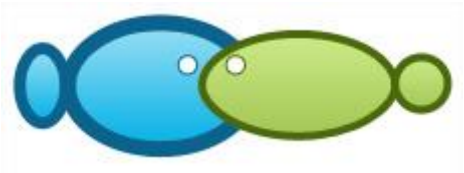

Bacterial population in intensive striped catfish *Pangasianodon hypophthalmus* ponds

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Abstract. Bacterial populations in intensive striped catfish (*Pangasianodon hypophthalmus*) ponds remain poorly understood. This study aimed to unravel the bacterial community structure in water and bottom sediment in catfish ponds during the rainy season, using next-generation sequencing technology. Next-generation sequencing data showed 23 phyla, 40 classes, 92 orders, 133 families and 242 genera in striped catfish ponds. 32 genera were common to both water and sediment, while 168 genera were confined to pond water and 42 genera were confined to bottom sediments. Pond water had higher biodiversity and harboured more genera than sediment. Proteobacteria, Fusobacteriota and Actinobacteriota were the most dominant phyla in water, while the four most dominant phyla in the sediment sample were Actinobacteriota, Myxococcota, Desulfobacterota and Proteobacteria. *Novosphingobium* was the most abundant genus in the water, while *Nitrospira* was the most abundant in sediment. In addition, the pathogenic bacteria *Aeromonas*, *Flavobacterium*, *Edwardsiella* and *Bacillus*, a common probiotic, were also detected in water and sediment, but with much lower abundances than *Novosphingobium* and *Nitrospira*.
Key Words: bacterial community, biodiversity, operational taxonomic unit, next-generation sequencing.

Introduction. Global fish production is estimated to have reached about 179 million tons in 2018, with a total first sale value estimated at USD 401 billion, of which 82 million tons, valued at USD 250 billion, came from aquaculture production. In 2018, inland aquaculture produced 51.3 million tons, of which striped catfish *Pangasianodon hypophthalmus* accounted for 2.3 million tons (4.3% of total finfish production) (FAO 2020). In Vietnam, fish production in 2020 was estimated at 8.4 million tons, almost half (4.56 million tons) of which came from aquaculture, including 1.56 million tonnes from pond culture of striped catfish in the Mekong Delta (MD) (VASEP 2020). As of 2020, there were 120 striped catfish hatcheries with 4,000 ha nursing ponds, with an annual production of up to 2 billion striped catfish larvae (VASEP 2020). In catfish culture, pond organic matter levels are normally high due to extraneous inputs such as feed and excreta. This drives a diverse community of bacteria and other microorganisms which mineralize the organic matter to inorganic forms (Moriarty 1997), some of which can be toxic, for example, nitrite, ammonia, and hydrogen sulphide (Chien 1992). The overall diversity and abundance of different microbial populations therefore play a key role in water quality (Giang et al 2008; Ut et al 2016) and the prevalence of endemic diseases (Dung et al 2008; Diep et al 2009; Pokhrel & Oanh 2021). However, information on the bacterial populations of catfish ponds is still sparse. Characterizing the bacterial populations of catfish pond water and sediments is an important first step to improve water quality and manage fish health more effectively (Zeng et al 2010; Tilia et al 2016).

In this study, high-throughput next-generation (NGS) technology was used to document the structure of bacterial populations in the water and sediment of striped catfish ponds in the Mekong Delta, Vietnam. This gene-sequencing technology enables cost-effective and time-efficient sequencing of genes and assessing genetic diversity in complex communities (Mardis 2008; Ploski 2016; Liu et al 2016), and it is used to describe the genetic structure of bacterial populations in a number of different

environments (Wu et al 2013; Tang et al 2014; Cardona et al 2016; Qin et al 2016; Huynh et al 2019).

Material and Method

Sampling site. Sediment and water samples were collected from 3 intensive catfish grow-out ponds in Can Tho city (Table 1). The catfish ponds sampled had a grow-out period of 4 months. Sampling was conducted in the rainy season. During sampling, information on pond management such as density, feed, water quality, and fish health management was also recorded.

Table 1

Sample collection and labeling samples from the intensive *Pangasianodon hypophthalmus*

Pond ID	*Water sample ID*	*Sediment sample ID*
Catfish pond 1	Catfish_Wat_1	Catfish_Sed_1
Catfish pond 2	Catfish_Wat_2	Catfish_Sed_2
Catfish pond 3	Catfish_Wat_3	Catfish_Sed_3

Wat-water sample; Sed-sediment sample; Catfish-striped catfish; 1,2 or 3 refers to one of the three ponds sampled.

Water samples. In each pond, integrated water sampling methods were applied. For this, the water column was sampled at 40-50 cm in depth, at five different sites in the pond, in the morning, using a Ruttner water sampler (Model 11.001, DK 8600 Silkeborg, Denmark). The five water samples from each pond were pooled in a 20 L-plastic bucket, then vigorously mixed, and a 2,000 mL subsample was collected into a sterilized borosilicate glass bottle. The samples were kept at 4°C for further bacterial analysis. Aliquots of the same samples were also used for the determination of total suspended solids (TSS), total ammonia nitrogen (TAN) and nitrite (NO_2^--N) using standard methods (APHA 2017). Water temperature, pH, and redox potential (ORP) were measured in-situ with a portable HANNA HI-9822 Multi-Parameter Water Quality Meter (Table 2).

Table 2

Physico-chemical water quality parameters and methods for analyses

Parameters	*Methods for preservation*	*Methods for analysis*
Temperature (°C)		HANNA HI-9822 Multi-Parameter Water
pH	Directly measured	Quality Portable Meter
ORP (mV)		
TSS (mg L^{-1})	Stored at 4°C	2540-D. Gravimetric method
TAN (mg L^{-1})	Stored at 4°C	4500-NH_3-F. Phenate method
NO_2^--N (mg L^{-1})	Stored at 4°C	4500-NO_2^--E. Colorimetric method

Sediment samples. Sediment samples of approximately 300 g wet weight were collected from five different sites using a device for sampling pond sediment as described by Somsiri et al (2006). Samples from each pond were collected, homogenized and then placed into labeled aseptic sample bags, transported to the laboratory in an icebox and then stored at 4ºC for further analysis.

DNA sample extraction. Total genomic DNA was extracted from each water sample using a DNeasy PowerWater Kit (Qiagen, Valencia, California, USA). Bacterial DNA was extracted from sediment using a DNeasy PowerSoil Pro Kit (Qiagen, Valencia, California, USA). In both cases, the procedures specified by the manufacturer were followed. The extracted DNA was then stored at -20ºC until use for metagenomic sequencing analysis (Huynh et al 2019).

16S metagenomics sequencing and data processing. Tag pyrosequencing of the V1-V9 region of the 16S rRNA gene was amplified using general bacteria primers: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Miller et al 2013). Primers were 5'-barcode tagged, and each specific barcode was assigned a specific DNA sample. The final PCR products were quantified and pooled together based on equal molarity. A negative control was also included to ensure there was no contamination of reagents. TruSeq DNA Sample Preparation Kits (Illumina) were used for the library construction and then the prepared library quantified using TruSeq Nano DNA Sample Preparation Kits (Illumina), before loading on to the up-to-date NGS sequencer, Illumina MiSeq® platform. The final pooled library was sequenced on Illumina Miseq/Miniseq with a 300-cycle flow cell. The sequencing was performed with PhiX spike-in. All the procedures for sequencing using the Illumina platform were performed at the KTEST High-Tech Analytical Center, Ho Chi Minh City, Vietnam.

Taxonomic analysis. Adapter primers and low-quality sequences were removed from the raw reads using Cutdapt and Trimmomatic Amplicon sequence variants (ASVs) were inferred from raw reads using the QIIME2-DADA2 pipeline. Chimeric sequences were also removed with the QIIME2-DADA2 pipeline. Taxonomy assignment was performed using classify-consensus-blast from QIIME2 (2020.2.0) with the SILVA database. SILVA provides comprehensive, quality checked and regularly updated datasets of aligned small (16S/18S, SSU) and large (23S/28S LSU) subunits of ribosomal RNA (rRNA) sequences for all three domains of life (Bacteria, Archaea and Eukarya). In 16S metagenomics operational taxonomic unit (OTU) approaches, the non-chimeric sequences from trimmed sequences after the quality control step were analyzed with the QIIME software package, version 1.80, using closed-reference OTU picking with the Greengenes 16S rRNA Taxonomy Database base pre-clustered at 97% identity (gg_13_8) (DeSantis et al 2006). Finally, the OTUs were classified for taxonomic assignment in the Greengenes database. The singletons were removed, and the composition visualization, alpha-diversity and beta-diversity analyses were performed with QIIME2 (2020.2.0). If applicable, taxa that had significant abundance among different groups were identified by LefSe (Segata et al 2011) using default settings. Taxonomic levels from phylum to genus and the result of clustering were visualized on principal components analysis (PCA) plot. In addition to PCA, a heatmap representation of genus-level bacterial composition and the abundance of genus for samples was also performed using a web tool for visualizing clustering of multivariate data (http://biit.cs.ut.ee/clustvis/) (Metsalu & Vilo 2015; Huynh et al 2019).

Table 3

Summary of read processing

Sample ID	*Raw sequences (paired)*	*Input*	*Filtered*	*Percentage of input passed filter*	*Denoised*	*Non-chimetric*
Catfish_Wat_1	251,905	298,024	296,799	99.59	288,969	26,1483
Catfish_Wat_2	251,247	113,684	102,870	90.49	95,650	92,925
Catfish_Wat_3	132,972	49,799	44,889	90.14	40,173	39,315
Catfish_Sed_1	250,349	98,842	91,509	92.58	71,375	71,316
Catfish_Sed_2	250,901	106,916	99,412	92.98	78,579	78,390
Catfish_Sed_3	249,087	75,803	70,448	92.94	53,358	53,358

Evaluation of shared microbiota community. To determine the microbial community shared between sediment and water samples, the presence/absence of OTUs in each sample was analyzed. OTU to be shared was considered when it appeared in both sediment and water samples. Additionally, unique OTUs are not shared between the sediment and water samples. These OTUs could represent transient bacterial populations because they could appear in only one of the samples. Hence, to avoid putative transitory microorganisms in the samples, only OTUs that had reads in all the sediment or water

sample replicates were used to determine the microbial community shared between sediment and water. In this regard, only OTUs that had reads in all the replicates of sediment sample but did not have reads in water sample were selected as unique OTUs. Similarity and difference of species between sediment and water samples are shown with Venn diagrams based on the previous study by Huynh et al (2019).

Operational taxonomic units (OTUs) present in all sediment and water sample replicates were considered to constitute the resident microbial community common to both sediment and water. This avoided including putative transitory microorganisms, particularly in water samples, in the microbial community common to both sediment and water. OTUs present in all sediment sample replicates, but not present in water samples, were considered to be unique. Similarities and differences in species between sediment and water samples were characterized in Venn diagrams (Huynh et al 2019).

Statistical analysis. Indices of microbial community diversity, including species, number of species, Margalef's species richness (d), Pielou's evenness (J), Shannon diversity indexes, PCA eigenvector plots and cumulative dominance (%) plot were calculated using Plymouth Routines in Multivariate Ecological Research (PRIMER) version 6.1.5 (Clarke & Gorley 2006). The correlations between the bacterial community structure and the environment factors were explored using the Pearson correlation analysis. The statistical analyses were conducted using SPSS (version 22.0).

Results

Water quality parameters. Table 4 shows the results for pond water quality. Temperature was in a suitable range for striped catfish. TSS, TAN and NO_2^--N levels were high in all the three ponds. All ponds had a relatively low pH compared to the normal requirements for freshwater fish.

Table 4

Water quality parameters in *Pangasianodon hypophthalmus* ponds

Pond ID	*pH*	*Temp. (^oC)*	*ORP (mV)*	*TSS (mg L^{-1})*	*TAN (mg L^{-1})*	*NO_2^--N (mg L^{-1})*
Catfish pond 1	6.7	28.1	114	207	1.74	1.47
Catfish pond 2	6.8	28.4	131	175	2.06	1.03
Catfish pond 3	6.7	27.9	96	188	1.65	0.98

Temp-temperature; Catfish-striped catfish; Pond-grow-out pond; 1, 2 or 3-one of the three ponds sampled.

Total taxa in striped catfish pond water and sediment. After removing singletons, non-redundant sequences were generated. The sequences were clustered into OTUs at 97% similarity level. The Green genes gg_13_8 reference OTU collection bank was used as a reference. OTUs obtained were assigned to 23 phyla, 40 classes, 92 orders, 133 families and 242 genera with taxonomic names in the striped catfish ponds (Figure 1).

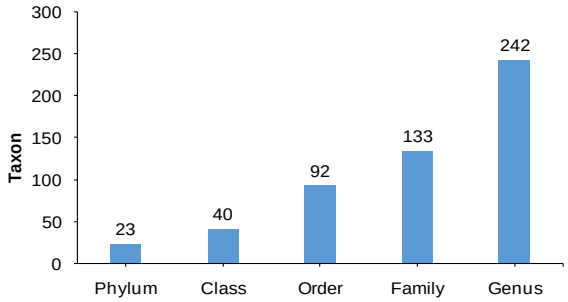

Figure 1. *Pangasianodon hypophthalmus* taxa in water and sediment.

A total of 200 OTUs were identified in striped catfish pond water (Figure 2). The genera *Meiothermus*, *Ochrobactrum*, *Negativibacillus*, *Komagataeibacter* and *Xanthomonas* were

unique to pond water and were not found in the sediment. In contrast, only 74 OTUs were found in the sediment (Figure 3), of which the genera *Oligoflexus*, *Silvanigrella*, *Anaerolinea*, *Azoarcus* and *Haliangium* were confined to the sediment and were not found in the pond water.

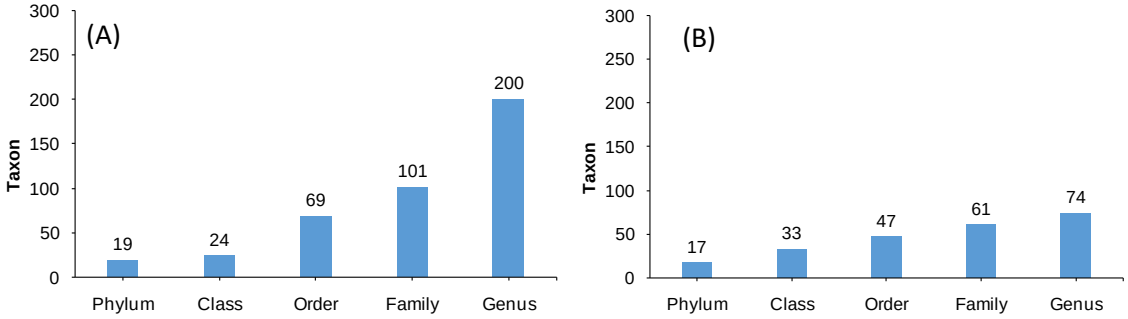

Figure 2. *Pangasianodon hypophthalmus* taxa in pond water (A) and sediment (B).

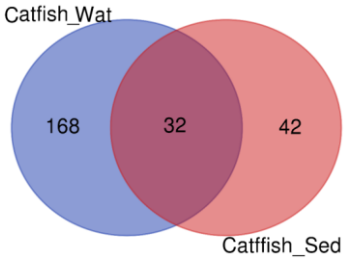

Figure 3. Venn diagram displaying similarities and differences in observed genera between *Pangasianodon hypophthalmus* water and sediment.

Shared genera between striped catfish pond water and sediment. In total, 32 OTUs were presented in both water and sediment, while 168 OTUs were restricted to pond water and 42 OTUs were found only in sediment.

Relative abundance at phylum and genera in pond water and sediment. The following phyla were more abundant in pond water than in sediment: Proteobacteria (water, 68%; sediment, 15%), Bacteroidota (water, 12%; sediment, 4%), Firmicutes (water, 6%; sediment, 4%) and Fusobacteriota (water, 8%; sediment, 2%) (Figure 4).

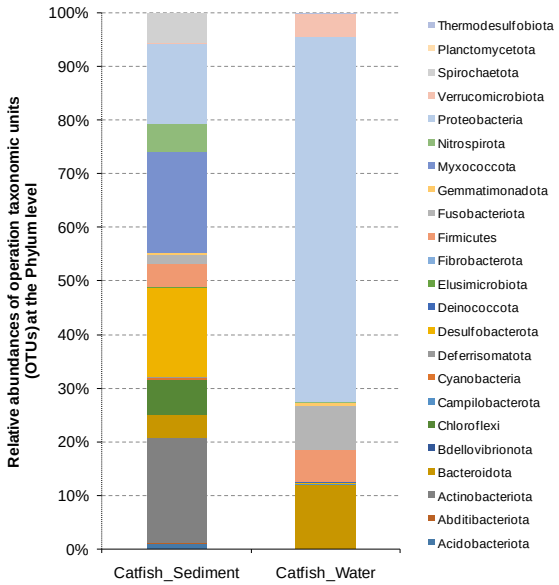

Figure 4. Relative abundances of operational taxonomic units (OTUs) at phylum level in *Pangasianodon hypophthalmus* ponds.

At the generic level, the following genera were more abundant in pond water than in the sediment: *Novosphingobium* (water, 42%; sediment, 1%), *Cetobacterium* (water, 23%; sediment, 3%), *Bacillus* (water, 2%, sediment, 0.5%) (Figure 5). On the other hand, the following genera were more abundant in the sediment than in the pond water: *Desulfovibrio* (sediment, 0.6%; water, 0.1%) *Nitrospira* (sediment, 22%; water, <1%) (Figure 5). Data were derived from an OTU table with OTUs of >0.1% of the total read count to simplify visualization of the result.

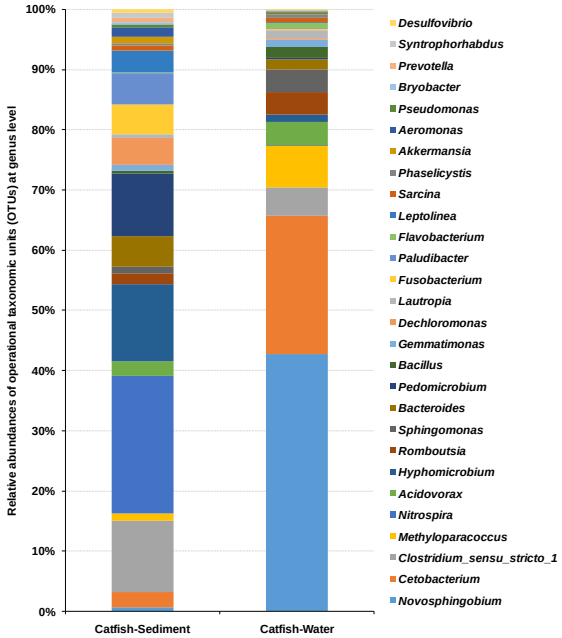

Figure 5. Relative abundances of shared OTUs at genus level in *Pangasianodon hypophthalmus* pond.

Principal component analysis (PCA) and heatmap analysis. Genera with a relative abundance of >0.1% were used for PCA and heatmap analyses. The contributions of principal component 1 (PC1) and PC2 were 71.8% and 15.9%, respectively, and together they explained 87.7% of the variation in the dataset (Figure 6).

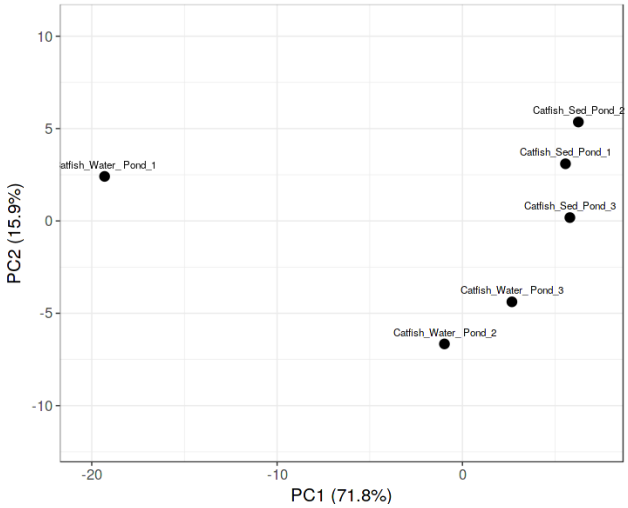

Figure 6. Principal components analysis of genera (>0.1% relative abundance) between water and sediment in *Pangasianodon hypophthalmus* ponds. Water: water sample; Sed: sediment sample; 1, 2 or 3 refers to one of the three ponds sampled.

PCA of microbiota revealed striped catfish pond clustered into distinct groups. Catfish_Water_Pond_1 clustered distinctively from the other samples (Figure 6),

confirming its dissimilarity with the other Catfish_Water samples. In addition, Catfish_Water_Pond_1 had the highest relative abundancy of genera (Figure 7).

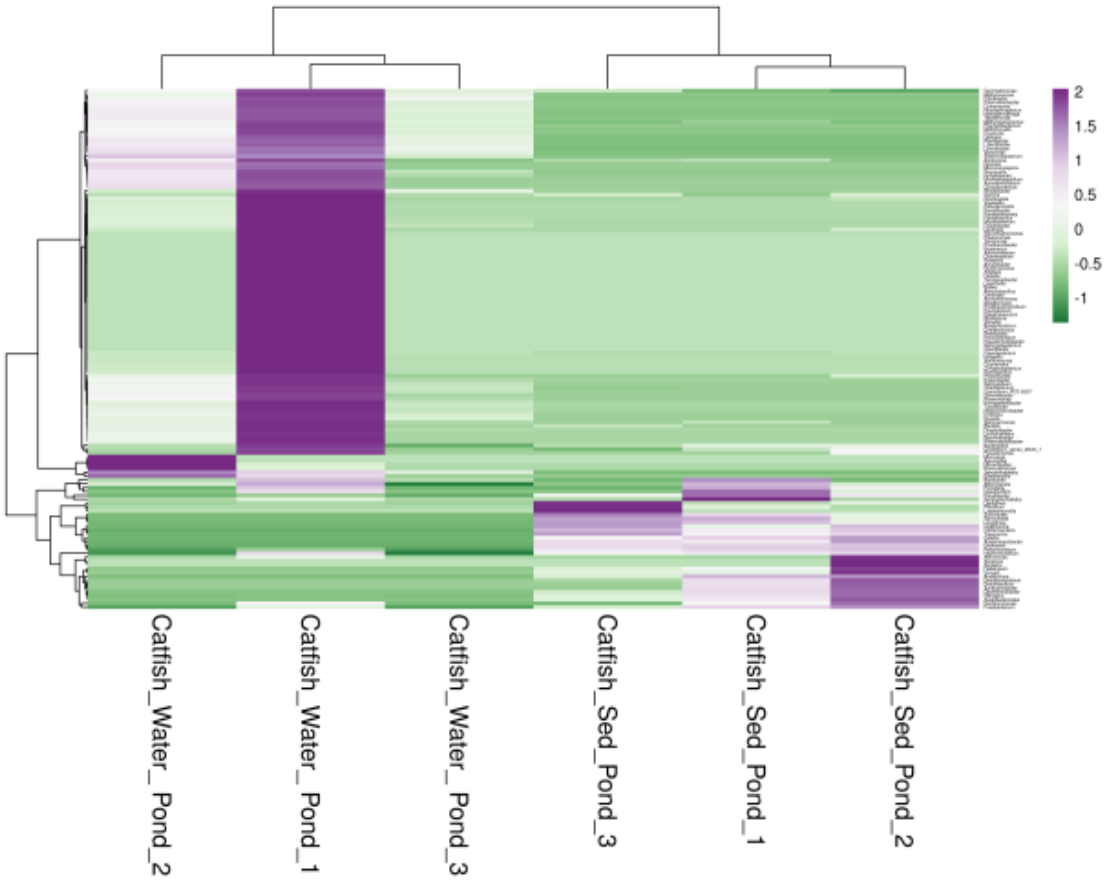

Figure 7. Heatmap of genera (>0.1% relative abundance) in ponds between *Pangasianodon hypophthalmus* pond water and sediment. Water: water sample; Sed: sediment sample; 1, 2 or 3 refers to one of three ponds sampled.

Biodiversity similarity analysis. Catfish_Water_Pond_2 and Catfish_Water_Pond_3 had a Bray Curtis similarity index of 57.1% (Figure 8), indicating a very similar bacterial composition. On the other hand, the Bray Curtis similarity index between Catfish_water_Pond_1 and Catfish_water_Pond_3 was only 24.3% (Figure 8), indicating a significant difference in bacterial populations between these two ponds. Interestingly, the obtained results revealed that the bacteria population in water was quite different from that in the sediment, with the similarity of less than 5% (Figure 8).

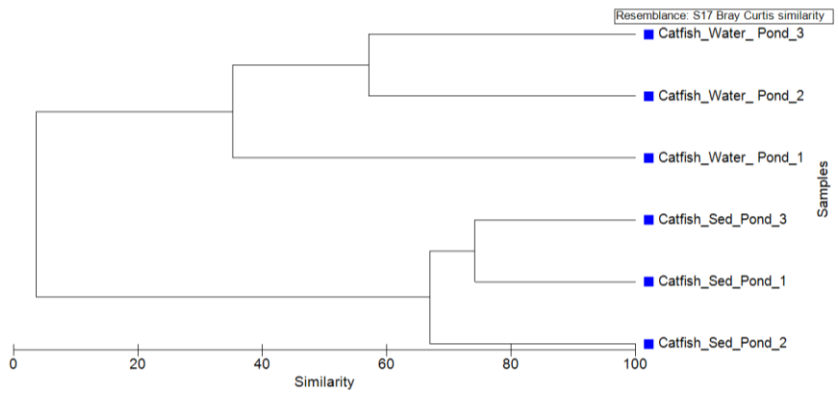

Figure 8. Similarity of genera in water and sediment among *Pangasianodon hypophthalmus* ponds. Water: water sample; Sed: sediment sample; 1, 2 or 3 refers to one of the three ponds sampled.

Biodiversity indices. Generally, pond water had greater species richness (d) than sediment (Table 5). In water, the highest count number of the genera (N), Margalef's species richness (d) and Shannon-Wiener index (H') were detected in the Catfish_Water_Pond_1, followed by Catfish_Water_Pond_2. In contrast, Margalef's species richness and Shannon-Wiener index were both lower in the pond sediment (Table 5). These data indicate that the pond water overall biodiversity was higher than in the sediment.

Table 5

Indices of *Pangasianodon hypophthalmus* pond microbial diversity

Sample	*N*	*d*	*J'*	*H'*
Catfish_Water_Pond_1	118,063	16.35	0.7347	3.863
Catfish_Water_Pond_2	39,591	8.029	0.7586	3.379
Catfish_Water_Pond_3	16,309	4.639	0.7486	2.866
Catfish_Sed_Pond_1	14,841	4.581	0.7975	3.036
Catfish_Sed_Pond_2	19,016	5.684	0.7616	3.079
Catfish_Sed_Pond_3	12,806	4.124	0.7307	2.695

Total number of individuals in the sample; d-Margalef's species richness; J'-Pielou's evenness; H'-Shannon-Wiener index.

Dominance plot for catfish pond microbiota. The plot of k-dominance curves of bacterial populations in pond water and sediment is shown in Figure 9. Catfish_Sed_Pond_3 had a higher species dominance, shown by the curve with a higher slope, which indicates a high environmental stress. The higher curve in Catfish_Sed_Pond_3 indicates less microbial diversity and less species evenness (J') (Table 5). The curves between Catfish_Water_Pond_2 and Catfish_Sed_Pond_1, Catfish_Sed_Pond_2 and Catfish_Sed_Pond_1 and Catfish_Sed_Pond_3 and Catfish_Sed_Pond_1 intersections (Figure 9) illustrate differences in the dominance and species richness, indicating a codominance. Non-compatibility happens once there is a marked difference in the number of genera and when one assemblage shows a higher incidence of codominance compared to other assemblages. The crossings show a slight variation in the number of genera.

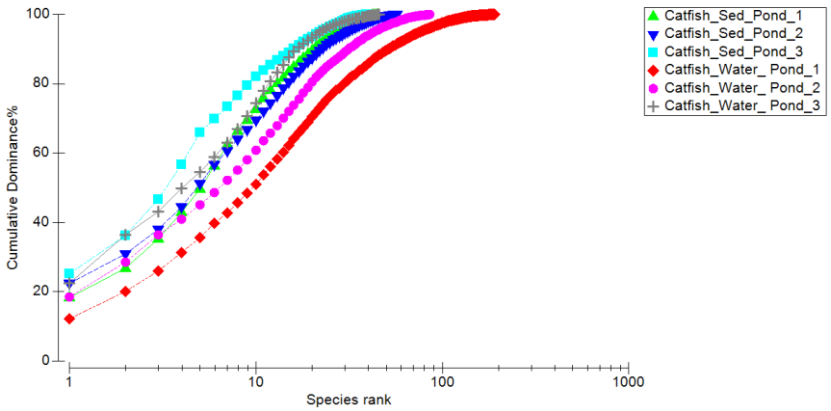

Figure 9. Plot of k-dominance curves of bacteria population in *Pangasianodon hypophthalmus* pond water and sediment. 1, 2 or 3 refers to one of the three ponds sampled.

Catfish_Water_Pond_1 has a lower slope. The curves Catfish_Sed_Pond_1, Catfish_Sed_Pond_2 and Catfish_Water_Pond_3 are grouped closer to each other, thus showing they are more similar to each other than they are to the other curves. The k-dominance curves stop at different points show that microbial species richness differs between the three ponds, both in the sediment and in the water (Figure 9).

Correlations between the bacterial community and environment factors. The top 30 most abundant genera in the water in three catfish ponds were selected to evaluate the correlation, in their community, with environmental variables (Table 6). Of all the specific classified genera identified, *Bacillus* (R=1.000, p<0.05), Clostridium_sensu_stricto_1 (R=1.000, p<0.05), *Emticicia* (R=0.998, p<0.05), *Methylomonas* (R=1.000, p<0.05), *Mycobacterium* (R=1.000, p<0.01), *Nubsella* (R=0.999, p<0.05), *Romboutsia* (R=0.999, p<0.05), *Runella* (R=1.000, p<0.01), Turicibacter (R=1.000, p<0.05) and *Xanthomonas* (R=0.998, p<0.05) were positively correlated with NO_2^--N.

Table 6

Pearson correlation coefficient of dominant genera with environmental variables

Genus	*pH*	*Temp.*	*ORP*	*TSS*	*TAN*	*NO_2^--N*
Acidovorax	-0.033	0.367	0.485	0.617	0.176	0.922
Bacillus	-0.437	-0.044	0.088	0.884	-0.240	1.000*
Cetobacterium	-0.282	0.123	0.252	0.794	-0.075	0.990
*Clostridium*_sensu_stricto_1	-0.445	-0.052	0.079	0.888	-0.248	1.000*
Comamonas	-0.208	0.197	0.324	0.746	0.001	0.976
Emticicia	-0.360	0.040	0.171	0.842	-0.158	0.998*
Gemmobacter	-0.092	0.312	0.434	0.662	0.118	0.943
Hydrogenophaga	-0.175	0.231	0.356	0.723	0.034	0.968
Limnobacter	-0.141	0.264	0.388	0.699	0.068	0.959
Limnohabitans	-0.251	0.154	0.282	0.774	-0.043	0.984
Luteolibacter	-0.217	0.188	0.316	0.752	-0.009	0.978
Methylocystis	-0.298	0.106	0.235	0.804	-0.092	0.992
Methyloparacoccus	-0.276	0.128	0.257	0.790	-0.070	0.989
Methylomonas	-0.438	-0.044	0.087	0.884	-0.240	1.000*
Mycobacterium	-0.418	-0.023	0.109	0.874	-0.219	1.000**
Novosphingobium	-0.177	0.228	0.354	0.724	0.032	0.968
Nubsella	-0.465	-0.075	0.056	0.898	-0.270	0.999*
Polynucleobacter	-0.224	0.182	0.309	0.756	-0.016	0.979
Phenylobacterium	-0.309	0.094	0.224	0.811	-0.104	0.993
Ramlibacter	-0.157	0.248	0.373	0.710	0.053	0.963
Reyranella	-0.051	0.350	0.470	0.631	0.159	0.929
Romboutsia	-0.384	0.015	0.145	0.855	-0.183	0.999*
Rhodobacter	-0.631	-0.270	-0.142	0.967	-0.455	0.968
Runella	-0.418	-0.023	0.108	0.874	-0.219	1.000**
Sphingomonas	-0.341	0.061	0.191	0.830	-0.137	0.997
Stenotrophomonas	-0.492	-0.105	0.026	0.911	-0.299	0.997
Turicibacter	-0.395	0.002	0.133	0.861	-0.195	1.000*
Variovorax	-0.063	0.339	0.460	0.640	0.147	0.933
Xanthomonas	-0.473	-0.084	0.047	0.902	-0.279	0.998*

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

Discussion. An aquaculture pond has many types of bacteria, most of which obtain energy by processing organic matter (Boyd 2019). This microbial ecosystem comprises a complex mixture of obligate aerobic bacteria that require oxygen, facultative aerobic bacteria that can live in aerobic or anaerobic conditions, and obligate anaerobic bacteria that cannot live in the presence of oxygen, including chemotrophs that use inorganic compounds such as nitrate, manganese, iron and sulphate as electron acceptors (Boyd 2019). Among other things, this bacterial community plays a key role in processing fecal and other organic waste, and thus improving the water quality in aquaculture ponds, and the composition and abundance of gut microbiota in fish (Del'Duca et al 2015; Jing et al 2021). Since solid excrement and uneaten feed accumulate on the pond's bottom, the upper layers of the pond's bottom might be expected to have a greater diversity and abundance of bacteria than the water column above. For example, Liu et al (2020)

reported that the number of valid sequences in water samples ranged from 274,585 to 350,362, while the number of valid sequences in sediment samples ranged from 298,070 to 356,672. After assignment of the effective sequence tags to different phylogenetic bacterial taxa, those authors concluded that sediment samples had a higher abundance and diversity of microbial community than water samples from the grass carp, tilapia and hybrid snakehead fish ponds. However, in contrast, we found that bacterial biodiversity in pond water was almost twice than in the sediment. Differences between datasets could be related to the number of samples, fish species, pond design, methods of sampling and analytical procedures (primers, clone library, regions of the 16S rRNA amplified etc.).

A number of studies of bacterial diversity and abundance have found Proteobacteria to dominate the bacterial communities of water in fish ponds (Qin et al 2016; Liu et al 2019). We also found Fusobacteriota to be an important part of this bacterial community, but unlike Qin et al (2016), our study did not find Cyanobacteria or Bacteroidetes to be important components in the water of striped catfish ponds. In our study, the four most dominant phyla in the sediment were Myxococcota, Desulfobacterota, Proteobacteria, Spirochaetota and Bacteroidota.

At the level of genus, *Novosphingobium* had higher abundance in pond water than in sediment, with the relative abundance of 42.6 and 0.65%, respectively. *Novosphingobium* is an aerobic gram-negative genus that reduces nitrate to nitrite (Tiirolla et al 2005). In addition, pond water also contained an abundance of the genus *Cetobacteri*um (23%), a member of the family Fusobacteriaceae and a common component of the microbiota of other freshwater fish ponds (Ramirez et al 2018). The most common genus in the sediment was *Nitrospira* (22.9%), a group of chemolithoautotrophic bacteria that oxidize nitrite to nitrate (Daims & Wagner 2018), with some strains being reported to be capable of full nitrification from ammonium to nitrite to nitrate (Daims et al 2015). The sulphate reducing genus *Desulfovibrio* was also common, an indication that the sediment of catfish ponds contains high levels of sulphur and high organic loading and anaerobic conditions, creating excellent conditions for sulfate reduction and sulfide generation (Boyd & Tucker 1998), as implied by the Venn diagram in Figure 3 and by the UPGMA analysis in Figure 8. The potential for development of anoxia at or near the pond bottom increases with the pond depth (De Silva & Phuong 2011). Our values for the Shannon-Wiener biodiversity index (H') in both water and sediment were towards the lower end of the range reported by Qin et al (2016) and Jing et al (2021), and much lower than the reported values of 7.793 for grass carp pond water and 9.533 for large mouth black bass pond water (Liu et al 2020).

The high Bray Curtis similarity index of 74.2% for sediments in Catfish_Sed_Pond_1 and Catfish_Sed_Pond_3 could be associated with their similar pH, TSS and TAN levels, and suggest that the amount of uneaten feed settling on the bottom is much the same in both ponds. Additionally, striped catfish pond water is more diverse and has more species richness (d) than sediment. Catfish_Water_Pond_1 has more species richness, and this could be because of the high level of TSS providing attachment sites to various particle-associated bacteria species. *Anaeromyxobacter* genus, which uses nitrate as a terminal electron acceptor, was abundant in Catfish_Water_Pond_1, indicating that the end products of the nitrification process were high; thus, a high microbial activity in the water of Catfish_Water_Pond_1 can be assumed.

In this study, the Illumina MiSeq sequencing analysis of water and sediment samples yielded 705,927 filtered sequences, with 596,787 non-chimeric sequences and assigned to 200 genera (19 phyla) and 74 genera (17 phyla) in water and sediment samples, respectively. Martınez-Porchas & Vargas-Albores (2017) reported that the Illumina MiSeq sequencing analysis of fifty water samples yielded 745,337 sequences in the V4-V5 hypervariable regions of the 16S rRNA gene. This is not consistent with the results obtained in the present work due to the difference in the 16S rRNA gene amplified region. The most common bacterial diseases of striped catfish are hemorrhagic disease caused by *Aeromonas hydrophila* and bacillary necrosis of Pangasius (BNP) caused by *Edwardsiella ictaluri* (Dung et al 2008; Pokhrel & Oanh 2021). In this study, at the genus level, *Aeromonas* and *Edwardsiella* were detected in the sediment, but their relative abundances were low in water (0.032 and 0.073% of the total reads, respectively).

Aeromonas had 0.45% relative abundance in the sediment. Another pathogen, *Flavobacterium columnare*, had a relative abundance of 1.02% in the pond water and 0.1% in sediment. This pathogen is the aetiological agent causing white patch disease in farmed catfish *P. hypophthalmus* fingerlings, leading to high mortality rates in commercial hatcheries and ponds in the Mekong Delta. Unfortunately, the OTUs at species level of these pathogenic bacteria were unassigned in this study.

Among the phylum Firmicutes, the members of the genus *Bacillus* are probably the most extensively studied, as beneficial microorganisms with application in aquaculture. *Bacillus* as probiotics possesses characteristics including their ability to produce spores and metabolites which are effective against a wide range of pathogenic microbes. *Bacillus* species have demonstrated great ability in the maintenance of water quality in aquaculture (Hlordzi et al 2020). In this study, relative abundances of *Bacillus* in water and sediment of ponds were 1.84% and 0.52%, respectively. The findings from our study indicate that the dominances of *Novosphingobium*, *Cetobacterium*, *Desulfovibrio*, *Nitrospira* or *Bacillus* play an important role in water quality improvement, in the striped catfish ponds. Further studies on isolation and selection of potentially probiotic bacteria for water treatment in striped catfish are imperatively needed.

Conclusions. The bacterial community structure differed between water and sediment of striped catfish ponds. Microbial biodiversity is more even in striped catfish pond sediment than in water. A total of 23 phyla, 40 classes, 92 orders, 133 families and 242 genera were assigned. The majority of the OTUs were shared between water and sediment and comprised 32 OTUs. The pathogenic bacteria genera *Aeromonas*, *Flavobacterium* and *Edwardsiella* were detected in both water and sediment with low relative abundances. *Novosphingobium* is the most abundant genus in water, while the most abundant one in sediment is *Nitrospira*. In addition, the potentially probiotic genus *Bacillus* was also observed in pond water and sediment. In addition, this study provided evidence that changes in microbial communities reflect the status of environmental quality. To our best knowledge, this is the first paper to unravel the bacterial population in the striped catfish ponds in the Mekong Delta, Vietnam.

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