16S rRNA-Based metagenomic analysis of microbial communities associated with wild *Labroides dimidiatus* from Karah Island, Terengganu, Malaysia

Nurul Ashyikin Noor Ahmad[a], Muhammad Danish-Daniel[a,b], Okomoda Victor Tosin[c], Nur Ariffin Asma Bt.[a,d,*]

[a] *School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[b] *Institute of Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[c] *Department of Fisheries and Aquaculture, University of Agriculture Makurdi, PMB, 2373, Benue State, Nigeria*
[d] *Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

A R T I C L E   I N F O

A B S T R A C T

This study was designed to evaluate the bacterial composition of the *Labroides dimidiatus* and its surrounding water. Fish and carriage water samples were obtained from corals of the Karah Island in Terengganu Malaysia. DNA was extracted and the bacteria communities on the skin mucus and stomach as well as water sample were classified (to family level) using the 16S rRNA-based metagenomics analysis. 1,426,740 amplicon sequence reads corresponding to 508 total operational taxonomic units were obtained from the three metagenomics libraries in this study. The *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria* and *Fusobacteria* were the most dominant bacterial phyla in all samples. A total of 36 different classes and 132 families were identified, many of which had shared presence in all samples while others were exclusive to different sample. Thirty-three of these were identified as pathogenic zoonotic bacterial. The results obtained indicate a strong influence of host environment on the composition of its microbiota. Knowing the composition of the microbiota is the first step toward exploring proper management of this ornamental fish in captivity.

1. Introduction

Interest in ornamental fish keeping is rapidly growing globally. It is currently considered the second largest global hobby next to photography [1]. However, majority of the marine ornamental fishes are caught from the wild [2]. Hence, understanding the microbial community associated with wild ornamental fishes could provide useful information about the husbandry requirement, health management, as well as help dictate effective biosecurity measure for these fishes to be raised in captivity. Gerzova et al., [3] had earlier suggested the fact that ornamental fishes are grossly understudied with respect to microbial communities, and could be potential sources of pathogenic infections. This is a public health concern in the pet industry due to the possibility of cross infections (between fishes) and zoonotic infections (between fish and man). The knowledge of the susceptibility of these fish to different microbes in the wild could help dictate measures that would prevent transfer of potentially pathogenic bacteria from infected fish to others fish group reared together in captivity and vice versa.

The *Labroides dimidiatus* is a very popular marine ornamental fish accounting for about 36% of the total ornamental imports to the United States of America [4]. This species is part of a larger group of fishes commonly known as the "Bluestreak cleaner wrasse". The group name reflects their "cleaning behavior" in the ocean; hence, they play an important ecological role in the coral reefs [5–7]. Generally, they specialize in removing ecto-parasites, diseased and injured tissue, as well as unwanted food particles from the fishes that visit them [8]. This "cleaning" attribute has also been exploited in the Aquaculture industries in some part of the world. For instance in Norway, different wrasse species are commonly used for the control of sea-lice in the Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) industries [9]. Hence, the environmental condition as well as the life cycles of these fishes could predispose them to a wide range of microbial infections.

However, there is paucity of information on the bacteria composition associated with many Bluestreak cleaner wrasse

species. One of the first steps to ensuring a heathy husbandry condition for aquarium fishes is to have a good understanding of the microbiome balance [10]. More so, knowledge of the bacterial communities (pathogenic or not) would help understand the complex relationship between the bacteria and its host (fish). In this study therefore, the bacterial composition on the skin and in the stomach of *L. dimidiatus* as well as that around in the surrounding water were investigated. We employed the metagenomics technique combined with 16S rRNA gene and the Next Generation Sequencing (NGS) analyses to identify and compare the diversities of bacteria observed.

2. Materials and methods

2.1. Sample collection and preparation

Samples of *L. dimidiatus* (between 0.5–2.8 g) were obtained from different location at the coral area of the Karah Island, Terengganu Malaysia. The fish caught were immediately transported to the Anatomy and Physiology Laboratory in the School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu for analysis. Ten healthy fish were tranquilized with 150 mg/1 solutions of tricaine methane sulphonate (MS222) [11] and killed by pitching. They were gently washed thrice with sterile seawater to remove the dirt on the fish without compromising the microbial colonies on the skin. The skin mucus was collected by dorsolateral scraping of the surface of the dead *L. dimidiatus* specimens with the aid of a sterile scalpel [12]. The collected mucus (i.e. ten samples from the ten fish) was placed in 1.5 ml microcentrifuge tubes, centrifuged for 10 min (12, 000 × g for at 4 °C) and stored at −80 °C until DNA analysis was done. The technique for sample collection from the fish stomach used in this study was a modification of earlier technique reported by Balcázar et al., [12]. The stomach of the ten fish samples were carefully removed and flashes frozen in 50 ml sterile falcon tubes, and then stored at −80 °C until DNA extraction was done. Water samples for analysis (ten in number) were earlier collected simultaneously during fish sampling using the method adopted by Smith et al., [13]. In brief, water samples were collected in sterilized blue cap bottles, store in cold storage boxes and transported to the laboratory for analysis.

2.2. DNA extraction

DNA was extracted using the NucleoSpin® Tissue Kit (Machery-Nagel, Germany) following the manufacturer's protocol. Samples of the skin mucus were first centrifuged for 5 min (13000rcf) before DNA was extracted. 25 mg of the collected fish stomach was homogenised in 50–75 μL phosphate buffer saline (PBS). The water sample on the other hand was conditioned following the method specified by Wolf et al., [14] before DNA extraction was done. After DNA extraction from the different samples, the quality (102.69 to 381.87 ng/ul) and concentration (1.8–2.08) was determined (using

biodrop machine) and found to within recommended range before storage at −20 °C.

2.3. 16S rRNA bacterial Amplification

The 16S rRNA gene in this study was amplified using the universal bacterial primer set 63 F (5"-CAGGCCTAACACATG-CAAGTC-3") and 1389R (5"-ACGGGCGGTGTGTACAAG-3") [15]. A reaction volume of 50 μL was used for the PCR containing DNA template, 1× Reaction Buffer, 2.5 mM MgCl$_2$, 200 μM of each dATP, dCTP, dGTP and dTTP (Vivantis Technologies, Malaysia), 0.5 μM of each primer and 2 U of Taq polymerase (Vivantis Technologies, Malaysia). The PCR condition were programmed for a 2 min initial denaturation at 95 °C, followed by 24 cycles of denaturation (at 95 °C for 30 s), annealing (at 53 °C for 1 min), and extension (at 72 °C for 2 min) and a final extension at 72 °C for 10 min. The reaction was performed on a PTC-0200 G thermo cycler (Bio-Rad Laboratories, Inc., USA). Beside PCR clean up done to purify the PCR product in this study, it is important to state that a negative control was included in the PCR amplification to ensure there was no contamination of reagents.

2.4. Illumina library generation: amplicon PCR

In a second PCR, 1 μL of amplicon was used. By following the method described by Bartram et al., [16], the V3 hypervariable region of the 16S rRNA genes was selected for this method because of its taxonomic resolution [17], conserved flanking regions [18], and length [19] (≥170 to 190 nucleotides), which is compatible with paired-end 125-base read assembly. Hence, the V3 region of the 16S rRNA gene was amplified using modified 341 F and 518R primers [18]. In addition, the V3 specific priming regions primers were complementary to the standard Illumina forward and reverse primers (Table 1). Hence, the primers contained a 6-bp indexing sequence to allow for multiplexing. Amplification primers were designed with Illumina adapters. PCR amplifications were carried out for each sample, using a reaction volume of 25 μL containing 12.5 μL of 2X KAPA HiFi HotStart Ready Mix, 0.75 μL of each 10μM Forward and Reverse primers, 1 μL of 12.5 ng/μL DNA templates and PCR-grade water up to 25 μL. The PCR conditions for amplification were as follows: 95 °C initial denaturation for 3 min, followed by 15 cycles of denaturation (at 98 °C for 20 s), annealing (at 67 °C for 15 s) and extension (at 72 °C for 15 s), and a final extension at 72 °C for 1 min. The PCR was performed in a DNA Engine thermocycler (Bio- Rad, Mississauga, Ontario, Canada).

Using a gel electrophoresis (2% agarose), the PCR products of the correct size were separated from the primers/primer dimers and recovered using a Gel Extraction and PCR Purification Combo Kit, Spin-column (BioTeke Corporation, Beijing, China). The purified PCR products were then sequenced using Illumina MiSeq Desktop Sequencer (Illumina, Inc.) (Service provided by the Science Vision Sdn. Bhd., Malaysia).

Table 1
Primers profiles used for the Illumina library construction.

Sample	Primer name	Oligonucleotide sequence (5'–3')	Reference
All samples	V3_F	aatcatacggcgaccaccgagatctacactctttccctacacgacgctcttccgatctCCTACGGGAGGCAGCAG	Bartram et al., (2011)
Skin mucus	V3_7R	caagcagaagacggcatacgagat**GATCTG**gtgactggagttcagacgtgtgctcttccccgatctATTACCGCGGCTGCTGG	Bartram et al., (2011)
Stomach content	V3_5R	caagcagaagacggcatacgagat**CACTGT**gtgactggagttcagacgtgtgctcttccccgatctATTACCGCGGCTGCTGG	Bartram et al., (2011)
Carriage Water	V3_13R	caagcagaagacggcatacgagat**CGTACT**gtgactggagttcagacgtgtgctcttccccgatctATTACCGCGGCTGCTGG	Bartram et al., (2011)
All samples	341 F	CCTACGGGAGGCAGCAG	Muyzer et al., 1993
All samples	518R	ATTACCGCGGCTGCTGG	Muyzer et al., 1993

Lowercase letters denote adapter sequences necessary for binding to the flow cell, underlined lowercase are binding sites for the Illumina sequencing primers, bold uppercase highlight the index sequences (the first 12 indexes were obtained from Illumina) and regular uppercase are the V3 region primers (341 F on for the forward primers and 518R for the reverse primers) (Bartram et al., 2011).

2.5. 16S rRNA-Based taxonomic analysis

The generated multi-million reads were trimmed and assembled using Mothur software [20]. Overlapping regions within Illumina paired-end reads were aligned to generate "contigs." If a mismatch was discovered, the paired end sequences involved in the assembly were discarded. All sequences with ambiguous base calls were also discarded. Sequences were then assigned taxonomic affiliations based on naïve Bayesian classification (RDP classifier) [21].

After trimming, screening and alignment of the sequences; they were assigned to operational taxonomic units of six samples of 16S rRNA gene fragments. The sequenced data was connected to server and the fastq file was downloaded. A tab-delimited "oligos" file containing the primer and barcode information was created. Then, the Greengenes reference files from the Mothur website (http://www.mothur.org/wiki/Taxonomy_outline) was downloaded, and the data analysis was performed. The operational taxonomic units (OTU) of the bacterial colonies were defined by a pairwise similarity cutoff of 97% using the Ribosomal Database Project [22] pyrosequencing pipeline.

3. Results

The total DNA successfully purified from skin mucus, stomach and water samples was appropriate for the subsequent PCR amplification (DNA yield ranged between 102.69–381.87 ng/μL; DNA purity ranged from 1.8 to 2.08). A near-full length of ˜1400bp was successfully amplified in all samples for the 16S rRNA gene. Similarly, a ˜330bp fragment was amplified for the nested PCR (with V3 regions) in all samples examined. The secondary amplification created a single amplicon of conserved region for compatibility with Illumina index and sequencing adapters.

3.1. Bacteria composition in Labroides dimidiatus from Karah Island, Terengganu Malaysia

Total number of amplicon sequences reads obtained from the skin mucus and stomach of *L. dimidiatus* as well as the carriage water was 1,426,740 reads (Table 2). The highest reads of 731,548 was obtained from the stomach samples, followed by the skin mucus (455,709 reads), while the carriage water had lower reads of the study (239,483 reads). In contrast, the operational taxonomy units (OTU) in the carriage water was more diverse (227) than those of the skin mucus (164) or stomach samples (117).

3.2. Phyla of associated Bacteria isolated in Labroides dimidiatus from Karah Island, Terengganu Malaysia

Taxonomic analysis of the V3 16S rRNA gene amplicon reads yielded a total of twelve classifiable phyla (Table 3). Five of which were dominant in the entire sample namely; *Proteobacteria* (57.7–65.9%), *Bacteroidetes* (7.7–13.4%), *Firmicutes* (10.4–15.4%), *Actinobacteria* (7.3–12.0%) and *Fusobacteria* (1.3–1.8%). The skin mucus harbored the higher percentage of *Proteobacteria, Bacteroidetes* and *Fusobacteria*. Similarly, the highest percentage of *Actinobacteria* was observed in the stomach. While, the highest percentage of *Firmicutes* observed were identified both in the stomach and the

Table 3
Percentage of bacterial phyla associated with *Labroides dimidiatus* from Karah Island, Terengganu Malaysia.

Phyla[2]	Skin mucus	Stomach content	Carriage water
Proteobacteria	65.9	60.7	57.7
Bacteroidetes	13.4	7.7	12.8
Firmicutes	10.4	15.4	15.4
Actinobacteria	7.3	12.0	8.4
Fusobacteria	1.8	1.7	1.3
Acidobacteria	0.0	0.9	2.2
Spirochaetes	0.0	0.9	0.0
Unclassified	0.6	0.9	0.4
Nitrospira	0.0	0.0	0.4
Planctomycetes	0.6	0.0	0.4
TM7	0.0	0.0	0.4
Tenericutes	0.0	0.0	0.4

[2] Phylum taxonomy and abundance were classified based on a confidence using the RDP Classifier. Values are given as a percentage.

carriage water. However, the carriage water samples contained the highest number of phyla [11] when compared to those identified in the stomach (8 phyla) and skin mucus (7 phyla). In addition to the five abundant and the unclassified phyla identified across all samples, *Acidobacteria*, *planctomycetes*, *Nitrospira*, TM7 and *Tenericutes* were isolated in the carriage water; however, at very low quantities (0.4%). Also, the *Spirochaetes* phylum was a unique phyla detected only in stomach of *L. dimidiatus*.

3.3. Distribution of bacterial community at class level

In this study, a total of 36 different classes of bacteria were identified in this study. The associated bacteria classes in the carriage water were more diverse than those in the skin mucus as well as those in the stomach of *L. dimidiatus*. However, most of these classes were about 1% or less of the total bacterial isolated, hence, limiting their resolution (Fig. 1). In general, the *Gammaproteobacteria* represented about 29.7%. While the *Alphaproteobacteria* and *Betaproteobacteria* were found at 29.7% and 17.91%. *Deltaproteobacteria* and *Epsilonproteobacteria* in the phyla *Proteobacteria* were detected at 2.6% and less than 1% respectively. *Actinobacteria*, *Bacilli*, *Flavobacteria*, were also found at more than 5%. However, *Clostridia*, *Sphingobacteria*, *Deltaproteobacteria*, *Fusobacteria* were found at less than 5%.

3.4. Distribution of bacterial community at order level

To assess the microbial composition, the most abundant class observed; *Proteobacteria* was categorized into different order level, while the remaining "orders" were grouped as "other" (Figs. 2, 3 and 4). The *Gammaproteobacteria*, were the largest chunk of *Proteobacteria* observed in this study, and with the largest number of orders isolated. The most prominent members include the *Alteromonadales* and *Oceanospirillales*. The *Betaproteobacteria* in all the sample groups were dominated by the *Burkholderiales*, while, the *Alphaproteobacteria* were composed primarily of *Rhizobiales*, *Rhodobacterales*, *Caulobacterales* and *Sphingomonadales*. Similarly, the *Bdellovibrionales*, *Desulfovibrionales*, and *Myxococcales* are commonly orders of the *Deltaproteobacteria* found in the carriage water (Fig. 4) and the skin (Fig. 2). However, only the *Myxococcales*

Table 2
Characteristics of 16S rRNA metagenomic libraries of *Labroides dimidiatus* from Karah Island, Terengganu Malaysia.

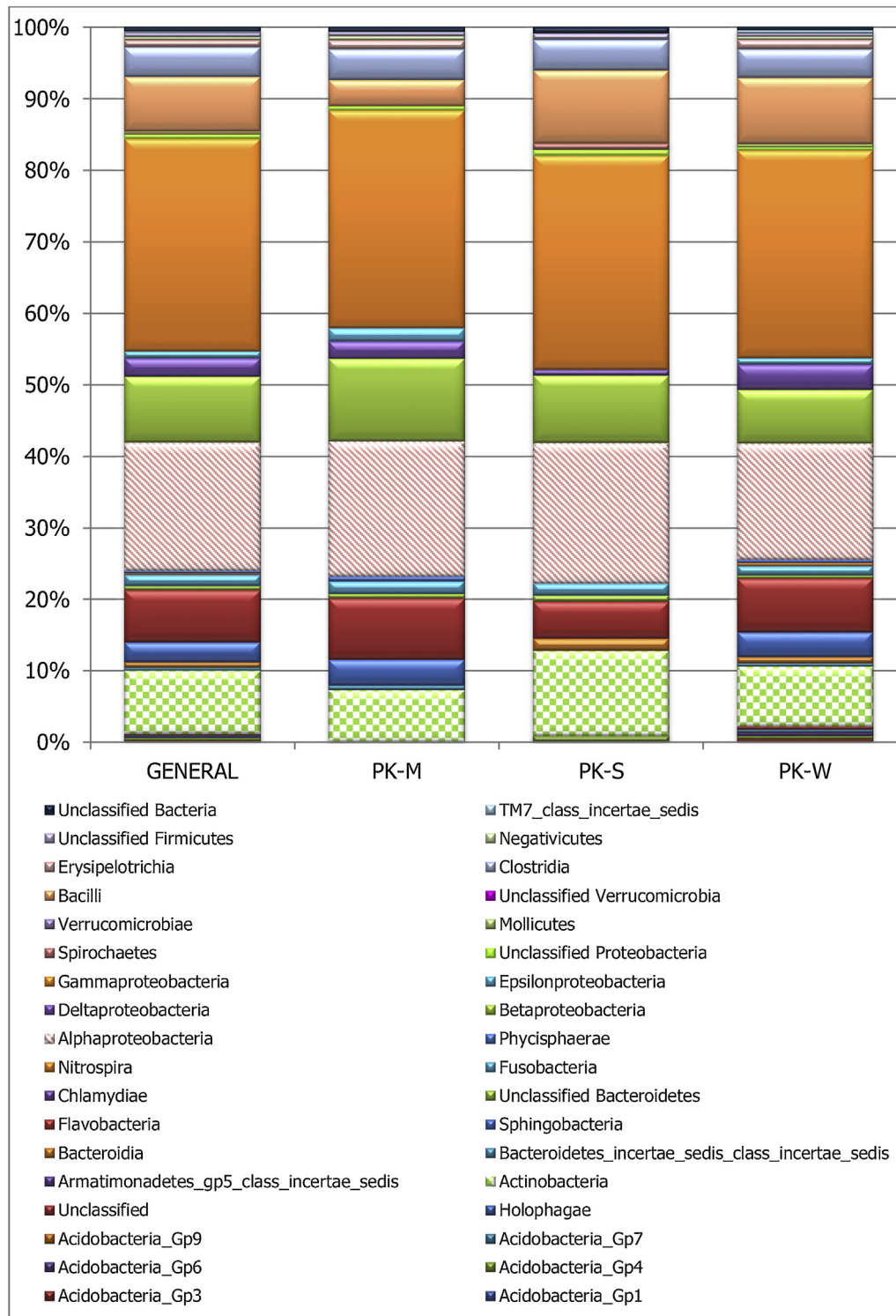|	Skin mucus	Stomach content	Carriage water
Amplicon sequences	455,709	731,548	239,483
Total of single-reads OTUs	164	117	227
Percentage of total single reads OTUs (%)	37.104	26.471	51.357

Fig. 1. Taxonomic diversity and relative abundance the different classes of Bacteria associated with *Labroides dimiditus* from Karah Island, Terengganu Malaysia.
PK = Karah Island, M = skin mucus, S = stomach W = carriage water. General = PK-M + PK-S + PK-W (i.e. the cumulative Bacteria classes associated with the *Labroides dimiditus* as observed in the mucus, stomach and carriage water).

was observed in the stomach for this group of bacterial (Fig. 3). Among the dominate orders grouped as "others" in this study are the *Bacillales*, *Flavobacteriales*, *Actinomycetales*, and *Sphingobacteriales*. Both *Flavobacteriales* and *Sphingobacteriales* are members of the phylum *Bacteroidetes* while *Actinomycetales* is a member of the *Actinobacteria*. These two phyla were earlier reported to be among the dominant isolated phylum after *Proteobacteria* in this study.

3.5. Distribution of bacterial community in Labroides dimidatus by family

A total of 82 and 59 families of bacteria were identified in the skin mucus and stomach respectively, while 116 bacteria families were identified in the carriage water. Forty-three of these families (32.58% of total identified microbes) were present in all three
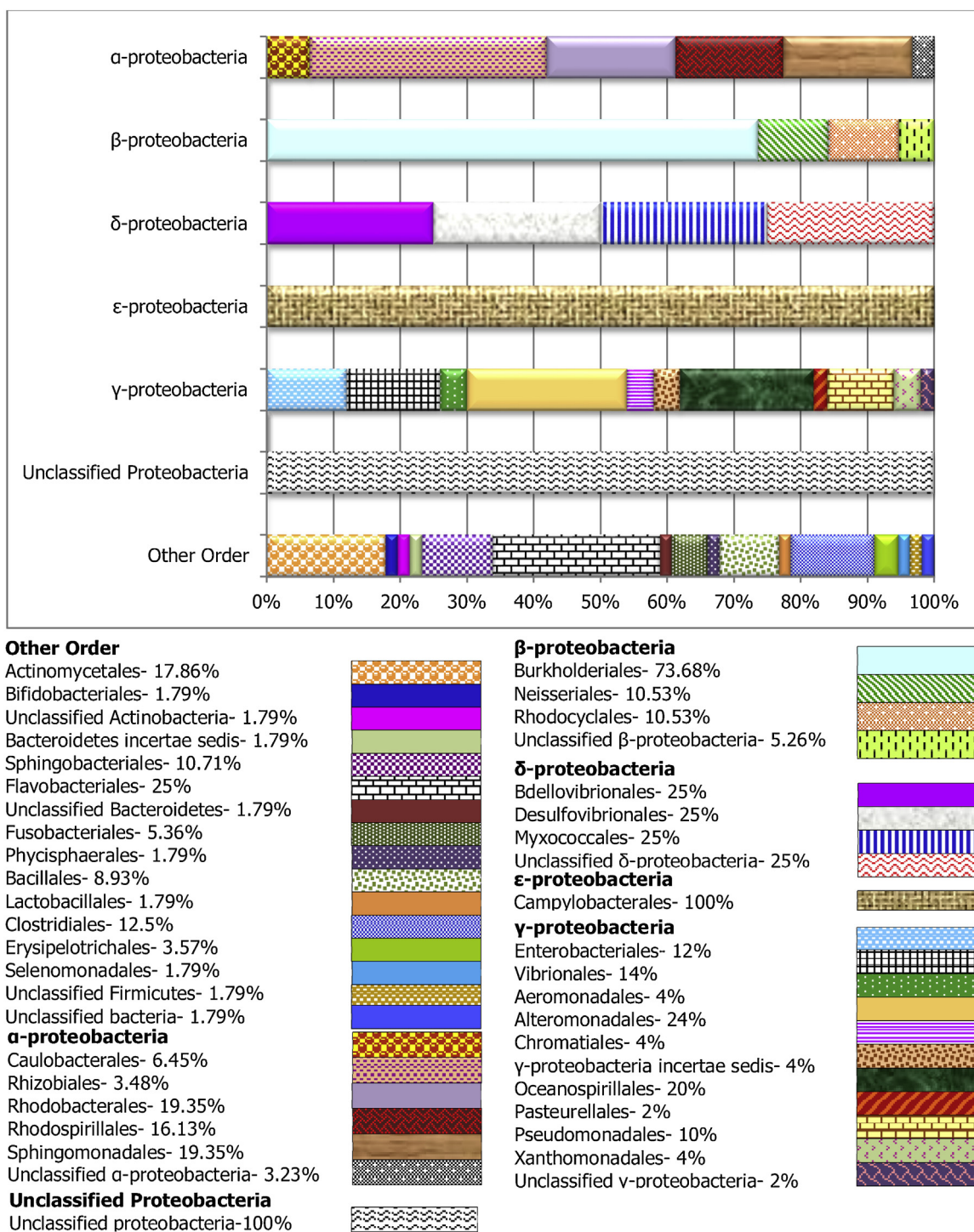
Other Order
Actinomycetales- 17.86%
Bifidobacteriales- 1.79%
Unclassified Actinobacteria- 1.79%
Bacteroidetes incertae sedis- 1.79%
Sphingobacteriales- 10.71%
Flavobacteriales- 25%
Unclassified Bacteroidetes- 1.79%
Fusobacteriales- 5.36%
Phycisphaerales- 1.79%
Bacillales- 8.93%
Lactobacillales- 1.79%
Clostridiales- 12.5%
Erysipelotrichales- 3.57%
Selenomonadales- 1.79%
Unclassified Firmicutes- 1.79%
Unclassified bacteria- 1.79%
α-proteobacteria
Caulobacterales- 6.45%
Rhizobiales- 3.48%
Rhodobacterales- 19.35%
Rhodospirillales- 16.13%
Sphingomonadales- 19.35%
Unclassified α-proteobacteria- 3.23%
Unclassified Proteobacteria
Unclassified proteobacteria-100%

β-proteobacteria
Burkholderiales- 73.68%
Neisseriales- 10.53%
Rhodocyclales- 10.53%
Unclassified β-proteobacteria- 5.26%
δ-proteobacteria
Bdellovibrionales- 25%
Desulfovibrionales- 25%
Myxococcales- 25%
Unclassified δ-proteobacteria- 25%
ε-proteobacteria
Campylobacterales- 100%
γ-proteobacteria
Enterobacteriales- 12%
Vibrionales- 14%
Aeromonadales- 4%
Alteromonadales- 24%
Chromatiales- 4%
γ-proteobacteria incertae sedis- 4%
Oceanospirillales- 20%
Pasteurellales- 2%
Pseudomonadales- 10%
Xanthomonadales- 4%
Unclassified γ-proteobacteria- 2%

Fig. 2. Taxonomic diversity and relative abundance at the order level of bacteria associated with skin mucus sample of *Labroides dimiditus* from Karah Island, Terengganu Malaysia. Each bar denotes individually orders in *Alpha-, Beta-, Delta-, Epsilon-,* and *Gamma-proteobacteria* respectively, while the last bar (others) shows orders in others phylum.

samples (Table 4, Fig. 5), while three (2.27%) were common only on the skin mucus and in the stomach of *L. dimidiatus* (Table 5, Fig. 5). Similarly, six other bacterial families (4.55%) found in the stomach were present in the carriage water samples (Table 6, Fig. 5). However, thirty bacteria families (22.73%) were shared between the skin mucus and carriage water (Table 7, Fig. 5). It is worth mentioning that, some thirty-seven families (28.03%) were found exclusively in the carriage water (Table 8 Fig. 5). However, only seven families (5.30%) were exclusive in the stomach (Table 9, Fig.

5) while, another six (4.55%) were only present in the skin mucus of the *L. dimidiatus* (Table 10, Fig. 5). A total of thirty-three pathogenic zoonotic bacterial were isolated in this study.

4. Discussion

Metagenomics method used in this study successful identified a number of bacterial associated with *L. dimidiatus*. To our knowledge, this is the first of such study aimed at characterising
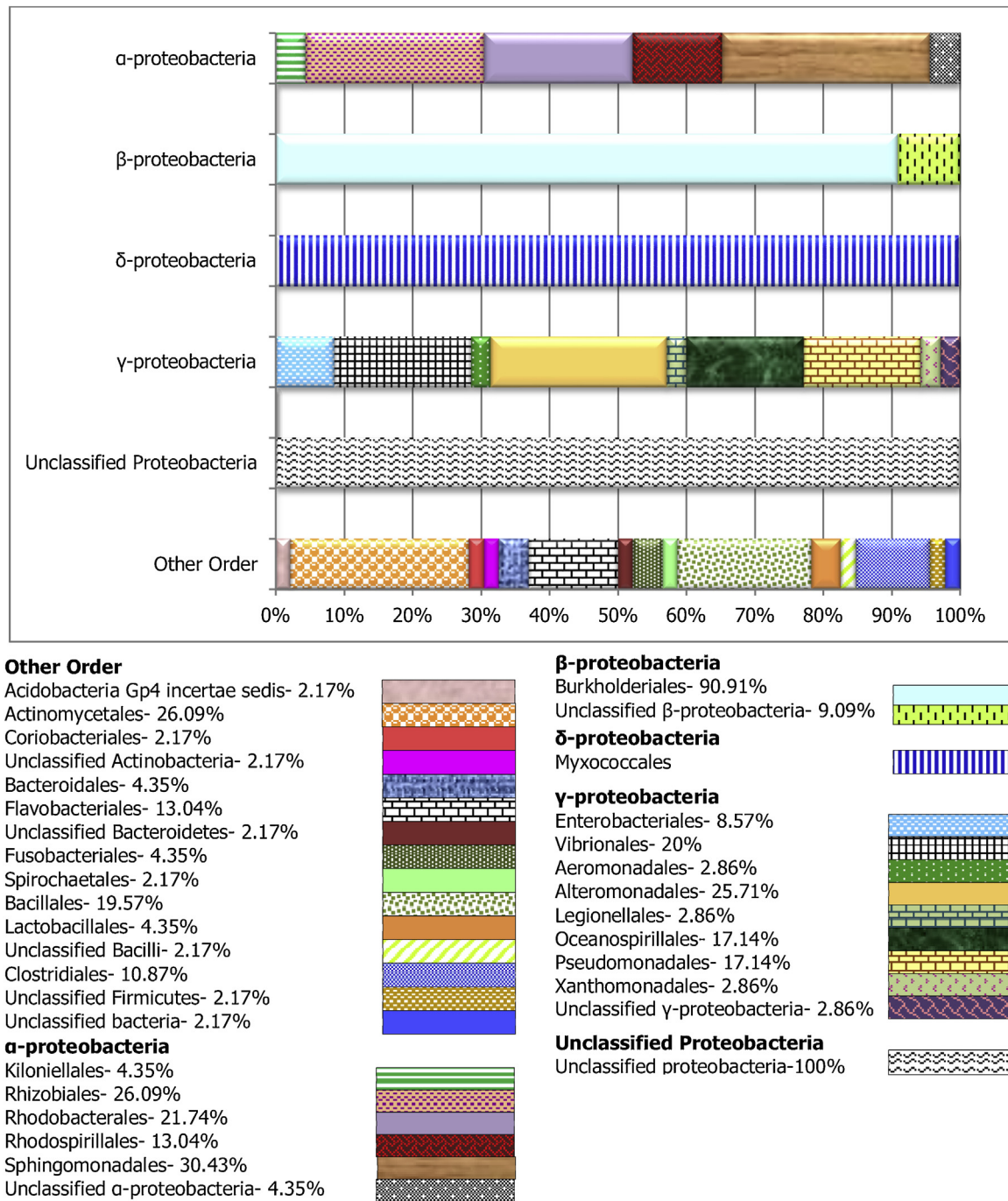
Other Order
Acidobacteria Gp4 incertae sedis- 2.17%
Actinomycetales- 26.09%
Coriobacteriales- 2.17%
Unclassified Actinobacteria- 2.17%
Bacteroidales- 4.35%
Flavobacteriales- 13.04%
Unclassified Bacteroidetes- 2.17%
Fusobacteriales- 4.35%
Spirochaetales- 2.17%
Bacillales- 19.57%
Lactobacillales- 4.35%
Unclassified Bacilli- 2.17%
Clostridiales- 10.87%
Unclassified Firmicutes- 2.17%
Unclassified bacteria- 2.17%

α-proteobacteria
Kiloniellales- 4.35%
Rhizobiales- 26.09%
Rhodobacterales- 21.74%
Rhodospirillales- 13.04%
Sphingomonadales- 30.43%
Unclassified α-proteobacteria- 4.35%

β-proteobacteria
Burkholderiales- 90.91%
Unclassified β-proteobacteria- 9.09%

δ-proteobacteria
Myxococcales

γ-proteobacteria
Enterobacteriales- 8.57%
Vibrionales- 20%
Aeromonadales- 2.86%
Alteromonadales- 25.71%
Legionellales- 2.86%
Oceanospirillales- 17.14%
Pseudomonadales- 17.14%
Xanthomonadales- 2.86%
Unclassified γ-proteobacteria- 2.86%

Unclassified Proteobacteria
Unclassified proteobacteria-100%

Fig. 3. Taxonomic diversity and relative abundance at the order level of bacteria associated with stomach sample of *Labroides dimiditus* from Karah Island, Terengganu Malaysia. Each bar denotes individually orders in *Alpha-*, *Beta-*, *Delta-*, *Epsilon-*, and *Gamma-proteobacteria* respectively, while the last bar (others) shows orders in others phylum.

the microbiota of wild *L. dimidiatus* using the Next Generation Sequencing technology. This method allows a more complex view of the composition of the microbiota of fish and its environment with high precision of taxonomic classification using thousands of reads [23]. The five most common phyla, in this study (*Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria* and *Fusobacteria*) had earlier been reported by Gerzova et al., [3] in carriage water of ornamental fish from three different continents. Similar finding have been reported by Boutin et al., [24]. Larsen et al. [25], had earlier isolated these groups of bacterial in the skin of mullet *Mugil cephalus*, red snapper *Lutjanus campechanus*, spotted seatrout *Cynoscion nebulosus*, sand seatrout *Cynoscion arenarius*, Atlantic

croaker *Micropogonias unduluatus*, and pinfish *Lagodon rhomboides*. Similarly, Gerzova et al., [3] had suggested that these are common phyla in the stomach of channel catfish *Ictalurus punctatus*, largemouth bass *Micropterus salmoides*, and bluegill *Lepomis macrochirus* and some ornamental fishes. It is a known fact that microbes form symbiotic relationships with their host organisms and offers several advantages [26]. However, the origins of most fish microbes have not yet been studied; hence, this phenomenon is poorly understood [27].

The findings of this study suggest that the structure of fish microbiomes from the Karah Island, Terengganu, Malaysia were largely influenced by the environments water where they are
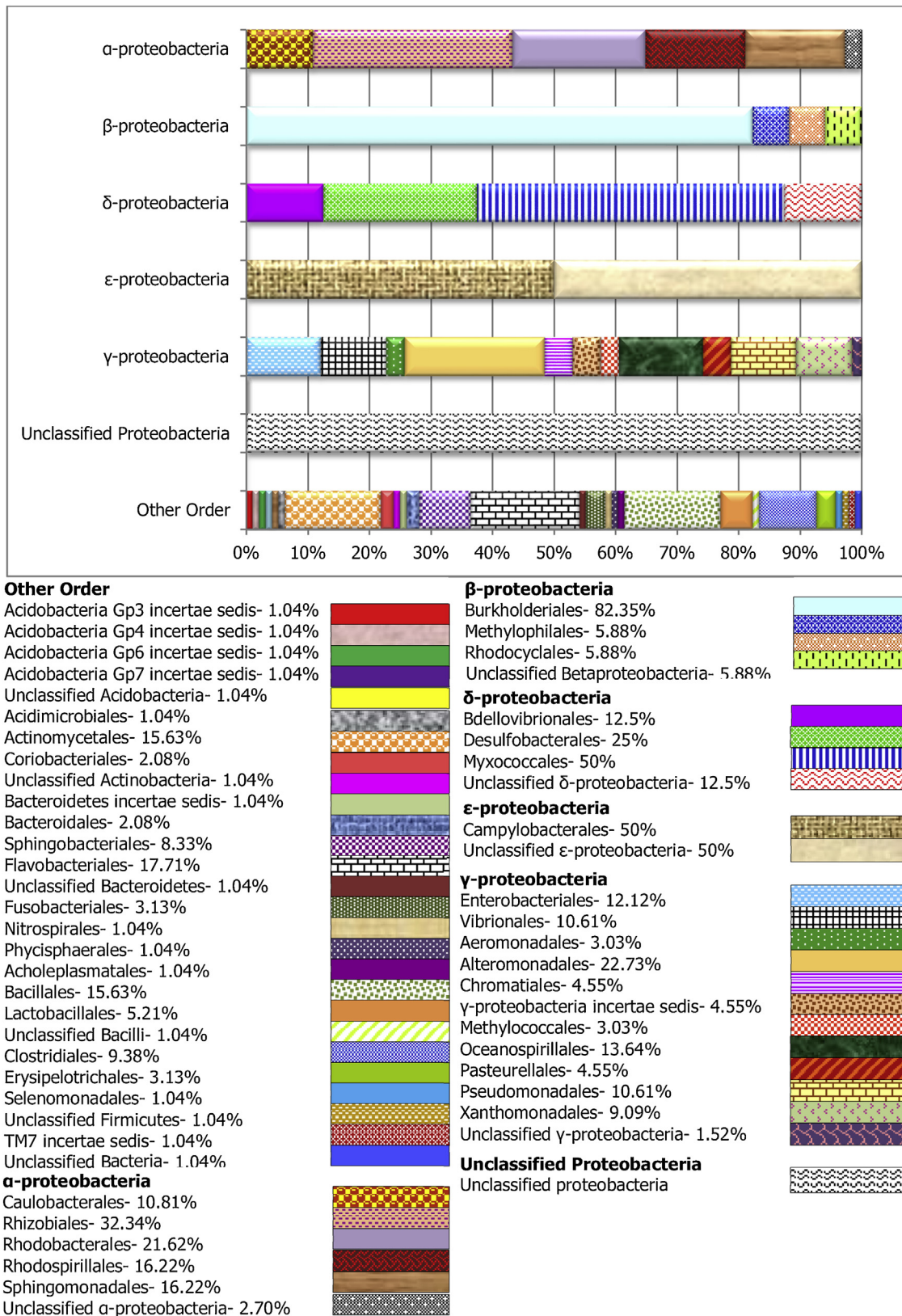
Fig. 4. Taxonomic composition and relative abundance at the order level of bacteria associated with carriage water from Karah Island, Terengganu Malaysia. Each bar denotes individually orders in *Alpha-*, *Beta-*, *Delta-*, *Epsilon-*, and *Gamma-proteobacteria* respectively, while the last bar (others) shows orders in others phylum.

found. Fish skin is constantly in contact with the aquatic environment, hence, the largely synchronised bacteria composition on the skin mucus of the *L. dimidiatus* and that of the carriage water in the study was expected. Georgala, [28] and Horsley [29], had earlier suggested that the external bacteria communities of many fishes are strong reflection of the microbial community present in the surrounding water. Beyond the bios of the surrounding waters, distribution of microbial communities in

Table 4
Shared bacteria families' in the skin mucus and stomach of *Labroides dimidiatus* as well as the water from Karah Island, Terengganu Malaysia.

| Serial Number	Class	Family
1	*Alphaproteobacteria*	*Acetobacteraceae*
2	*Clostridia*	*Lachnospiraceae*
3	*Actinobacteria*	*Micrococcaceae*
4	*Alphaproteobacteria*	*Bradyrhizobiaceae*
5	*Betaproteobacteria*	*Burkholderiaceae*
6	*Betaproteobacteria*	*Burkholderiales_incertae_sedis*
7	*Alphaproteobacteria*	*Bradyrhizobiaceae*
8	*Betaproteobacteria*	*Burkholderiaceae*
9	*Betaproteobacteria*	*Burkholderiales_incertae_sedis*
10	*Actinobacteria*	*Corynebacteriaceae*
11	*Actinobacteria*	*Propionibacteriaceae*
12	*Actinobacteria*	*Unclassified Actinomycetales*
13	*Actinobacteria*	*Unclassified Actinobacteria*
14	*Flavobacteria*	*Flavobacteriaceae*
15	*Flavobacteria*	*Unclassified Flavobacteriales*
16	*Unclassified Bacteroidetes*	*Unclassified Bacteroidetes*
17	*Fusobacteria*	*Fusobacteriaceae*
18	*Alphaproteobacteria*	*Hyphomicrobiaceae*
19	*Alphaproteobacteria*	*Methylobacteriaceae*
20	*Alphaproteobacteria*	*Unclassified Rhizobiales*
21	*Alphaproteobacteria*	*Rhodobacteraceae*
22	*Alphaproteobacteria*	*Unclassified Rhodospirillales*
23	*Alphaproteobacteria*	*Erythrobacteraceae*
24	*Alphaproteobacteria*	*Sphingomonadaceae*
25	*Alphaproteobacteria*	*Unclassified Sphingomonadales*
26	*Alphaproteobacteria*	*Unclassified Alphaproteobacteria*
27	*Betaproteobacteria*	*Comamonadaceae*
28	*Betaproteobacteria*	*Oxalobacteraceae*
29	*Betaproteobacteria*	*Unclassified Burkholderiales*
30	*Betaproteobacteria*	*Unclassified Betaproteobacteria*
31	*Gammaproteobacteria*	*Unclassified Oceanospirillales*
32	*Gammaproteobacteria*	*Moraxellaceae*
33	*Gammaproteobacteria*	*Pseudomonadaceae*
34	*Gammaproteobacteria*	*Xanthomonadaceae*
35	*Gammaproteobacteria*	*Unclassified Gammaproteobacteria*
36	*Unclassified Proteobacteria*	*Unclassified Proteobacteria*
37	*Bacilli*	*Bacillaceae_1*
38	*Bacilli*	*Staphylococcaceae*
39	*Bacilli*	*Unclassified Bacillales*
40	*Clostridia*	*Peptostreptococcaceae*
41	*Clostridia*	*Unclassified Clostridiales*
42	*Clostridia*	*Unclassified Firmicutes*
43	*Unclassified bacteria*	*Unclassified Bacteria*
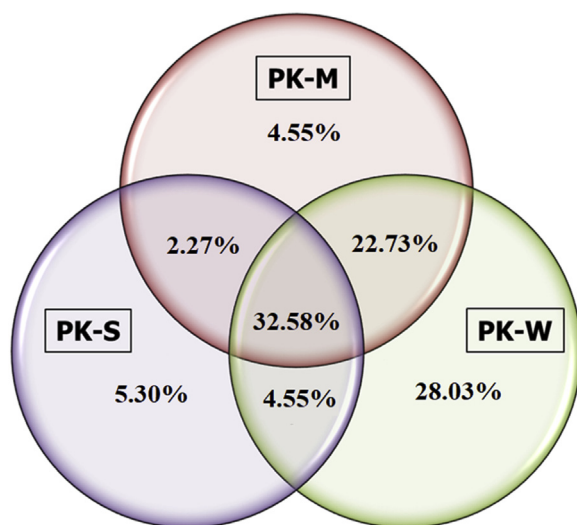
Fig. 5. Venn diagram of the percentages of shared and unique bacteria families identified in three different types of samples collected in this study. PK = Karah Island, M = skin mucus, S = stomach, W = carriage seawater.

the stomach may also be largely influenced by the type of diet fed by the fish [30]. Schmidt et al. [31], and Gajardo et al., [32] had earlier justified this assumption in their study with *Salmo salar*. This position cannot be suggested for the current study, as no attempt was made to characterise the microbes of the feed fed by the fish in the wild. However, the mutualism interaction between *L. dimidiatus* and its client (i.e. the fishes it helps remove parasites and bacteria infection from) could present a new pathogenic infection partway in this fish [33]. Similarly, this could be a peculiar form of parasitic transmission pattern in other cleaner wrasses fishes.

Out of the many genera isolated in this study, *Alteromonas* and *Vibrio* were two of the dominant genera of the Gammaproteobacteria as observed in all samples. This has been reported prevalent in many other marine environments [34–36]. *Alteromonas* has also been reported in some species of fish reared in a re-circulatory aquaculture system [37,38]. The bacteria group *Alteromonas* have great carbon cycling potential, hence are composite of member of the marine master recyclers [39,40]. Also, the genus *Vibrio*, have been reported in other members of wrasse [41–44]. Several species of *Vibrio* are typically associated with marine and freshwater environments [45]. The *Vibrio* infections can spread rapidly when fish are confined in very high stocking densities a scenario found in commercial aquaculture systems where morbidity may reach 100% in affected facilities [13]. *Vibrio* sp. are pathogenic to human, and responsible for disease in wild and reared organisms, which includes fish, molluscs, crustaceans, rotifers, and corals, [46]. Another genus of interest in abundance isolated in the skin mucus of *L. dimidiatus* was the *Sandarakinotalea*. The presence of this bacteria group may be due to the burrowing activity of the fish in the sand for the purpose of predatory avoidance. This has been earlier demonstrated in the study reported by Slobodkin and Fishelson, [47].

Out of the 132 microbes identified in this study, 32% were shared/found in all site examined (i.e. mucus, stomach and the carriage water). Members of the *Gammaproteobacteria* were the most commonly shared bacteria among all the samples in this study. So were the members of the *Alphaproteobacteria*, *Betaproteobacteria*, *Actinobacteria*, *Flavobacteria*, *Fusobacteria*, *Bacilli* and *Clostridia*. Usually, shared bacteria communities within a population, are reflection of symbiotic association possibly because of metabolic benefits between the bacteria and it host [48]. Most of the bacteria identified are involved in nutrient cycling. *Rhodobacteraceae* for instance is deeply involved in sulfur and carbon biogeochemical cycling and symbiosis with aquatic micro- and macro-organisms [49,50]. Carbon cycling and biodegradative capabilities are widespread characteristic within the members of the *Alpha-* and *Gammaproteobacteria*. These include the *Alteromonadaceae*, *Pseudomonadaceae*, *Sphingomonadaceae*, and *Vibrionaceae* as well as *Cytophagaceae, Flavobacteriaceae* and *Bacteroidetes*, which are members of the *Cytophaga-Flavobacteria-Bacteroides* (CFB) clade [51].

Lactobacillus spp., *Lactococcus* spp., *Enterococcus* spp., *Shewanella* spp., *Bacillus* spp., *Aeromonas* spp., Vibrio, *Enterobacter* spp., *Pseudomonas* spp., *Clostridium* spp., and *Kocuria* spp. identified in this study are common probiotic bacteria used in the aquaculture industries [52,53]. These probiotic bacteria have been isolated in several healthy fish species such as Indian carp, *Labeo rohita*, *Nile tilapia*, *Oreochromis niloticus*, gilt-head bream, *Sparus aurata* [54–56]. They function to improve growth, immune responses, resistance to pathogenic bacteria, and expression of different genes involved in inflammation, development and digestion processes in the fish host [54–56]. In this study, 28% of the identified microbes were found unique to the carriage water, while about 5% were unique to the mucus and stomach of the fish. The observation of some bacteria exclusive to different samples or

Table 5
Shared bacteria families' in the skin mucus and stomach of *Labroides dimidiatus* from Karah Island, Terengganu Malaysia.

Serial Number	Class	Family
1	*Bacilli*	*Carnobacteriaceae*
2	*Actinobacteria*	*Brevibacteriaceae*
3	*Actinobacteria*	*Dermacoccaceae*

Table 6
Shared bacteria families' in the stomach of *Labroides dimidiatus* and water from Karah Island, Terengganu Malaysia.

Serial Number	Class	Family
1	*Actinobacteria*	*Coriobacteriaceae*
2	*Alphaproteobacteria*	*Rhizobiaceae*
3	*Deltaproteobacteria*	*Nannocystaceae*
4	*Actinobacteria*	*Microbacteriaceae*
5	*Deltaproteobacteria*	*Unclassified Myxococcales*
6	*Bacilli*	*Paenibacillaceae_1*

Table 7
Shared bacteria families' in the skin mucus of *Labroides dimidiatus* and water from Karah Island, Terengganu Malaysia.

Serial Number	Class	Family
1	*Betaproteobacteria*	*Rhodocyclaceae*
2	*Deltaproteobacteria*	*Phaselicystidaceae*
3	*Epsilonproteobacteria*	*Helicobacteraceae*
4	*Erysipelotrichia*	*Erysipelotrichaceae*
5	*Negativicutes*	*Veillonellaceae*
6	*Actinobacteria*	*Actinomycetaceae*
7	*Actinobacteria*	*Nocardiaceae*
8	*Alphaproteobacteria*	*Caulobacteraceae*
9	*Gammaproteobacteria*	*Pasteurellaceae*
10	*Clostridia*	*Clostridiales_Incertae_Sedis_XI*
11	*Acidobacteria_Gp4*	*Acidobacteria_Gp4_family_ incertae_sedis*
12	*Actinobacteria*	*Nocardioidaceae*
13	*Bacilli*	*Lactobacillaceae*
14	*Bacteroidetes_incertae_ sedis_class_incertae_sedis*	*Bacteroidetes_incertae_ sedis_family_incertae_sedis*
15	*Sphingobacteria*	*Flammeovirgaceae*
16	*Sphingobacteria*	*Cytophagaceae*
17	*Phycisphaerae*	*Phycisphaeraceae*
18	*Alphaproteobacteria*	*Hyphomonadaceae*
19	*Flavobacteria*	*Cryomorphaceae*
20	*Alphaproteobacteria*	*Rhodospirillaceae*
21	*Deltaproteobacteria*	*Bacteriovoracaceae*
22	*Deltaproteobacteria*	*Unclassified Deltaproteobacteria*
23	*Gammaproteobacteria*	*Colwelliaceae*
24	*Gammaproteobacteria*	*Ferrimonadaceae*
25	*Gammaproteobacteria*	*Ferrimonadaceae*
26	*Gammaproteobacteria*	*Moritellaceae*
27	*Gammaproteobacteria*	*Chromatiaceae*
28	*Gammaproteobacteria*	*Unclassified Chromatiales*
29	*Gammaproteobacteria*	*Alcanivoracaceae*
30	*Gammaproteobacteria*	*Gammaproteobacteria_family_ incertae_sedis*

Table 8
Bacteria families' exclusive in water from Karah Island, Terengganu Malaysia.

Serial Number	Class	Family
1	*Gammaproteobacteria*	*unclassified Xanthomonadales*
2	*Bacilli*	*Paenibacillaceae_2*
3	*Bacilli*	*Enterococcaceae*
4	*Acidobacteria_Gp3*	*Acidobacteria_Gp3_family_ incertae_sedis*
5	*Acidobacteria_Gp6*	*Acidobacteria_Gp6_family_ incertae_sedis*
6	*Acidobacteria_Gp7*	*Acidobacteria_Gp7_family_ incertae_sedis*
7	*unclassified Acidobacteria*	*unclassified Acidobacteria*
8	*Actinobacteria*	*Acidimicrobiaceae*
9	*Bacteroidia*	*Porphyromonadaceae*
10	*Alphaproteobacteria*	*unclassified Caulobacterales*
11	*Deltaproteobacteria*	*Desulfobacteraceae*
12	*Deltaproteobacteria*	*Desulfobulbaceae*
13	*Gammaproteobacteria*	*Methylococcaceae*
14	*Mollicutes*	*Acholeplasmataceae*
15	*Bacilli*	*Alicyclobacillaceae*
16	*Bacilli*	*Bacillales_Incertae_Sedis_XII*
17	*Bacilli*	*Thermoactinomycetaceae_1*
18	*Bacilli*	*Leuconostocaceae*
19	*Bacilli*	*unclassified Lactobacillales*
20	*Clostridia*	*Ruminococcaceae*
21	*Actinobacteria*	*Pseudonocardiaceae*
22	*Sphingobacteria*	*Sphingobacteriaceae*
23	*Alphaproteobacteria*	*Bartonellaceae*
24	*Betaproteobacteria*	*Alcaligenaceae*
25	*Betaproteobacteria*	*Methylophilaceae*
26	*Gammaproteobacteria*	*Unclassified Pseudomonadales*
27	*Bacilli*	*Planococcaceae*
28	*Bacilli*	*Streptococcaceae*
29	*Gammaproteobacteria*	*Hahellaceae*
30	*Bacilli*	*Bacillaceae_2*
31	*Clostridia*	*Clostridiaceae_1*
32	*Bacteroidia*	*unclassified Bacteroidales*
33	*Nitrospira*	*Nitrospiraceae*
34	*TM7_class_incertae_ sedis*	*TM7_family_incertae_sedis*
35	*Sphingobacteria*	*Chitinophagaceae*
36	*Alphaproteobacteria*	*Phyllobacteriaceae*
37	*Gammaproteobacteria*	*Sinobacteraceae*

Table 9
Bacteria families' exclusive to the stomach of *Labroides dimidiatus* collected from Karah Island, Terengganu Malaysia.

Serial Number	Class	Family
1	*Actinobacteria*	*Streptomycetaceae*
2	*Bacteroidia*	*Prevotellaceae*
3	*Bacilli*	*Bacillales_Incertae_Sedis_XI*
4	*Gammaproteobacteria*	*Legionellaceae*
5	*Epsilonproteobacteria*	*Unclassified Epsilonproteobacteria*
6	*Alphaproteobacteria*	*Kiloniellaceae*
7	*Spirochaetes*	*Unclassified Spirochaetales*

Table 10
Bacteria families' exclusive to the skin mucus of *Labroides dimidiatus* collected from Karah Island, Terengganu Malaysia.

Serial Number	Class	Family
1	*Actinobacteria*	*Dermatophilaceae*
2	*Actinobacteria*	*Bifidobacteriaceae*
3	*Betaproteobacteria*	*Neisseriaceae*
4	*Epsilonproteobacteria*	*Unclassified Campylobacterales*
5	*Sphingobacteria*	*Unclassified Sphingobacteriales*
6	*Epsilonproteobacteria*	*Campylobacteraceae*

environment is not new. Earlier study by Kormas et al., [57], Bakke et al., [58], Estruch et al. [59], and Li et al. [60], have showed that aquaculture microbiota composition in the fish was different from those found in the surrounding environment. Cardinale et al., [61], however, had earlier concluded that the differences in the structure of bacteria communities of the environment and the host organism might simply be opportunistic infection, rather than symbiotic partnership.

Fish can also be a source of human infections caused by pathogens transmitted from fish or the aquatic environment. Depending on season, and the level of exposure pathogenic bacteria could be transmitted to human. Thirty-three of such zoonotic pathogenic bacteria were observed in this study. Notable among them is the *Mycobacteria*. This is one of the most common fish-borne bacteria pathogens known to man. It can cause

granulomatous inflammation of the human skin [62]. Similarly, *Erysipelothrix spp.* is transmissible from fish to humans, and causes infection of cutaneous wounds. This typically results in localized, painful, self-limiting cellulitis, with purple discoloration and oedema ('fish rose') [63,64]. In addition to these two, *Streptococcus* spp, *Photobacterium* spp, and *Vibrio* spp, are other fish-borne zoonosis bacteria associated with *L. dimidiatus* worth mentioning in this study. Gram positive bacteria such as *Clostridium*, *Lactococcus* and *Staphylococcus* were only found in the stomach and water sample while *Streptococcus* is found in all samples of *L. dimidiatus*. *Clostridium* is commonly commensal in the intestines of marine animals and in the sediments of the environment/decaying organic matter where paralytic neurotoxin is produced [65]. *Lactococcus* and *Streptococcus* are also zoonotic fish-borne bacteria found in a wide variety of temperate and warm water fishes [66,67]. Pathogens of gram negative bacteria such as *Aeromonas* spp, *Edwardsiella* spp., *Enterobacteriaceae*, *Francisella* spp., *Pseudomonas*, and *Vibrio* spp. isolated mostly in this study have been implicated for zoonotic infections of fish and humans in previous studies [68–71].

Earlier studies by Rasmussen & Sorensen, [72], Horner-Devine et al. [73], Leflaive et al., [74] Sala et al. [75,76], had concluded that functional bacteria composition in a systems are further driven by changes in the environmental conditions such as nutrient availability and pollutants. Similarly, changes in salinity, season and geographic location could severely influence the composition of free-living and symbiotic bacteria communities in the fish [77,78]. This means that changes in the dynamics of the external environment such as those obtained when wild fishes are raise in captivity could result in substantial differences in the bacteria community than what was reported in this study. In line with this assumption, Smith et al. [13], had suggested the possibility of ornamental fish raised in captivity harbouring novel microbial communities and pathogens that could pose potential risks to the pet industry, fish trade, humans and other fish species. Hence, there is need to intensify research in this regards for biosecurity purposes and to ensure suitable husbandry of aquarium held wild ornamental fishes such as *L. dimidiatus*.

4.1. Limitations of the study

The collected samples of fish used for this study were gently washed thrice with sterile seawater to remove the dirt on the fish. This was done with the hope that the microbial colonies on the skin were not compromised. However, this is likely not the case. In correction of the limitation in this study, it is recommended that DNA be extracted from the sterilized water used to wash the fish's skin in future studies.

Acknowledgements

References

[1] N.D. Balachandran, T.A.K. Thipramalai, T. Balasubramanian, T. Kapila, A study on the effect of using mangrove leaf extracts as a feed additive in the progress of bacterial infections in marine ornamental fish, J. Coast. Life Med. 1 (3) (2013) 217–224.

[2] E.J. Noga, in: E.J. Noga (Ed.), Fish Disease : Diagnosis and Treatment, second, ed, Wiley-Blackwell, US, 2010, pp. 5–7.

[3] L. Gerzova, P. Videnska, M. Faldynova, K. Sedlar, I. Provaznik, A. Cizek, I. Rychlik, Characterization of microbiota composition and presence of selected antibiotic resistance genes in carriage water of ornamental fish, PLoS One 9 (8) (2014)e103865, doi:http://dx.doi.org/10.1371/journal.pone.0103865.

[4] A.L. Rhyne, M.F. Tlusty, P.J. Schofield, Revealing the appetite of the marine aquarium fish trade: the volume and biodiversity of fish imported into the United States, PLoS One 7 (5) (2012) e35808.

[5] UNEP-WCMC, SRG 42/8/a International Trade in Aquatic Ornamental Species, Prepared for the European Commission Directorate General E – Environment ENV.E.2. – Development and Environment, 2007.

[6] UNEP-WCMC, Consultation Process on Monitoring of International Trade in Ornamental Fish, European Commission Directorate General E – Environment ENV.E.2. – Development and Environment, 2008.

[7] C. Wabnitz, M. Taylor, E. Green, T. Razak, The Global Trade in Marine Ornamental Species from Ocean to Aquarium, UNEP-WCMC, Cambridge, UK, 2003.

[8] G.W. Potts, The ethology of *Labroides dimidiatus* (cuv & val) Labridae, pisces on Aldabra, Anim. Behav. 21 (1973) 250–291.

[9] E. Rimstad, D. Basic, S. Gulla, B. Hjeltnes, S. Mortensen, Risk Assessment of Fish Health Associated With the Use of Cleaner Fish in Aquaculture. Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food and Environment. VKM Report 2017:32, ISBN: 978-82-8259-289-5, ISSN: 2535-4019, Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway, 2017.

[10] G.D. Gómez, J.L. Balcázar, A review on the interactions between gut microbiota and innate immunity of fish. Federation of European Microbiological Societies, Immunol. Med. Microbiol. 52 (2008) 145–154.

[11] E.J. Wagner, T. Jensen, R. Arndt, M.D. Routedge, Q. Brddwisch, Effects of rearing density upon cut throat trout haematology, hatchery performance, fin erosion and general health and condition, Prog. Fish-Cult. 59 (1997) 173–187.

[12] J.L. Balcázar, N.M. Lee, J. Pintado, M. Planas, Phylogenetic characterization and in situ detection of bacterial communities associated with seahorses (*Hippocampus guttulatus*) in captured, Syst. Appl. Microbiol. 33 (2010) 71–77.

[13] K.F. Smith, V. Schmidt, G.E. Rosen, L. Amaral-Zettler, Microbial diversity and potential pathogens in ornamental fish aquarium water, PLoS One 7 (9) (2012) e39971.

[14] J. Wolf, A. Prüss-Ustün, O. Cumming, Review of the evidence relating drinking-water and sanitation to diarrhoea: a meta-regression, Trop. Med. Int. Health 19 (8) (2014) 928942.

[15] Y. Hongo, H. Yuzawa, M. Ohkuma, T. Kudo, Evaluation of primers and PCR conditions for the analysis of 16S rRNA genes from a natural environment. Federation of European Microbiological Societies, Microbiol. Lett. 221 (2003) 299–304.

[16] A.K. Bartram, M.D. Lynch, J.C. Stearns, G. Moreno-Hagelsieb, J.D. Neufeld, Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads, Appl. Environ. Microbiol. 77 (11) (2011) 3846–3852.

[17] S.M. Huse, L. Dethlefsen, J.A. Huber, D.M. Welch, D.A. Relman, M.L. Sogin, Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing, PLoS Genet. 4 (2008)e1000255.

[18] G. Muyzer, E.C. de Waal, A.G. Uitterlinden, Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, Appl. Environ. Microbiol. 59 (1993) 695–700.

[19] G.B. Gloor, R. Hummelen, J.M. Macklaim, R.J. Dickson, A.D. Fernandes, R. MacPhee, Microbiome profiling by Illumina sequencing of combinatorial sequence-tagged PCR products, PLoS One 5 (2010)e15406.

[20] P.D. Schloss, S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, R.A. Lesniewski, B.B. Oakley, D.H. Parks, C.J. Robinson, J.W. Sahl, B. Stres, G.G. Thallinger, D.J. Van Horn, C.F. Weber, Introducing mothur: open-source, platform-independent, community- supported software for describing and comparing microbial communities, Appl. Environ. Microbiol. 75 (23) (2009) 7537–7541.

[21] Q. Wang, G.M. Garrity, J.M. Tiedje, J.R. Cole, Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy, Appl. Environ. Microbiol. 73 (2007) 5261–5267.

[22] J.R. Cole, Q. Wang, E. Cardenas, J. Fish, B. Chai, R.J. Farris, A.S. Kulam-Syed-Mohideen, D.M. McGarrell, T. Marsh, G.M. Garrity, J.M. Tiedje, The ribosomal database project: improved alignments and new tools for rRNA analysis, Nucleic Acids Res. 37 (2009) 141–145.

[23] A.S. Whiteley, S. Jenkins, I. Waite, N. Kresoje, H. Payne, B. Mullan, Microbial 16S rRNA ion tag and community metagenome sequencing using the ion torrent (PGM) platform, J. Microbiol. Methods 91 (2012) 80–88, doi:http://dx.doi.org/10.1016/j.mimet.2012.07.008.

[24] S. Boutin, L. Bernatchez, C. Audet, N. Derôme, Network analysis highlights complex interactions between pathogen, host and commensal microbiota, PLoS One 8 (12) (2013) e84772.

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

[26] J. Kennedy, J.R. Marchesi, A.D.W. Dobson, Marine metagenomics: strategies for the discovery of novel enzymes with biotechnological applications from marine environments (Review), Microb. Cell Fact. 7 (27) (2008) 1–8.

[27] A.M. Larsen, Z. Tao, S.A. Bullard, C.R. Arias, Diversity of the skin microbiota of fishes: evidence for host species specificity. Federation of European Microbiological Societies, Microbiol. Ecol. 85 (2013) 483–494.

[28] D.L. Georgala, The bacterial flora of the skin of North Sea cod, J. Gen. Microbiol. 18 (1958) 84–91.

[29] R.W. Horsley, A review on the bacterial flora of teleosts and elamobrachs, includings methods for its analysis, J. Fish Biol. 10 (1977) 529–553.

[30] A. Landeira-Dabarca, C. Sieiro, M. Álvarez, Change in food ingestion induces rapid shifts in the diversity of microbiota associated with cutaneous mucus of Atlantic salmon Salmo salar, J. Fish Biol. 82 (2013) 893–906.

[31] V. Schmidt, L. Amaral-Zettler, J. Davidson, S. Summerfelt, C. Good, The influence of fishmeal-free diets on microbial communities in Atlantic salmon Salmo salar recirculation aquaculture systems, Appl. Environ. Microbiol. 82 (2016) 4470–4481, doi:http://dx.doi.org/10.1128/AEM.00902-16.

[32] K. Gajardo, A. Jaramillo-Torres, T. Kortner, D. Merrifield, J. Tinsley, A. Bakke, Alternative protein sources in the diet modulate microbiota and functionality in the distal intestine of Atlantic salmon (Salmo salar), Appl. Environ. Microbiol. 83 (2017) e02615–16, doi:http://dx.doi.org/10.1128/AEM.02615-16).

[33] A.S. Grutter, Parasite removal rates by the cleaner wrasse Labroides dimidiatus, Inter-Res. Mar. Ecol. Programme Ser. 130 (1996) 61–70.

[34] P. Juteau, R. Larocque, D. Rho, A. LeDuy, Analysis of the relative abundance of different types of bacteria capable of toluene degradation in a compost biofilter, Appl. Microbiol. Biotechnol. 52 (1999) 863–868.

[35] K. Watanabe, M. Teramoto, S. Harayama, An outbreak of nonflocculating catabolic populations caused the breakdown of a phenol-digesting activated-sludge process, Appl. Environ. Microbiol. 65 (1999) 2813–2819.

[36] N.J. Palleroni, A.M. Port, H.K. Chang, G.J. Zylstra, Hydrocarboniphaga effusa gen. nov., sp nov., a novel member of the gamma-Proteobacteria active in alkane and aromatic hydrocarbon degradation, Int. J. Syst. Evol. Microbiol. 54 (2004) 1203–1207.

[37] A.J. Fjellheim, K.J. Playfoot, J. Skjermo, O. Vadstein, Vibrionaceae dominates the microflora antagonistic towards Listonella anguillarum in the intestine of cultured Atlantic cod (Gadus morhua L.) larvae, Aquaculture 269 (2007) 98–106.

[38] T. van der Meeren, L. Brunvold, R.-A. Sandaa, Ø. Bergh, T. Castberg, R. Thyrhau, A. Mangor-Jensen, Water quality and microbial community structure in juvenile Atlantic cod (Gadus morhua L.), Aquaculture 316 (2011) 111–120, doi:http://dx.doi.org/10.1016/j.aquaculture.2011.03.016.

[39] C.E. Nelson, E.K. Wear, Microbial diversity and the lability of dis- solved organic carbon, Proc. Natl. Acad. Sci. 111 (2014) 7166–7167.

[40] A. Buchan, G.R. LeCleir, C.A. Gulvik, J.M. González, Master recy-clers: features and functions of bacteria associated with phytoplankton blooms, Nat. Rev. Microbiol. 12 (2014) 686–698, doi:http://dx.doi.org/10.1038/nrmicro3326.

[41] S. Jensen, O.B. Samuelsen, K. Andersen, L. Torkildsen, C. Lambert, G. Choquet, C. Paillard, Ø. Bergh, Characterisation of strains of Vibrio splendidus and V. Tapetis isolated from corkwing wrasse Symphodus melops suffering vibriosis, Dis. Aquat. Org. 53 (2003) 25–31.

[42] Ø. Bergh, O.B. Samuelsen, Susceptibility of corkwing wrasse Symphodus melops, goldsinny wrasse Ctenolabrus rupestris, and Atlantic salmon Salmo salar smolt, to experimental challenge with Vibrio tapetis and Vibrio splendidus isolated from corkwing wrasse. wrasse, Aquac. Int. 15 (2007) 11–18.

[43] L.S. Harkestad, Experimental Infections of Corkwing, Symphodus melops, with the Vibrio tapetis Strains CECT 4600, LP2 and NRP45. (In Norwegian). Msc Thesis, University of Bergen, Department of Biology, 2011 136 pages.

[44] D. Colquhoun, S. Duodu, H.K. Nilsen, C. Sviland, Ø Vågnes, Helsesituasjonen hos marin fisk 2011 (In Norwegian), in: A.B. Olsen, H. Hellberg (Eds.), (2012) Fiskehelserapporten 2011, vol.2012, Veterinærinstituttet, 2012, pp. 33–38.

[45] A.V. Nilsen, H. Viljugrein, M.V. Røsaeg, D. Colquhoun, Rensefiskhelse - kartlegging av dødelighet og dødelighetsårsaker (Cleaner fish health - a survey of mortalities and causes of death; in Norwegian), The Norwegian Veterinary Institute's Report Series, 2014, pp. 12.

[46] B. Gomez-Gil, C.C. Thompson, Y. Matsumura, T. Sawabe, T. Iida, R. Christen, F. Thompson, T. Sawabe, The famlily Vibrionaceae, in: E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt, F. Thompson (Eds.), The Prokaryotes–Gammaproteobacteria, Springer, Berlin Heidelberg, 2014, pp. 660–713.

[47] L.B. Slobodkin, L. Fishelson, The effect of the cleaner-fish Labroides dimidiatus on the point diversity of fishes on theReef front at Eilat, Am. Nat. 108 (961) (1974) 369–376. May - Jun., 1974 http://www.jstor.org/stable/2459898.

[48] M. Grube, M. Cardinale, J. Vieira de Castro, H. Müller, G. Berg, Species-specific structural and functional diversity of bacterial communities in lichen symbioses, ISME J. 3 (2009) 1105–1115.

[49] T. Brinkhoff, H.-A. Giebel, M. Simon, Diversity, ecology, and genomics of the Roseobacter clade: a short overview, Arch. Microbiol. 189 (2008) 531–539.

[50] M. Pujalte, T. Lucena, M.A. Ruvira, D.R. Arahal, M.C. Macián, The family Rhodobacteraceae, in: E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt, F. Thompson (Eds.), The Prokaryotes- Alphaproteobacteria and Betaproteobacteria, Springer, Berlin Heidelberg, 2014, pp. 439–512.

[51] L.P. Wackett, Bacteria in sand, Environ. Microbiol. 15 (2013) 2144–2145, doi: http://dx.doi.org/10.1111/1462-2920.12176.

[52] T. Péréz-Sánchez, I. Ruiz-Zarzuela, I. deBlas, J.L. Balcázar, Probiotics in aquaculture: a current assessment, Rev. Aquac. 6 (2014) 133–146.

[53] S.K. Nayak, Probiotics and immunity: a fish perspective (Review), Fish Shellfish Immunol. 29 (2010) 2–14.

[54] S.S. Giri, V. Sukumaran, M. Oviya, Potential probiotic Lactobacillus plantarum VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, Labeo rohita, Fish Shellfish Immunol. 34 (2013) 660–666.

[55] A. Del'Duca, C.D. Evangelista, D.C. Galuppo, A.P. Cesar, Evaluation of the presence and efficiency of potential probiotic bacteria in the gut of tilapia (Oreochromis niloticus) using the fluorescent in situ hybridization technique, Aquaculture 391 (2013) 115–121.

[56] R. Cerezuela, J. Meseguer, M.A. Esteban, Effects of dietary inulin, Bacillus subtilis and microalgae on intestinal gene expression in gilthead seabream (Sparus aurata L.), Fish Shellfish Immunol. 34 (2013) 843–848.

[57] K.A. Kormas, A. Meziti, E. Mente, A. Frentzos, Dietary differences are reflected on the gut prokaryotic community structure of wild and commercially reared sea bream (Sparus aurata), Microbiol. Open 3 (5) (2014) 718–728.

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

[59] G. Estruch, M.C. Collado, D.S. Pe-aranda, A. Tomás Vidal, M. Jover Cerdá, Pérez, G. Martínez, Impact of fishmeal replacement in diets for gilthead sea bream (Sparus aurata) on the gastrointestinal microbiota determined by pyrosequencing the 16S rRNA gene, PLoS One 10 (2015)e0136389, doi:http://dx.doi.org/10.1371/journal.pone.0136389.

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

[61] B.J. Cardinale, D.S. Srivastava, J.E. Duffy, J.P. Wright, A.L. Downing, M. Sankaran, C. Jouseau, Effects of biodiversity on the functioning of trophic groups and ecosystems, Nature 443 (2006) 989–992.

[62] D.T. Gauthier, Bacterial zoonoses of fishes: a review and appraisal of evidence for linkages between fish and human infections, Vet. J. 203 (2015) 27–35.

[63] A.C. Reboli, W.E. Farrar, Erysipelothrix rhusiopathiae: an occupational pathogen, Clin. Microbiol. Rev. 2 (1989) 354–359.

[64] Q. Wang, B.J. Chang, T.V. Riley, Erysipelothrix rhusiopathiae, Vet. Microbiol. 140 (2010) 405–417.

[65] N.H. Bean, P.M. Griffin, Foodborne disease outbreaks in the United States, 1973–1987: pathogens, vehicles, and trends, J. Food Prot. 53 (1990) 804–817.

[66] J.F.W. Chan, P.C.Y. Woo, J.L.L. Teng, S.K.P. Lau, S.S.M. Leung, F.C.C. Tam, K.Y. Yuen, Primary infective spondylodiscitis caused by Lactococcus garvieae and a review of human L. Garvieae infections, Infection 39 (2011) 259–264.

[67] J.H. Kim, J. Go, C.R. Cho, J.I. Kim, M.S. Lee, S.C. Park, First report of human acute acalculous cholecystitis caused by the fish pathogen Lactococcus garvieae, J. Clin. Microbiol. 51 (2013) 712–714.

[68] A. Hejazi, F.R. Falkiner, Serratia marcescens, J. Med. Microbiol. 46 (1997) 903–912.

[69] E. McCoy, J. Morrison, V. Cook, J. Johnston, D. Eblen, C. Guo, Foodborne agents associated with the consumption of aquaculture catfish, J. Food Prot. 74 (2011) 500–516.

[70] M. Weir, A. Rajic, L. Dutil, N. Cernicchiaro, F.C. Uhland, B. Mercier, N. Tusevljak, Zoonotic bacteria, antimicrobial use and antimicrobial resistance in ornamental fish: a systematic review of the existing research and survey of aquaculture-allied professionals, Epidemiol. Infect. 140 (2012) 192–206.

[71] A. Camus, A. Berliner, T. Clauss, N. Hatcher, D. Marancik, Serratia marcescens associated ampullary system infection and septicaemia in a bonnethead shark, Sphyrna tiburo (L.), J. Fish Dis. 36 (2013) 891–895.

[72] L.D. Rasmussen, S.J. Sorensen, Effects of mercury contamination on the culturable heterotrophic, functional and genetic diversity of the bacterial community in soil, FEMS Microbiol. Ecol. 36 (2001) 1–9.

[73] M.C. Horner-Devine, K.M. Carney, B.J.M. Bohannan, An ecological perspective on bacterial biodiversity, Proc. R. Soc. Lond., B, Biol. Sci. 271 (2004) 113–122.

[74] J. Leflaive, M. Danger, G. Lacroix, E. Lyautey, C. Oumarou, L. Ten-Hage, Nutrient effects on the genetic and functional diversity of aquatic bacterial communities, FEMS Microbiol. Ecol. 66 (2008) 379–390.

[75] M.M. Sala, M. Estrada, J.M. Gasol, Seasonal changes in the functional diversity of bacterioplankton in contrasting coastal environments of the NW Mediterranean, Aquat. Microb. Ecol. 44 (2006) 1–9.

[76] M.M. Sala, R. Terrado, C. Lovejoy, F. Unrein, C. Pedros-Alio, Metabolic diversity of heterotrophic bacterioplankton over winter and spring in the coastal Arctic Ocean, Environ. Microbiol. 10 (2008) 942–949.

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

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