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Microbial communities associated with the skin, gill, and gut of large yellow croaker (*Larimichthys crocea*)

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Abstract

The microbiota inhabiting the surface of fish mucosal tissue play important roles in the nutrition, metabolism and immune system of their host. However, most investigations on microbial symbionts have focused on the fish gut, but the microbiota associated with external mucosal tissues (such as the skin and gill) is poorly understood. This study characterised the traits and dynamic of microbial communities associated with the skin, gill and gut of large yellow croaker (*Larimichthys crocea*) culturing with net enclosures or pens at different sampling times (with seasonal transition). Results revealed the structure and function of microbial communities differed according to the mucosal tissues of large yellow croaker. The richness and diversity of microbiota in the skin were significantly higher than that in the gill and gut. Discriminative microbial taxa such as *Psychrobacter* in the skin, *Enterobacterales* in the gill, and *Fusobacterium* in the gut, and discriminative predictive functions were identified in the skin, gill and gut. Furthermore, different environmental-related factors (such as sampling time/season and culture method) had impacts on the fish microbiota differently. The diversity and composition of microbiota associated with the skin, gill and gut changed over time, and the difference in skin microbiota across sampling times was most significant among the three tissues. The culture method significantly impacted the diversity and composition of skin microbiota, but no significant difference was found in the gill and gut microbiota between net enclosure and net pen. These results indicated that the skin microbiota of large yellow croaker was more diverse and affected by environmental-related factors than other tissues. This study provides new insights into the structure, environmental response pattern, and relationship with host health of microbiota associated with the mucosal tissues of large yellow croaker.

Keywords Large yellow croaker, Skin, Gill, Gut, Microbiota, Environmental factors

Introduction

Vertebrate hosts naturally harbor diverse microbial communities that reside in different body sites. These symbiotic microbes evolve along with the host and support vital physiological functions, such as nutrient assimilation, energy metabolism, and development and maturation of the immune system [1, 2]. Similarly to other vertebrates, the microbiota of fish has also been demonstrated to be involved in host nutrition, metabolism and immune response [3–5]. Rapid development of culture-independent molecular based omics facilitates many studies on microorganisms that present on host mucosal

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surfaces, and finds that fish display a great variation in microbiota due to high diversity in host and environmental factors [6–9]. Especially fish live in variable aquatic environments, some factors that are habitat specific, include bacterioplankton composition and physicochemical parameters (such as temperature and salinity) in surrounding water, as well as the anthropogenic factors in aquaculture practices, can shape or alter the composition and structure of the microbiota with consequences for the performance of host-related physiological functions [10–12]. The climate change caused by global warming is also known to have large impact on the gut microbiota of fish [13]. Elucidating the structure and function of microbial communities associated with mucosal tissues in fish species, as well as understanding how these change with aquaculture practices, are essential to understanding the host-microbiota interactions in aquatic vertebrates, as well as to developing microbiota-based strategies for fish health and growth in aquaculture and fisheries management.

The skin, gill, and gut tissues are recognized as the major mucosal tissues and the principal microbial entry regions in fish [14]. Currently, the majority of research has focused on the composition and diversity of microbial communities associated with these tissues in fish [4]. The body site is considered to be a major driver of microbial diversity in fish species, and difference in microbiota composition in different fish mucosal tissues may be related to disparate host physiological functions [15–19]. The gut is the most widely studied anatomical site for microbial ecology since it is closely related to the nutritional metabolism and health of the host fish [20–22]. The composition of fish gut microbiota can be influenced by host selection, diet, and environmental factors [7, 10, 11, 23]. Compared to the stable conditions of the buffered gut environment, the skin and gill of fish are external mucosal tissues that are in direct contact with the surrounding water, and the microbial communities in skin and gill could be more susceptible to environmental changes [16, 24]. Despite extensive studies on fish gut microbiota, knowledge about the skin and gill microbiota, microbial variations among different body niches, as well as the extent to which fish-associated microbiota are influenced by environmental-related factors are still poorly understood.

The large yellow croaker (*Larimichthys crocea*) is an economically important marine fish species widely distributed in China [25]. In 2022, large yellow croaker aquaculture production achieved 257,683 tons, ranking 1st in mariculture fish production in China [26]. Given the importance of microbiota to host health, several studies have reported the composition and diversity of microbiota associated with the gut of large yellow croaker [27, 28]. However, no literature is on knowledge of microbiota associated with external mucosal tissues such as skin and gill of large yellow croaker. In the present study, we investigated the microbiota associated with three major mucosal tissues (skin, gill and gut) of large yellow croaker and microbiota in surrounding seawater using high throughput sequencing of the 16S rRNA gene, and assessed the effects of temporal environmental change (different sampling times with seasonal transition) and culture method (net enclosure and pen) on fish-associated microbiota. We hypothesized that the structure and function of microbial communities differ according to the mucosal tissues of large yellow croaker, and expected a greater influence of the environmental-related factors on fish skin and gill microbiota than gut microbiota.

Materials and methods

Sample collection

Four batches (E-Sep, E-Nov, P-Nov, and E-Jan) of adult large yellow croakers (body weight: 613 ± 131 g) were collected from a commercial fish farm located near the Dachen Island (Taizhou, China). The fish fingerlings were introduced from a breeding fish farm (Ningbo, China), and were reared near the Dachen Island with two different culture methods for at least 5 months. Batches E-Sep, E-Nov, and E-Jan were sampled in a net enclosure (50 m × 100 m, and tide level ranged from 10 to 15 m) on 14 Sep. 2023, 3 Nov. 2023, and 16 Jan. 2024, respectively. During the sampling period, the water temperature dropped from 26℃ to 11℃ (26℃, 23℃, and 11℃ in each sampling time) with the seasonal transition from autumn to winter. Batch P-Nov was sampled in a net pen (90 m circumference, and 8 m depth) on 3 Nov. 2023, which compared with Batch E-Nov in a net enclosure. The details of the sampling information are shown in Table 1. The skin mucus, gill and intestinal samples were collected from four fish individuals in each batch. The large yellow croaker individuals were caught randomly in each sampling site. After capture, the skin mucus of all fish was immediately collected by gently rubbing with a sterile cotton swab along the entire length of the lateral line. The gill (0.5 g) was removed from the gill chamber and the distal intestines (hindgut, 2 cm) were removed from the abdominal cavity with sterile scissors and tweezers. Sampling procedures in our study complied with the guidelines of the Institutional Animal Care and Use Committee on the care and use of animals for scientific purposes. Meanwhile, the seawater (1000 mL) was collected with a sampling vessel at 50 cm depth in the net pen or net enclosure, and was transferred into a sterile glass bottle. The seawater was transported with ice bags to the laboratory located in Taizhou University within four hours, and was filtered with a 0.2 μm nuclepore

Table 1 Details of samples collected in Dachen Island

Batch	Sampling time	Culture method	Water Temp	No. fish body weight (Mean ± SD)	No. fish gut	No. fish gill	No. fish skin	No. seawater
E-Sep	14 Sep. 2023	Net enclosure	26℃	602.6 ± 56.8 g	4	4	4	1
E-Nov	3 Nov. 2023	Net enclosure	23℃	555.5 ± 88.3 g	4	4	4	1
P-Nov	3 Nov. 2023	Net pen	23℃	675.3 ± 251.3 g	4	4	4	1
E-Jan	16 Jan. 2024	Net enclosure	11℃	620.3 ± 53.5 g	4	4	4	1

track-etched membrane. All collected gill and gut tissue samples, skin swabs and filter membranes were immediately transferred to centrifuge tubes and kept stored at − 80 °C until the DNA extraction.

DNA extraction, PCR amplification, and Miseq sequencing

The DNA extraction of the skin mucus, gill, hindgut and membranes from seawater samples was performed using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, USA). Briefly, using sterile materials, each sample of 100 − 250 mg chopped gut and gill tissues, skin swabs and water membranes was placed to a Disruptor Tube with 725 μL SLX-Mlus Buffer for vortex lysing 3 − 5 min, and then were incubated at 70℃ for 10 min added with 72 μL DS Buffer. Centrifuge at 10,000 g for 5 min at room temperature, and 400 μL supernatant was transferred into a clean 1.5 mL centrifuge tube. Subsequent protocols followed by the kit manufacturer's instructions for DNA extraction. The quantity and quality of the extracted DNA were examined with NanoDrop 2000 (Thermo Scientific, USA). The V3-V4 regions of 16S rRNA gene of the extracted DNA were amplified by PCR using the universal primer pair of 338F and 806R. PCR were programmed as follows: 3 min at 95℃; 29 cycles × (30 s at 95℃; 30 s at 53℃; 45 s at 72℃); 10 min at 72℃. The PCR products were detected by 2% lipid sugar gel electrophoresis and purified using the AxyPrepDNA Gel Extraction Kit (Axygen, USA). The purified PCR products were conducted to library construction following the guide of TruSeq™ DNA Sample Prep Kit (Illumina, USA). Then, the prepared libraries were sequenced on an Illumina MiSeq platform by Majorbio Bio-Pharm Technology Co., Ltd (Shanghai, China).

Sequencing data processing

The paired-end (PE) raw reads obtained by Illumina sequencing were quality-filtered using Fastp (v0.19.6) and merged using FLASH (v1.2.11). The assembled reads were quality-filtered by discarding reads with a Phred quality score below 20 over a window of 50 bp, a homopolymer run longer than 10 bases and a nucleotide difference of > 2 bases in the primer region. The effective tag sequences with a similarity of 97% were clustered into operational taxonomic units (OTUs) with UPARSE (v7.0.1001). Taxonomic assignment of the OTUs was performed by Ribosomal Database Project (RDP) classifier (v2.11) against the Silva 16S rRNA database (v138.1). The original generated OTU table was randomly subsampled to equal sequencing depth based on the minimum number of valid sequences in the samples (41,914), and the OTUs identified as chloroplast and mitochondria were removed. Then, the subsampled OTU table was used for subsequent analyses.

Bioinformatics and statistical analyses

Alpha-diversity including richness estimators of Ace and Chao1, and diversity estimators of Shannon and Simpson were estimated by Mothur (v1.30.2). Multiple group comparison test of alpha-diversity estimators was performed by Kruskal–Wallis H test and post-hoc pairwise Tukey–Kramer test. Distance matrix of beta-diversity was calculated by QIIME (v1.9.1) based on Bray–Curtis algorithm, and visualized by principal co-ordinates analysis (PCoA). The permutational multivariate analysis of variance (PERMANOVA) computed with 999 permutations was conducted to determine the significance of inter-group differences among community structures. The Kyoto Encyclopedia of Genes and Genomes (KEGG) functional profile of microbial community based on 16S rRNA gene data was predicted by PICRUSt2 (v2.2.0). The linear discriminant analysis (LDA) effect size (LEfSe) analysis was conducted to evaluate the significant differential abundance features in microbial taxa and KEGG functional pathways of interest based on the LDA score. The statistical significance was set at $P < 0.05$.

Results

Overall sequencing data

A total of 2,179,528 valid reads were obtained from all 52 samples after quality control and rarefaction. At the 97% similarity level, these sequences were clustered into 4,492 OTUs. The rarefaction curve for each sample tended to the plateau level, indicating the sufficient sequencing depth (Fig. S1). Overall, 2445, 1629, 731 and 1647

OTUs were identified in the fish skin, gill, gut and seawater, respectively, among which 510, 477 and 177 OTUs were shared between seawater and fish skin, gill and gut, respectively (Fig. 1A). The OTU numbers of the fish skin, gill, gut and seawater identified in each batch were shown in Table 2.

Diversity of the microbial communities

According to the fish mucosal tissues, alpha-diversity analysis of Ace, Chao1, Shannon, and Simpson estimators indicated that the richness and diversity of microbiota in fish skin were significantly higher than that in the fish gill and gut ($P < 0.05$), and were highest in the seawater (Fig. 1B-E). Differences in richness and diversity of microbiota in the fish skin, gill, gut and seawater were also observed among the batches (Table 2). We therefore explored the variation pattern with the alpha-diversity of microbiota associated with the different mucosal tissues over time and across culture method. According to the sampling times, the richness and diversity of microbiota of the three tissues changed significantly over time or with seasonal transition (E-Sep, E-Nov, and E-Jan, $P < 0.05$). The richness and diversity of fish skin microbiota in E-Sep were significantly higher than that in E-Nov and E-Jan ($P < 0.05$), the richness and diversity of fish gill microbiota in E-Sep were significantly higher than that in E-Jan ($P < 0.05$), while the richness of fish gut microbiota in E-Sep was significantly lower than that in E-Nov ($P < 0.05$). According to the culture methods, a significant difference was observed in the diversity (Simpson index) of fish skin microbiota between net enclosure and net pen (E-Nov and P-Nov, $P < 0.05$), but no difference was observed in the richness and diversity of fish gill and gut microbiota between the two culture methods ($P > 0.05$).

PCoA combined with PERMANOVA test showed that the microbiota composition in the fish skin, gill, gut and seawater were significantly different from each other ($R^2 = 0.2310$, $P = 0.001$, Fig. 2A). The microbiota was

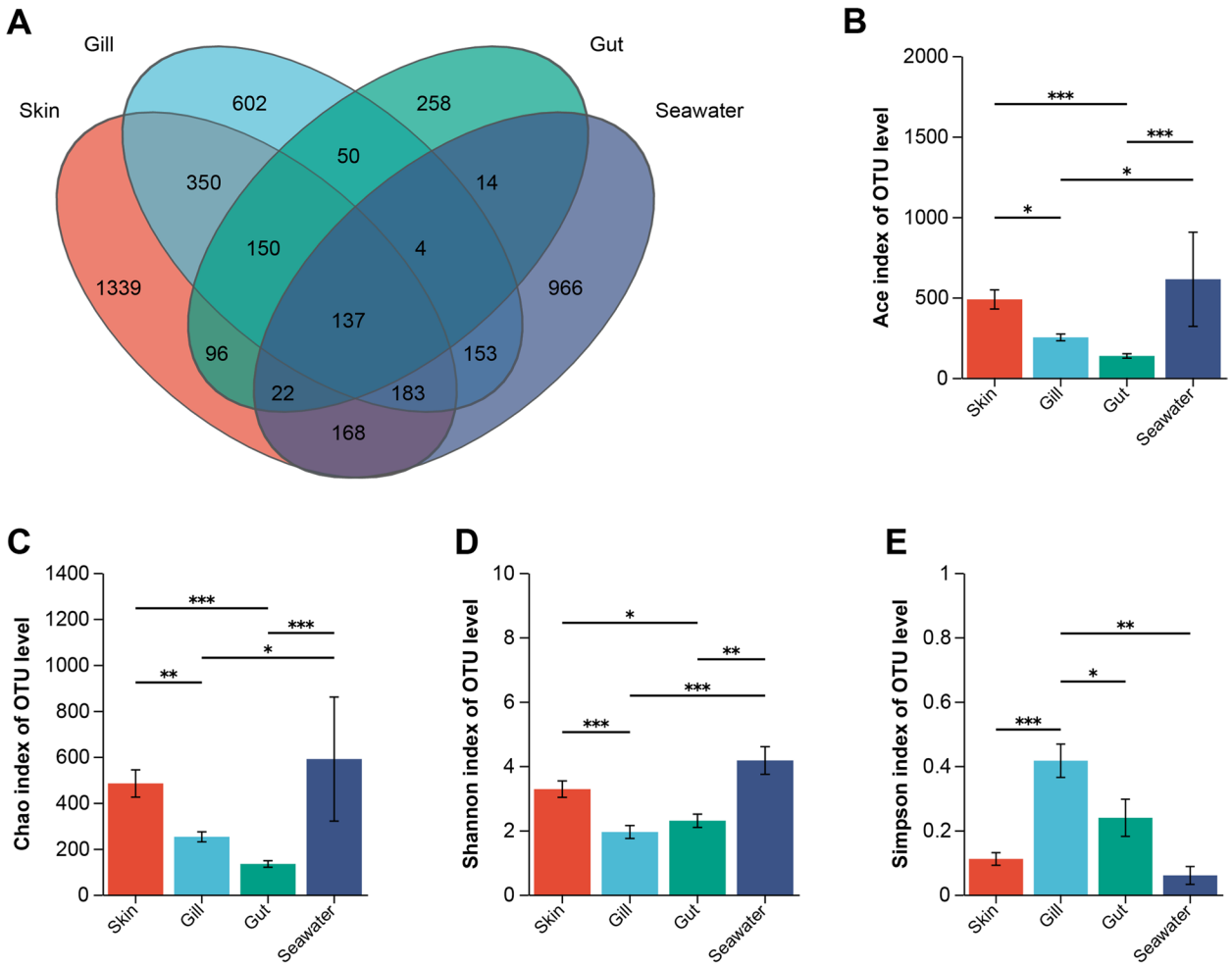

Fig. 1 OTU number and alpha-diversity estimators of microbiota in skin, gill, gut of large yellow croaker and seawater. **A** Venn plot of the OTUs distribution. **B** Ace index. **C** Chao1 index. **D** Shannon index. **E** Simpson index. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Table 2 Alpha-diversity of microbiota in the skin, gill, gut of large yellow croaker and seawater

Sampling site	Batch	Total OTUs	Observed OTUs	Ace	Chao1	Shannon	Simpson
Skin	E-Sep	1206	723 ± 68^{a}	779.20 ± 80.25^{a}	784.50 ± 78.99^{a}	4.71 ± 0.23^{a}	0.02 ± 0.01^{d}
	E-Nov	729	271 ± 247^{b}	310.60 ± 257.60^{b}	305.60 ± 252.60^{b}	2.16 ± 0.37^{d}	0.22 ± 0.04^{a}
	E-Jan	696	302 ± 28^{b}	310.90 ± 28.31^{b}	314.60 ± 28.89^{b}	3.51 ± 0.15^{b}	0.07 ± 0.02^{c}
	P		**0.018**	**0.031**	**0.018**	**0.007**	**0.007**
	E-Nov	729	271 ± 247	310.60 ± 257.60	305.60 ± 252.60	2.16 ± 0.37	0.22 ± 0.04^{a}
	P-Nov	830	424 ± 84	545.80 ± 83.26	529.30 ± 90.70	2.74 ± 0.34	0.13 ± 0.02^{b}
	P		**0.312**	**0.312**	**0.312**	**0.112**	**0.031**
Gill	E-Sep	886	319 ± 89^{a}	330.80 ± 96.60^{a}	332.80 ± 101.90^{a}	2.76 ± 0.60^{a}	0.24 ± 0.12^{b}
	E-Nov	512	217 ± 18^{ab}	234.30 ± 23.75^{ab}	235.50 ± 24.03^{ab}	2.33 ± 0.48^{a}	0.28 ± 0.14^{b}
	E-Jan	328	145 ± 15^{b}	153.50 ± 15.21^{b}	155.00 ± 14.11^{b}	1.07 ± 0.31^{b}	0.66 ± 0.11^{a}
	P		**0.010**	**0.012**	**0.015**	**0.023**	**0.024**
	E-Nov	512	217 ± 18	234.30 ± 23.75	235.50 ± 24.03	2.33 ± 0.48	0.28 ± 0.14
	P-Nov	627	259 ± 61	284.40 ± 50.50	282.80 ± 61.18	1.62 ± 0.52	0.48 ± 0.11
	P		**0.312**	**0.312**	**0.312**	**0.112**	**0.112**
Gut	E-Sep	176	79 ± 8	91.66 ± 16.75^{b}	87.67 ± 13.94^{b}	2.23 ± 0.09	0.17 ± 0.04^{ab}
	E-Nov	464	174 ± 92	193.20 ± 81.25^{a}	191.60 ± 84.05^{a}	2.83 ± 0.67	0.12 ± 0.03^{b}
	E-Jan	296	130 ± 30	138.10 ± 28.06^{ab}	136.10 ± 27.35^{ab}	1.52 ± 1.08	0.52 ± 0.34^{a}
	P		**0.023**	**0.031**	**0.021**	**0.219**	**0.017**
	E-Nov	464	174 ± 92	193.20 ± 81.25	191.60 ± 84.05	2.83 ± 0.67	0.12 ± 0.03
	P-Nov	241	108 ± 33	120.50 ± 23.96	117.40 ± 25.43	2.59 ± 0.73	0.15 ± 0.08
	P		**0.194**	**0.112**	**0.112**	**0.665**	**0.889**
Seawater	E-Sep	1054	1045	1460.05	1366.40	4.31	0.04
	E-Nov	220	220	223.98	225.14	3.86	0.05
	P-Nov	217	217	219.24	220.75	3.23	0.14
	E-Jan	538	538	545.71	545.65	5.27	0.01

The data with different superscripts within same column are significantly different ($P < 0.05$)

significantly differed according to the fish mucosal tissues ($R^2 = 0.2028$, $P = 0.001$), and the microbiota in seawater was also significantly different from that of the fish skin ($R^2 = 0.1460$, $P = 0.003$), gill ($R^2 = 0.1752$, $P = 0.001$) and gut ($R^2 = 0.1463$, $P = 0.004$), respectively. PCoA also displayed obvious separations of the microbiota composition in the fish skin ($R^2 = 0.9068$, $P = 0.001$, Fig. 2B), gill ($R^2 = 0.5209$, $P = 0.001$, Fig. 2C), and gut ($R^2 = 0.3260$, $P = 0.047$, Fig. 2D) among the batches. We therefore explored the variation pattern with the beta-diversity of microbiota associated with the different mucosal tissues over time and across culture method (Table 3). According to the sampling times, the microbiota composition of the three tissues changed significantly over time or with seasonal transition, which can explain 0.8760, 0.5960 and 0.3640 of the fish skin, gill and gut microbiota variation, respectively (E-Sep, E-Nov and E-Jan, $P < 0.05$). According to the culture methods, a significant difference was observed in the microbiota composition of the fish skin ($R^2 = 0.9397$, $P = 0.027$) between net enclosure and net pen (E-Nov and P-Nov), while no significant differences in the microbiota composition of the fish gill ($R^2 = 0.2231$, $P = 0.111$) and gut ($R^2 = 0.0506$, $P = 0\ 0.825$) were observed between the two culture methods.

Taxonomic composition of the microbial communities

The OTUs were annotated into 53 microbial phyla, 142 classes, 337 orders, 606 families, 1353 genera, and 2427 species. At the phylum level, Proteobacteria (45.18%-83.99%), Bacteroidota (3.56%-16.90%), Firmicutes (5.09%-9.72%) and Actinobacteriota (1.45%-9.06%) were consistently dominant across tissues and seawater. Gut tissue seemed most different from the other sample types with high abundances of Fusobacteriota (20.71%) and Spirochaetota (14.03%) (Fig. 3A). At the genus level, *Acinetobacter* (6.80%-16.83%) were consistently dominant across tissues and seawater. Some other genera that dominated the fish skin were *Psychrobacter* (19.19%), *Exiguobacterium* (5.76%) and *Empedobacter* (5.47%). Some other genera that dominated the fish gill were unclassified_o__*Enterobacterales* (36.05%), *2013Ark19i* (16.22%), *Achromobacter* (6.89%) and *Perlucidibaca* (2.76%). Some other genera that dominated the fish gut were *Brevinema* (14.03%), *Cetobacterium*

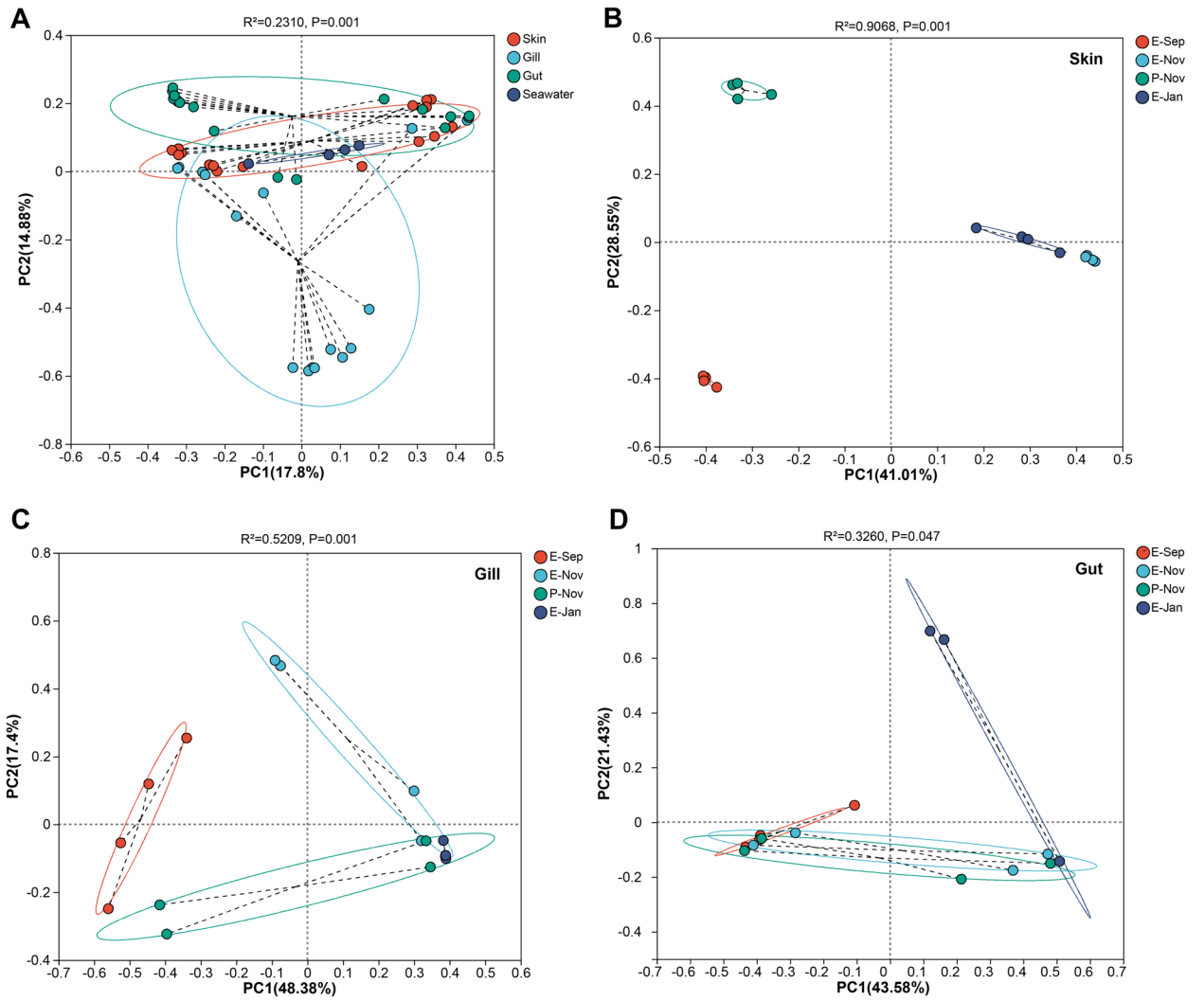

Fig. 2 Beta-diversity of the microbial communities at the OTU level. PCoA carried out on the Bray–Curtis distance among the fish skin, gill, gut and seawater samples (**A**), and among the different batches of fish skin (**B**), gill (**C**) and gut (**D**). PERMANOVA tests the statistical significance of variables ($P < 0.05$)

Table 3 Beta-diversity of microbiota in the skin, gill and gut of large yellow croaker tested with PERMANOVA

Factors	Sampling time/season (E-Sep, E-Nov, E-Jan)		Culture method (E-Nov, P-Nov)	
PERMANOVA	R^2	*P*	R^2	*P*
Skin	0.8760	0.001	0.9397	0.027
Gill	0.5921	0.001	0.2231	0.111
Gut	0.3640	0.023	0.0506	0.825

(10.71%), *Fusobacterium* (9.83%), *Perlucidibaca* (6.60%) and unclassified_f__*Barnesiellaceae* (6.36%). Some other genera that dominated the seawater were *Sphingomonas* (12.66%), *Pseudomonas* (5.40%) and *Brevundimonas* (5.33%) (Fig. 3B). LEfSe identified discriminative microbial taxa from phylum to genus levels (LDA score > 4) in the fish skin, gill and gut (Fig. 3C), in which such as *Psychrobacter* was significantly enriched in fish skin, *Enterobacterales* in the fish gill, and *Fusobacterium* in the fish gut. Moreover, the variation patterns with the composition of microbial communities over time and across culture method as well as some deviations from these patterns across different tissues (Fig. 4).

Function prediction of the microbial communities

The KEGG functional profiles of microbial communities in the fish skin, gill and gut predicted by PICRUSt2 included 6 categories in pathway level 1, 46 categories in pathway level 2, and 415 categories in pathway level 3. LEfSe identified discriminative KEGG functional

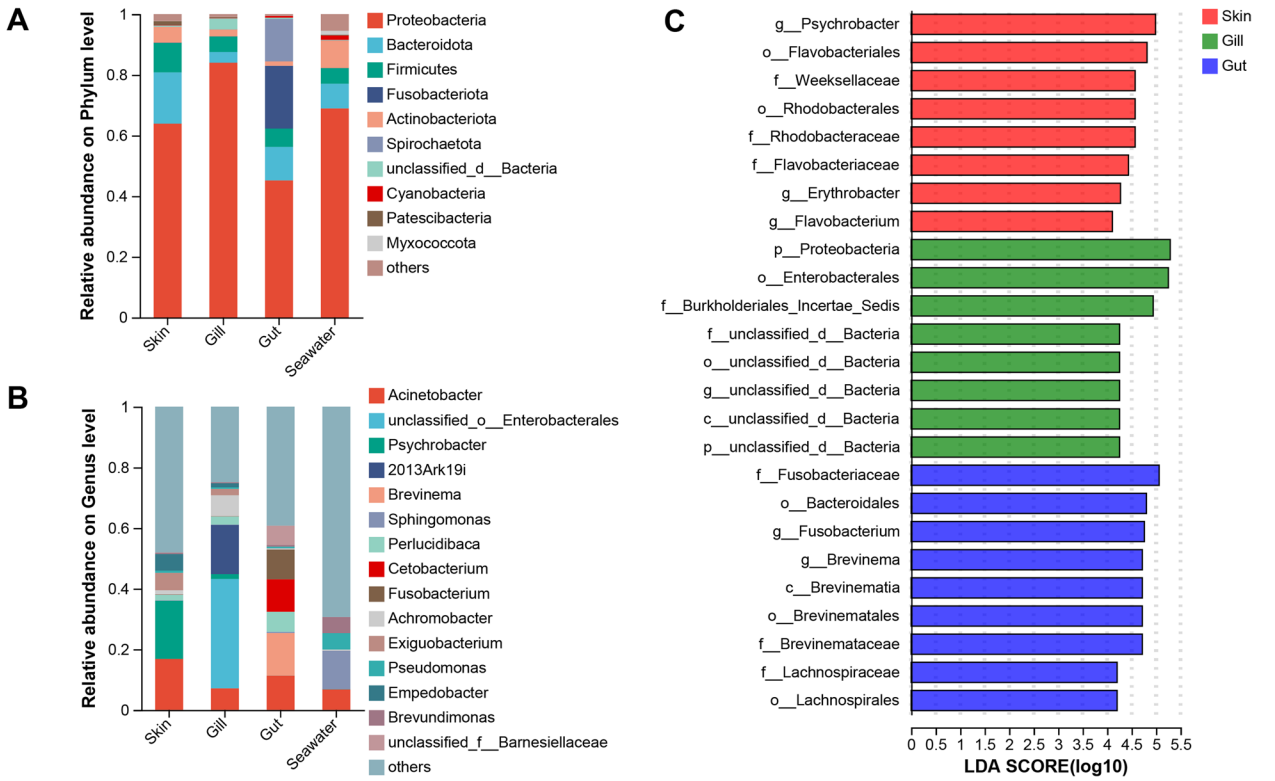

Fig. 3 Composition of microbial communities in skin, gill, gut of large yellow croaker and seawater. **A** At the phylum level. **B** At the genus level. **C** Histogram of the LDA score computed for differentially abundant features with cut-off LDA score > 4 for tissue comparison

pathways in level 2 (LDA score > 2) in the fish skin, gill and gut (Fig. 5). Pathways such as "amino acid metabolism", "xenobiotics biodegradation and metabolism", "lipid metabolism", "transport and catabolism" and "infectious disease: parasitic" were most abundant in the fish skin microbial communities. Pathways such as "cell motility", "signal transduction", "environmental adaptation", "endocrine and metabolic disease" and "circulatory system" were most abundant in fish gill microbial communities. Pathways such as "carbohydrate metabolism", "glycan biosynthesis and metabolism", "nucleotide metabolism", "biosynthesis of other secondary metabolites" and "immune system" were most abundant in fish gut microbial communities. Moreover, the variation patterns with the KEGG functional profiles of microbial communities were found over time and across culture method as well as some deviations from these patterns across different tissues (Fig. 6).

Discussion

Our study focused on the diversity, taxonomic composition and dynamic of microbial communities associated with skin, gill and gut of large yellow croaker. Consistent with our hypotheses, the structure and function of microbial communities differed according to the fish mucosal tissues, and fish skin microbiota was more diverse and variable over time (with seasonal transition) and across culture method than the gut microbiota.

The microbial communities differ according to the associated mucosal tissues

Fish body sites such as skin, gill and gut, evolve to allow colonization on the mucosal surfaces by a great diversity of symbiotic microbiota. Previous studies in other fish species, such as rainbow trout and sofie fish reported that the anatomical region is the major determinant of microbial communities, and a specific microbial assemblages exist in each region or tissue type [15–19]. Similar to previous study, we were also able to the distinguish between the microbial communities associated with the skin, gill and gut in large yellow croaker, despite some changes in temporal (sampling times) or spatial scales (culture methods). Higher richness and diversity of microbiota were observed in external mucosal tissues especially in the skin of large yellow croaker, as previous found that the fish skin had high microbial diversity [15, 18]. This pattern could be related to the extent of contact with sources of microbes in the surrounding seawater. In general, the skin and gill are tissues that in direct contact with the surrounding water, and the associated microbiota

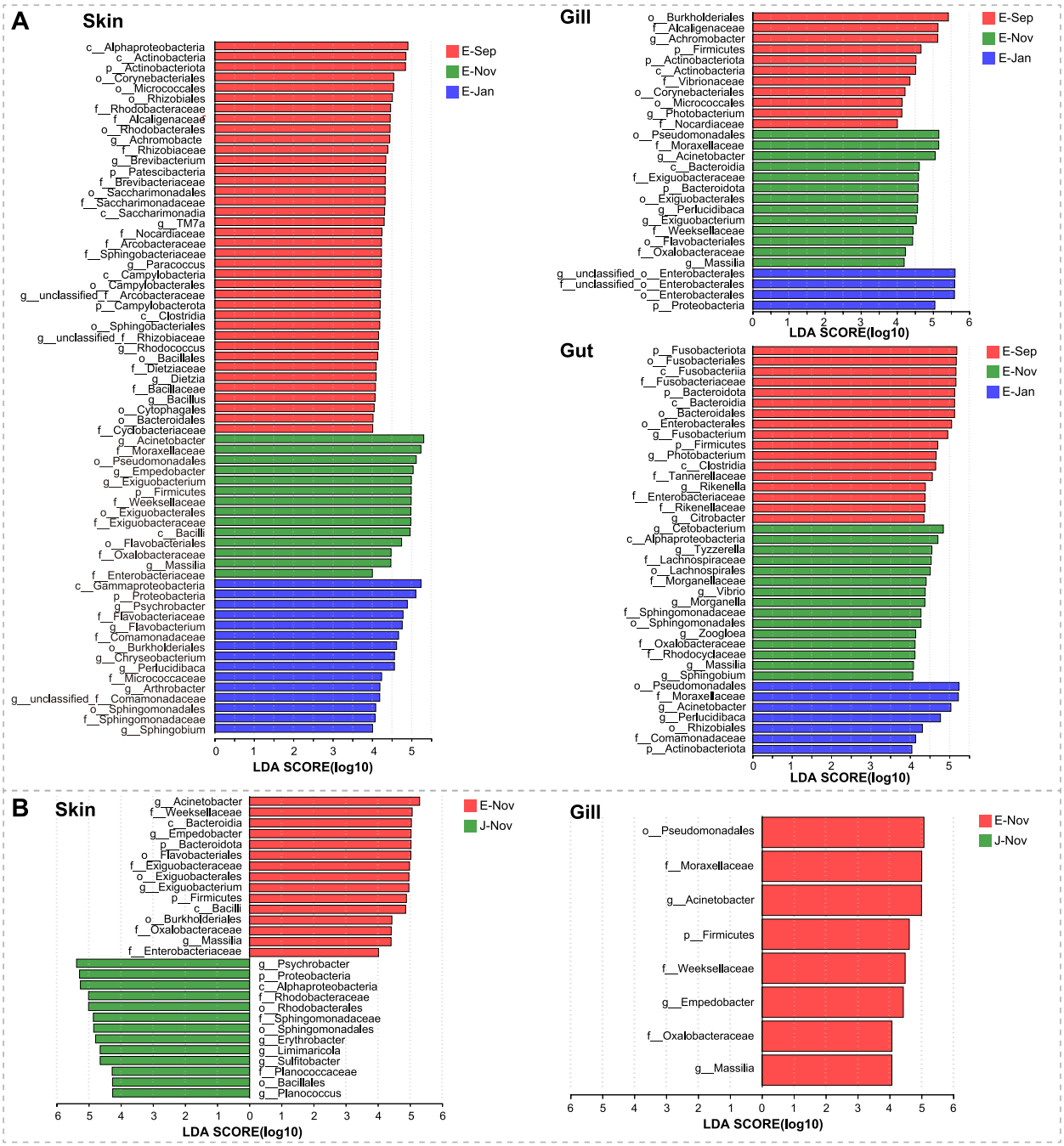

Fig. 4 Comparison of skin, gill, and gut microbial communities between the seasons (**A**) and among the culture methods (**B**). Histogram of the LDA score computed for differentially abundant features with cut-off LDA score > 4. No differentially abundant feature was identified in gut microbial communities among the culture methods

are considered to the partial reflection of the microbial diversity of the water flowing over these surfaces [8, 29]. In fact, this view is also supported by the highest richness and diversity of microbiota observed in seawater and more OTUs shared between skin or gill and seawater than gut in this study. In addition, we found that the microbiota composition associated with the skin, gill and gut of larger yellow croaker significantly differed from that of seawater, although the dominant microbial phyla and genera of fish had some overlap with that of seawater. This result suggests that the microbial communities associated with skin, gill and gut of large yellow croaker

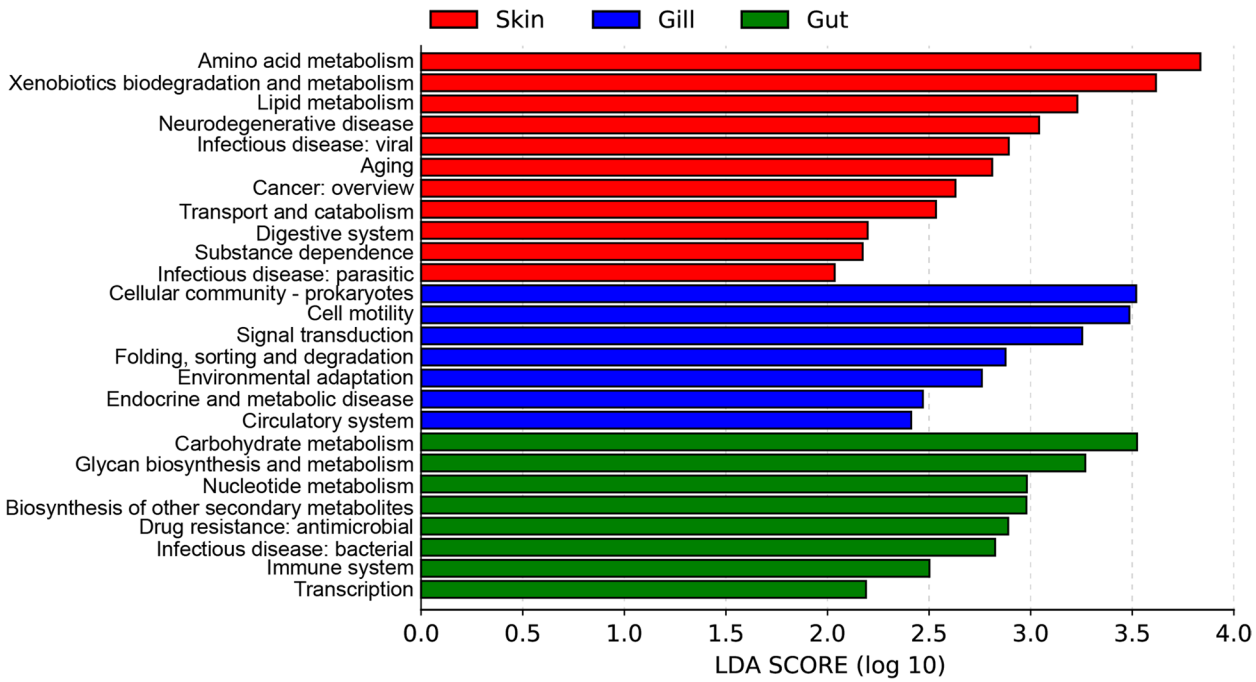

Fig. 5 Discriminative KEGG functional pathways in different tissues with cut-off LDA score > 2

are not simple reflections the microbiota of surrounding water, but may be the result of selective pressure of different tissues [24, 30]. Further, we found a series of discriminative microbial taxa associated with the skin, gill and gut of large yellow croaker, such as *Psychrobacter* in the skin, *Enterobacterales* in the gill, and *Fusobacterium* in the gut. These results are consistent with previous findings. For example, *Psychrobacter* are known as part of the normal surface microbiota of marine fish skin [31, 32]. *Enterobacteriaceae* (*Enterobacterales*) were found as dominant and core taxa in Nile tilapia and grey mullet gill [33]. *Fusobacterium* were reported as the representative taxa in gut of wild large yellow croaker in previous study [27]. In general, microbial symbionts are critical biological components for host fitness traits [1]. It is suggested that the skin, gill and gut of large yellow croaker may selectively enrich these taxa that are vital for the host health.

Effects of the environmental-related factors on the microbial communities

In order to explore the response patterns of microbiota associated with the skin, gill and gut of larger yellow croaker to environmental changes, we further analyzed the variation pattern with the microbial communities associated with the different mucosal tissues over time and across culture method. The temporal environmental change in this study include the alterations of environmental physicochemical parameters (especially seasonal changes in water temperature) and the seawater microbiota. Among which, the water temperature is a well-known major environmental factor affecting fish-associated microbiota [34–37]. In the present study, we found that the diversity and composition of microbial communities in the skin, gill and gut of large yellow croaker changed significantly over time, which could be largely explained by the seasonal water temperature changes. Difference in variation patterns with microbial communities over time were found across different tissues. The difference in skin microbial communities across sampling times was most significant among the three tissues. Moreover, the culture method had only significantly influenced on the diversity and composition of microbiota in the skin of large yellow croaker. These results are consistent with the conclusion that the fish skin microbiota is more affected by environmental factors than the gut microbiota in previous studies [15, 24, 30], as we predicted. By contrast, in addition to environmental factors, the gut microbiota is largely affected by host-related factors and diet [38, 39]. Previous studies reported that the gut microbial communities of large yellow croaker could be influenced by dietary factors, such as the protein ingredient composition and exogenous functional constitutes [40–42]. This pattern also reflects differences in the factors determining the composition of these microbial communities. We found that no significant

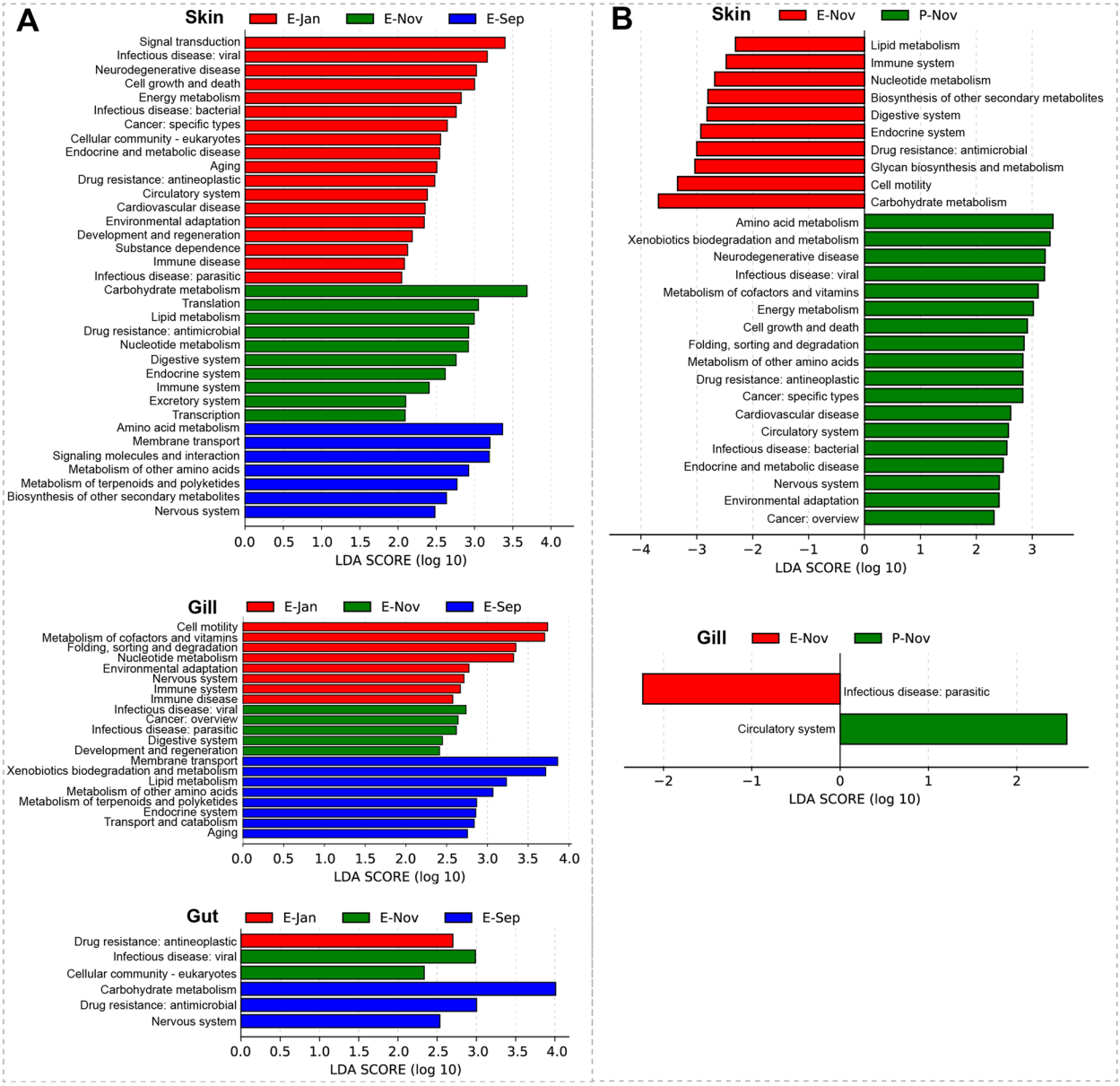

Fig. 6 Discriminative KEGG functional pathways in different sampling times (**A**) and culture methods (**B**) with cut-off LDA score > 2. No discriminative KEGG functional pathway was identified in gut microbial communities between the culture methods

difference in the gill and gut microbial communities of large yellow croaker between net enclosure and net pen, suggesting that the culture method is not the main environmental factor affecting the fish gill and gut microbiota. This result is similar to previous work that found no significant differences in fish gut microbial communities between a recirculating aquaculture system and net pens [12], and indicates that holding system does not have a large effect on the microbial community of fish guts. Compared with the temporal environmental change, we found that the culture method had less impact on the gut and gill microbiota of large yellow croaker. This result suggests that different environmental factors have different effects on the gill and gut microbial communities of large yellow croaker.

Functional profiles of the microbial communities

The symbiotic microbiota evolves along with the host and supports vital physiological functions via various approaches, such as digestion and absorption of nutrients, immune regulation, and resistance to pathogens infection [3–5]. Abundant KEGG functional pathways were

predicted in microbial communities associated with the skin, gill and gut of large yellow croaker, suggesting their potential functions in a variety of host metabolic and physiological processes. Multiple discriminative KEGG functional pathways in level 2 were identified in the skin, gill and gut of large yellow croaker, suggesting that they participate in different physiological functions of large yellow croaker. For example, the skin microbiota was enriched in pathways of "amino acid metabolism", "xenobiotics biodegradation and metabolism", "lipid metabolism", "transport and catabolism" and "infectious disease: parasitic". These predicted functions may be related to mucus secreted by the fish skin, for the skin mucus of fish has various components such as proteins, carbohydrates and lipids, and provides a the first line against the invading pathogens [43]. The skin microbiota may participate in the secretion of skin mucus components and immune defense function through those pathways. The gill microbiota was enriched in pathways related to interaction and communication processes such as "cell motility", "signal transduction", "environmental adaptation" and "circulatory system", as well as "endocrine and metabolic disease". These predictive functions may be associated with the fact that the fish gill is the major organ for respiratory gas exchange and excretion [44]. The gill microbiota may participate in respiratory gas exchange through circulatory system, or excretion function through cell motility. The gut microbiota was enriched in pathways of "carbohydrate metabolism", "glycan biosynthesis and metabolism", "nucleotide metabolism", "biosynthesis of other secondary metabolites" and "immune system". This result is consistent with the facts that the fish gut is the primary digestive organ and the constituent of the mucosal immune system [14, 45], and the microbiota in fish gut plays vital roles in immune regulation and assist in optimal nutrient absorption [21, 46]. Furthermore, the variation patterns with the KEGG functional profiles of microbial communities were found over time and across culture method, suggesting that microbiota could assist host fish adaptation to environmental changes through various metabolic pathways. Our study highlights the relationship between microbiota within different body niches of large yellow croaker and host health. However, the functions of these microbial communities of large yellow croaker need to be verified in the further studies.

Conclusion

In conclusion, we investigated the traits and dynamic of microbial communities associated with the skin, gill and gut of large yellow croaker. Our study showed the structure and function of microbial communities differed according to the mucosal tissues of large yellow croaker, and changed over time (with seasonal transition) and across culture method. Different environmental-related factors (such as sampling season and culture method) had impacts on microbial communities differently. In particular, as we expected, the skin microbiota of large yellow croaker was more diverse and affected by environmental-related factors than other tissues.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03695-6.

Supplementary Material 1.

Acknowledgements
Not applicable.

Authors' contributions
Jingan Wang conducted research conception, sample collection, data analysis, manuscript writing and funding acquisition. Chenghao Hu and Xiaojie Tong conducted sample collection and preparation. conducted sample collection and preparation. Yuan Gao and Renjie Liang conducted sample collection and funding acquisition. Chibo Liu and Kai Zhao conducted project administration and supervision.

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Data availability
The raw sequencing reads of 16s amplicon sequencing has been deposited in NCBI under the accession number PRJNA1100934.

Declarations

Ethics approval and consent to participate
A local ethics committee ruled that no formal ethics approval was required in this particular case. The fish samples were collected from a commercial fish farm upon obtaining their permission.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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