Mayhew TM, Myklebust R (2003) Electron microscopy of the intestinal microflora of fish. *Aquaculture* 227:395–415. <https://doi.org/10.1016/j.aquaculture.2003.05.001>
- Roberts RJ, Agius C, Saliba C, Bossier P, Sung YY (2010) Heat shock proteins (chaperones) in fish and shellfish and their potential role in relation to fish health: a review. *J Fish Dis* 33:789–801. <https://doi.org/10.1111/j.1365-2761.2010.01183.x>
- Ronkin D, Seroussi E, Nitzan T, Doron-Faigenboim A, Cnaani A (2015) Intestinal transcriptome analysis revealed differential salinity adaptation between two tilapia species. *Comp Biochem Physiol Part D Genomics Proteomics* 13:35–43. <https://doi.org/10.1016/j.cbd.2015.01.003>
- Ross DE, Marshall CW, May HD, Norman RS (2016) Comparative genomic analysis of *Sulfurospirillum cavolei* MES reconstructed from the metagenome of an electrosynthetic microbiome. *PLoS ONE* 11:e0151214–e0151214. <https://doi.org/10.1371/journal.pone.0151214>
- Salyers AA (1984) Bacteroides of the human lower intestinal tract. *Annu Rev Microbiol* 38:293–313. <https://doi.org/10.1146/annurev.mi.38.100184.001453>
- Schmidt VT, Smith KF, Melvin DW, Amaral-Zettler LA (2015) Community assembly of a euryhaline fish microbiome during salinity acclimation. *Mol Ecol* 24:2537–2550. <https://doi.org/10.1111/mec.13177>
- Schmitz M, Douxfils J, Mandiki SNM, Morana C, Baekelandt S, Kestemont P (2016) Chronic hyperosmotic stress interferes with immune homeostasis in striped catfish (*Pangasianodon hypophthalmus*, S.) and leads to excessive inflammatory response during bacterial infection. *Fish Shellfish Immunol* 55:550–558. <https://doi.org/10.1016/j.fsi.2016.06.031>
- Schmitz M, Mandiki SNM, Douxfils J, Ziv T, Admon A, Kestemont P (2016) Synergic stress in striped catfish (*Pangasianodon hypophthalmus*, S.) exposed to chronic salinity and bacterial infection: Effects on kidney protein expression profile. *J Proteomics* 142:91–101. <https://doi.org/10.1016/j.jprot.2016.04.046>
- Schmitz M, Baekelandt S, Bequet S, Kestemont P (2017) Chronic hyperosmotic stress inhibits renal Toll-Like Receptors expression in striped catfish (*Pangasianodon hypophthalmus*, Sauvage)

- exposed or not to bacterial infection. *Dev Comp Immunol* 73:139–143. <https://doi.org/10.1016/j.dci.2017.03.020>
- Schmitz M, Baekelandt S, Tran Thi LK, Mandiki SNM, Douxfils J, Nguyen TQ, Do Thi Thanh H, Kestemont P (2017) Osmoregulatory and immunological status of the pond-raised striped catfish (*Pangasianodon hypophthalmus* S.) as affected by seasonal runoff and salinity changes in the Mekong Delta. *Vietnam Fish Physiol Biochem* 43:39–49. <https://doi.org/10.1007/s10695-016-0266-7>
- Schmitz M, Ziv T, Admon A, Baekelandt S, Mandiki SNM, L'Hoir M, Kestemont P (2017) Salinity stress, enhancing basal and induced immune responses in striped catfish *Pangasianodon hypophthalmus* (Sauvage). *J Proteomics* 167:12–24. <https://doi.org/10.1016/j.jprot.2017.08.005>
- Scott GR, Baker DW, Schulte PM, Wood CM (2008) Physiological and molecular mechanisms of osmoregulatory plasticity in killifish after seawater transfer. *J Exp Biol* 211:2450–2459. <https://doi.org/10.1242/jeb.017947>
- Seck EH, Senghor B, Merhej V, Bachar D, Cadoret F, Robert C, Azhar EI, Yasir M, Bibi F, Jiman-Fatani AA, Konate DS, Musso D, Doumbo O, Sokhna C, Levasseur A, Lagier JC, Khelaifia S, Million M, Raoult D (2019) Salt in stools is associated with obesity, gut halophilic microbiota and *Akkermansia muciniphila* depletion in humans. *Int J Obes* 43:862–871. <https://doi.org/10.1038/s41366-018-0201-3>
- Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. *Genome Biol* 12:R60
- Sutriana A, Hashim R, Akter MN, Nor SAM (2018) Galactooligosaccharide and a combination of yeast and  $\beta$ -glucan supplements enhance growth and improve intestinal condition in striped catfish *Pangasianodon hypophthalmus* fed soybean meal diets. *Fish Sci* 84:523–533. <https://doi.org/10.1007/s12562-018-1195-4>
- Takei Y (2021) The digestive tract as an essential organ for water acquisition in marine teleosts: lessons from euryhaline eels. *Zool Lett* 7:10. <https://doi.org/10.1186/s40851-021-00175-x>
- Takei Y, Hwang P-P (2016) Homeostatic responses to osmotic stress. In: Schreck CB, Tort L, Farrell AP, Brauner CJ (eds) *Fish Physiology*. Elsevier, pp 207–249
- Team RC (2013) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available from: <http://www.R-project.org/>
- Teschler JK, Cheng AT, Yildiz FH (2017) The two-component signal transduction system VxrAB positively regulates *Vibrio cholerae* biofilm formation. *J Bacteriol* 199:e00139–e217
- Tine M, Bonhomme F, McKenzie DJ, Durand J-D (2010) Differential expression of the heat shock protein Hsp70 in natural populations of the tilapia, *Sarotherodon melanotheron*, acclimatised to a range of environmental salinities. *BMC Ecol* 10:11. <https://doi.org/10.1186/1472-6785-10-11>
- Tlaskalova-Hogenova H, Tuckova L, Mestecky J, Kolinska J, Rossmann P, Stepankova R, Kozakova H, Hudcovic T, Hrnecir T, Frolova L, Kverka M (2005) Interaction of mucosal microbiota with the innate immune system. *Scand J Immunol* 62:106–113. <https://doi.org/10.1111/j.1365-3083.2005.01618.x>
- Tyagi I, Tyagi K, Bhutiani R, Chandra K, Kumar V (2021) Bacterial diversity assessment of world's largest sewage-fed fish farms with special reference to water quality: a Ramsar site. *Environ Sci Pollut Res* 28:42372–42386. <https://doi.org/10.1007/s11356-021-13756-2>
- Usher ML, Talbot C, Eddy FB (1990) Effects of transfer to seawater on digestion and gut function in Atlantic salmon smolts (*Salmo salar* L.). *Aquaculture* 90:85–96. [https://doi.org/10.1016/0044-8486\(90\)90285-U](https://doi.org/10.1016/0044-8486(90)90285-U)
- Wexler HM (2007) Bacteroides: the good, the bad, and the nitty-gritty. *Clin Microbiol Rev* 20:593–621. <https://doi.org/10.1128/CMR.00008-07>
- Whittamore JM (2012) Osmoregulation and epithelial water transport: lessons from the intestine of marine teleost fish. *J Comp Physiol B* 182:1–39. <https://doi.org/10.1007/s00360-011-0601-3>
- Winkhofer KF, Tatzelt J, Haass C (2008) The two faces of protein misfolding: gain- and loss-of-function in neurodegenerative diseases. *EMBO J* 27:336–349. <https://doi.org/10.1038/sj.emboj.7601930>
- Wong S, Rawls JF (2012) Intestinal microbiota composition in fishes is influenced by host ecology and environment. *Mol Ecol* 21:3100–3102. <https://doi.org/10.1111/j.1365-294X.2012.05646.x>
- Wu J, Shi Y-H, Zhang X-H, Li C-H, Li M-Y, Chen J (2015) Molecular characterization of an IL-1 $\beta$  gene from the large yellow croaker (*Larimichthys crocea*) and its effect on fish defense against *Vibrio alginolyticus* infection. *Dong wu xue yan jiu = Zool Res* 36:133–141
- Xu T, Wu Y, Lin Z, Bertram R, Götz F, Zhang Y, Qu D (2017) Identification of genes controlled by the essential YycFG two-component system reveals a role for biofilm modulation in *Staphylococcus epidermidis*. *Front Microbiol* 8:724
- Xu Y, Wang N, Tan H-Y, Li S, Zhang C, Feng Y (2020) Function of *Akkermansia muciniphila* in obesity: interactions with lipid metabolism, immune response and gut systems. *Front Microbiol* 11:219
- Yang W-K, Hseu J-R, Tang C-H, Chung M-J, Wu S-M, Lee T-H (2009) Na<sup>+</sup>/K<sup>+</sup>-ATPase expression in gills of the euryhaline sailfin molly, *Poecilia latipinna*, is altered in response to salinity challenge. *J Exp Mar Bio Ecol* 375:41–50
- Yang W-K, Hsu A-D, Kang C-K, Lai IP, Liao P-S, Lee T-H (2018) Intestinal FXyD12 and sodium-potassium ATPase: A comparative study on two euryhaline medakas in response to salinity changes. *PLoS One* 13:e0201252
- Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL (2012) Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* 13:134. <https://doi.org/10.1186/1471-2105-13-134>
- Zhang M, Sun Y, Liu Y, Qiao F, Chen L, Liu W-T, Du Z, Li E (2016) Response of gut microbiota to salinity change in two euryhaline aquatic animals with reverse salinity preference. *Aquaculture* 454:72–80. <https://doi.org/10.1016/j.aquaculture.2015.12.014>
- Zhang T, Li Q, Cheng L, Buch H, Zhang F (2019) *Akkermansia muciniphila* is a promising probiotic. *Microb Biotechnol* 12:1109–1125. <https://doi.org/10.1111/1751-7915.13410>
- Zhao R, Symonds JE, Walker SP, Steiner K, Carter CG, Bowman JP, Nowak BF (2020) Salinity and fish age affect the gut microbiota of farmed Chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* 528:735539. <https://doi.org/10.1016/j.aquaculture.2020.735539>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.