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Effects of Water Temperature on the Growth, Antioxidant Capacity, and Gut Microbiota of *Percocypris pingi* Juveniles

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Abstract: It is necessary to determine the optimal temperature for *Percocypris pingi* growth in recirculating aquaculture systems. To describe the effects of temperature, we evaluated the growth, antioxidant enzyme activity, and gut microbiota structure of *P. pingi* at different temperatures, including 14, 18, 22, and 26 °C. Results showed that increases in body weight of individuals of the groups subjected to 18 and 22 °C temperatures were considerably higher than those in the groups subjected to temperatures of 14 and 26 °C between 20 and 60 d after the experiment started. Acid phosphatase activity in the liver and kidneys of *P. pingi* did not differ significantly among the various temperature groups ($p > 0.05$). A gradual restoration of the alkaline phosphatase and superoxide dismutase activities to variations in the surrounding temperature was observed in the liver and kidney of *P. pingi*. Interestingly, the water temperature did not affect the α-diversity or composition of the gut microbiota of *P. pingi*. In conclusion, water temperatures between 14 and 26 °C significantly impacted the growth of *P. pingi* ($p < 0.05$) but not the liver and kidney antioxidant capacity or the gut microbiota within 60 d.

Keywords: *Percocypris pingi*; gut microbiota; antioxidant enzyme activity; growth; water temperature

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1. Introduction

As an endemic species in the upstream regions of the Yangtze River, *Percocypris pingi* is an economically important freshwater fish in southeast China because of its high protein level, reduced fat content, and high nutritional value [1–3]. However, owing to the destruction of its natural habitat caused by environmental degradation, dam construction, and overfishing, its population has sharply declined in recent years. These issues have led the fish species to be included in the list of key protected wild animals in China (2021) [4]. Artificial reproduction and breeding are crucial for meeting consumer demand and preventing the depletion of natural *P. pingi* resources [5]. Currently, regarding reproduction and breeding, research has been conducted on the embryonic development of *P. pingi* [2], the impact of temperature, pH, and salinity on the survival of juvenile stages [6], and the allometric growth of *P. pingi* larvae [3].

As ectotherms, fish physiology is strongly affected by temperature [7,8]. The temperature usually affects: basal metabolism; fish energy storage by influencing nutrient digestion and assimilation; surplus energy investment in reproduction and growth; and energy intake via feeding [8,9]. The thermal safety margin and resilience have become important indicators for assessing fish adaptation to the surrounding aquatic environment owing to climate change, specifically global warming [10,11]. Although previous studies have shown that the tolerance range of larval *P. pingi retrodorslis* to temperature is 0–32 °C, the optimal growth temperature is 8–27 °C [6], and the tolerance range of adult *P. pingi* to temperature is 2–28 °C, and the optimal growth temperature is 20–25 °C [12]. The effect of temperature on the physiological processes and metabolism of *P. pingi* has not yet been explained thoroughly.

The gut microbiota plays a crucial role in various physiological processes in the host [13–17]. These elements are also integral to the health of host fish [18]. Understanding the effects of temperature on host–microbiota interactions would improve predictions of biodiversity responses to climate warming [19]. Recently, Ghosh et al. [18] reported that temperature changes to 18 and 8 °C from the control level of 13 °C triggered marked dysbiosis in the fecal microbiota of chum salmon, *Oncorhynchus keta*, and the opportunistic pathogenic *Vibrio* and *Tenacibaculum* sequence variants were highly abundant at high and low temperatures, respectively, and may impede host immunity. However, the influence of temperature change on the gut microbiota of *P. pingi* remains unclear.

To describe the effects of temperature on the physiology and gut microbiota of *P. pingi*, we compared the growth, antioxidant enzyme activity, and gut microbiota structure of *P. pingi* under different water temperatures. These results provide essential data for the breeding and ecological management of the fish species.

2. Materials and Methods

2.1. Experimental Design and Sample Collection

P. pingi juveniles with an initial body weight of 34.02 ± 1.20 g and body length of 12.83 ± 0.53 cm were collected from the Yangtze River Fisheries Research Institute and stocked in a recirculating aquaculture system at 18 °C before the experiment. In April 2018, 840 fish were selected and distributed evenly across four independent recirculating aquaculture systems at a starting temperature of 18 °C. Three conical polypropylene tanks (diameter = 80 cm, water volume = 300 L, and water flow rate = 0.286 L/s) were included in each recirculating aquaculture system, with a fish density of 70 individuals per tank. The water temperature was adjusted at four temperatures of 14, 18, 22, and 26 °C (named T1, T2, T3, and T4) at a rate of 1 °C/4 h using a 1.47 KW aquarium refrigerating and heating machine (temperature control accuracy 0.1 °C) in four independent recirculating aquaculture systems. After the target temperature was stable within a deviation of <0.5 °C for one week, fish were subjected to the acclimation procedure for 60 d. Fish were fed the formulated feed twice (09:00 and 17:00) per day. The remaining feed was drained after 2 h of feeding. The biochemical composition of the formulated feed comprised of crude protein, crude lipid, crude ash, crude fiber, lysine, total phosphorus, and moisture at ≥42.0, ≥5.0, ≥16.0, ≥1.0, ≥2.4, and ≤12.0%, respectively. During the experiment, the one-third volume of water was replaced with aerated tap water of the same volume and temperature in each tank daily. The photoperiod was 12L:12D. All fish were individually weighed 0, 20, 40, and 60 d after the start of the experiment to calculate the weight gain rate (WGR) and specific growth rate (SGR). WGR and SGR were calculated according to the method described by Zeng et al. [20].

When the target temperature was stable (deviation < 0.5 °C) for one week and before feeding, three random fish (named S1, S2, and S3) were collected from each tank and anesthetized with an overdose (70 mg/L) MS 222 (Syndel, Ferndale, WA, USA) [21,22], and the time was labeled as D0. Body weight was measured before tissue sampling. The exterior surfaces of the fish were swabbed using 75% ethanol before dissecting the ventral midline. Liver, kidney, and fecal contents in the intestine were collected using a sterile scalpel and forceps and then stored at −80 °C until further analysis. After feeding, three fish samples were collected at 20 (D20), 40 (D40), and 60 (D60) days, when the experiment was completed, using the same process as D0.

2.2. Determination of Antioxidant Enzyme Activities in the Liver and Kidney

The levels of superoxide dismutase (SOD), alkaline phosphatase (AKP), and acid phosphatase (ACP) in the liver and kidney of *P. pingi* samples were measured using the corresponding kits (A060-2 for ACP, A059-2 for AKP, and A001-1 for SOD; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) on a Chemray 240 automatic biochemical analyzer (Rayto, Shenzhen, China) according to the manufacturer's instructions.

2.3. Gut Microbiota Structure Analyses

Total microbiota genomic DNA was extracted from approximately 0.3 g of the freshly dissected gut of each fish using the FastDNA spin kit for soil (MP, Solon, OH, USA). DNA quality was evaluated using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), and DNA integrity was evaluated using 1.2% agarose gels. According to the concentration, the DNA was diluted to 10 ng/μL using sterile water for further amplification. The V4-V5 hypervariable region of 16S rDNA was amplified using the universal primer pair 515F and 909R, with a 12-nucleotide sample-specific barcode included at the 5′-end of the 515F sequence to distinguish samples, as previously described [23,24]. The amplicons were purified and sequenced using the HiSeq platform at Guangdong Meilikang Bio-Science Ltd. (Foshan, China).

Raw reads were merged using FLASH 1.2.8 and subsequently processed using QIIME 1.9.0 [25], as previously described [26]. Briefly, all the merged sequences were assigned to each sample based on their barcode sequences, and trimmed barcodes and primer sequences were removed using QIIME 1.9.0 software. Low-quality and chimeric sequences were removed using QIIME 1.9.0 and UCHIME, respectively. Subsequently, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) with 97% identity using UPARSE [27]. The taxonomy of each OTU was assigned using the RDP classifier [28] in the gg_13_8 database. Alpha-diversity indices and weighted and unweighted UniFrac distances were calculated using the QIIME version 1.9.0.

2.4. Data Analyses

The results are presented as the mean ± standard error. Nonparametric multivariate analysis of variance was used to test for differences between microbial communities using the R vegan package [29]. RDA with the Monte Carlo method was conducted using the R vegan package. Pearson's correlation analysis was conducted using R psych, reshape2, and corrplot packages. Heatmap plots and boxplots were drawn using the R pheatmap and ggpubr packages, respectively. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Effects of Water Temperature on the Growth of P. pingi

During the experiment, the dissolved oxygen was 7.1–8.6 mg/L, ammonia nitrogen was 0.3–0.7 mg/L, nitrite nitrogen was 0.02–0.08 mg/L, and pH was in the range of 7.5–8.1. None of the fish died during the experiment. The body weight of *P. pingi* in each temperature group increased with culture time. The body weights of the individuals in the groups subjected to 18 and 22 °C increased significantly between D20 and D60 compared to those in the groups subjected to 14 and 26 °C ($p < 0.05$). However, there was no significant difference between the 18 and 22 °C groups and between the 14 and 26 °C groups ($p > 0.05$; Table 1).

The weight gain rates of the 14, 18, and 22 °C groups reached a maximum at D40 and decreased at D60. The weight gain rate of the group subjected to 26 °C was the highest at D20, the lowest at D40, and increased at D60. The weight gain rates of the 18 and 22 °C groups were significantly higher than those of the 14 and 26 °C groups between D0 and D40 ($p < 0.05$). There was no significant difference in the weight gain rate between the 18 and 22 °C groups and between the 14 and 26 °C groups ($p > 0.05$). The weight gain rate of the group subjected to 22 °C was significantly higher than that of the groups subjected to 14 and 26 °C between D40 and D60, and there was no significant difference between the other groups (Table 1).

Table 1. Body weight and weight gain rate of juvenile *Percocypris pingi* at different temperatures. Significant differences in means between the four culture temperature groups are indicated using distinct shoulder letters in the same row ($p < 0.05$).

Item	Culture Time (d)	Culture Temperature (°C)			
		14	18	22	26
Body weight (g/ind.)	0	33.61 ± 0.77	34.65 ± 0.45	34.43 ± 0.97	33.39 ± 0.93
	20	35.95 ± 0.64 ^{a}	40.84 ± 0.97 ^{b}	40.34 ± 0.68 ^{b}	36.11 ± 1.00 ^{a}
	40	39.94 ± 1.21 ^{a}	48.33 ± 1.17 ^{b}	46.84 ± 1.12 ^{b}	37.42 ± 1.16 ^{a}
	60	42.58 ± 0.48 ^{a}	53.25 ± 1.41 ^{b}	52.79 ± 1.09 ^{b}	40.02 ± 1.36 ^{a}
Weight gain rate (%)	20	6.97 ± 0.55 ^{a}	17.87 ± 3.02 ^{b}	17.22 ± 2.26 ^{b}	8.19 ± 4.05 ^{a}
	40	11.08 ± 1.39 ^{a}	18.34 ± 0.74 ^{b}	16.13 ± 1.40 ^{b}	3.62 ± 0.35 ^{c}
	60	6.66 ± 2.24 ^{a}	10.17 ± 0.76 ^{ab}	12.7 ± 2.79 ^{b}	6.96 ± 1.96 ^{a}
Specific growth rate (%)	20	0.34 ± 0.03 ^{a}	0.82 ± 0.13 ^{b}	0.79 ± 0.10 ^{b}	0.39 ± 0.19 ^{a}
	40	0.53 ± 0.07 ^{a}	0.84 ± 0.03 ^{b}	0.75 ± 0.06 ^{b}	0.18 ± 0.02 ^{c}
	60	0.32 ± 0.11 ^{a}	0.48 ± 0.03 ^{a,b}	0.60 ± 0.13 ^{b}	0.33 ± 0.09 ^{a}

3.2. Effects of Water Temperature on the Activities of Three Antioxidant Enzymes in the Liver and Kidney of P. pingi

There was no significant difference in ACP activity in the liver and kidney of *P. pingi* among the different temperature groups. At D40 of culture, the AKP activities of the liver and kidney in the 22 °C group were significantly higher than those of the other groups. Moreover, the SOD activity in the kidneys of *P. pingi* individuals in the group subjected to 22 °C was also significantly higher than that in other groups ($p < 0.05$; Figure 1). At D60 of culture, the SOD activity in the livers of *P. pingi* individuals in the group subjected to 22 °C was significantly higher than that in the group subjected to 26 °C, and the SOD activity in the kidneys of the individuals in the group subjected to 22 °C was significantly higher than that in the group subjected to 18 °C ($p < 0.05$; Figure 1). These results implied that AKP and SOD activities in the liver and kidney of *P. pingi* gradually adapted to changes in ambient temperature.

3.3. Effect of Water Temperature on the Gut Microbiota Structure of Percocypris pingi

After removing low-quality sequences, 1,527,925 high-quality sequences were obtained from 48 gut microbiota samples of *P. pingi* cultured at different temperatures. To eliminate the interference of various sample sequencing depths on the subsequent analysis results, we randomly selected 22,306 sequences from each sample for subsequent analysis. Although 65 phyla were detected in these sequences, *Crenarchaeota*, *Euryarchaeota*, *Parvarchaeota*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Cyanobacteria*, *Firmicutes*, *Fusobacteria*, *Planctomycetes*, *Proteobacteria*, SBR1093, *Tenericutes*, and *Verrucomicrobia* dominated the gut microbiota, containing 97.33 ± 0.20% of the high-quality sequences analyzed in this study (Figure 2A). Although their relative abundances varied between different temperature groups at distinct time points during culture, only the relative abundances of *Actinobacteria* and *Verrucomicrobia* in the group subjected to 18 °C decreased gradually with culture time, whereas the relative abundance of *Fusobacteria* gradually increased (Figures 3 and 4).

A total of 12,090 OTUs were identified. However, an average of 1763.83 ± 55.67 OTUs were detected in each sample (Figure 2B). At the beginning of the experiment, there was no significant difference in the average weight and activities of ACP, AKP, and SOD in the liver and kidney of *P. pingi* samples between different temperature groups (Table 1), and the α-diversity indices of the gut microbiota were significantly different between the different temperature groups (Figure 2B–E). Particularly, the richness, Shannon, and Simpson indices of the gut microbiota in the groups subjected to 26 °C were significantly higher than those in the groups subjected to 18 °C and 22 °C, and the ACE index was significantly higher than that of the group subjected to 22 °C. Moreover, the richness, ACE, and Shannon indices of the gut microbiota in the group subjected to 14 °C were significantly higher than

those in the group subjected to 18 °C ($p < 0.05$; Figure 2B–E). Although these α-diversity indices changed significantly during the experiment, they did not correlate with culture temperature (Figure 2B–E). These results suggest that culture temperature did not affect the α-diversity of the *P. pingi* gut microbiota.

At the genus level, 1212 genera were detected in the gut microbiota, of which 47 were dominant (Figure 5). Although the relative abundances of the most dominant genera were significantly different between the groups ($p < 0.05$; Figure 5), only *Bacteroides*, *Cetobacterium*, *Novosphingobium*, *Sediminibacterium*, and *Escherichia* weakly correlated with temperature (Figure 6). The ratio of the relative abundance of *Bacteroides* in the 22 °C and 26 °C groups was reversed with the passage of culture time (Figures 6 and 7). The relative abundance of *Cetobacterium* in the group subjected to 18 °C exhibited an upward trend with the increase in culture time, whereas that of *Novosphingobium* and *Sediminibacterium* gradually declined (Figures 6 and 7). Moreover, the relative abundance of *Escherichia* in the group subjected to 26 °C group exhibited an upward trend with the increase in culture time (Figures 6 and 7).

To analyze the correlation between the gut microbiota composition of *P. pingi* and the culture conditions (temperature and sampling time) and the activities of ACP, AKP, and SOD in the liver and kidney of *P. pingi*, RDA with the Monte Carlo method was used to analyze the correlation between OTU and the dominant genus composition of *P. pingi* gut microbiota and the indicators. The results showed that only sample time and kidney ACP activity were significantly correlated with the composition of *P. pingi* gut microbiota (Figure 8A,B). Pearson correlation analysis showed that the sample time was significantly positively correlated with the relative abundances of *Cetobacterium* and *Curvibacter* but significantly negatively correlated with the relative abundances of *Mycoplasma*, *Streptococcus*, *Corynebacterium*, and *Laceyella*. Culture temperature was significantly positively correlated with the relative abundance of *Escherichia* and *Vitreoscilla* and was significantly negatively correlated with *Bacteroides*. ACP activity in the liver was significantly positively correlated with the relative abundance of *Exiguobacterium* spp. Liver SOD activity was significantly negatively correlated with the relative abundance of *Aeromonas* and *Vitreoscilla*. Kidney ACP and AKP activities were significantly positively correlated with the relative abundances of *Aeromonas* and *Curvibacter*, respectively (Figure 8C,D).

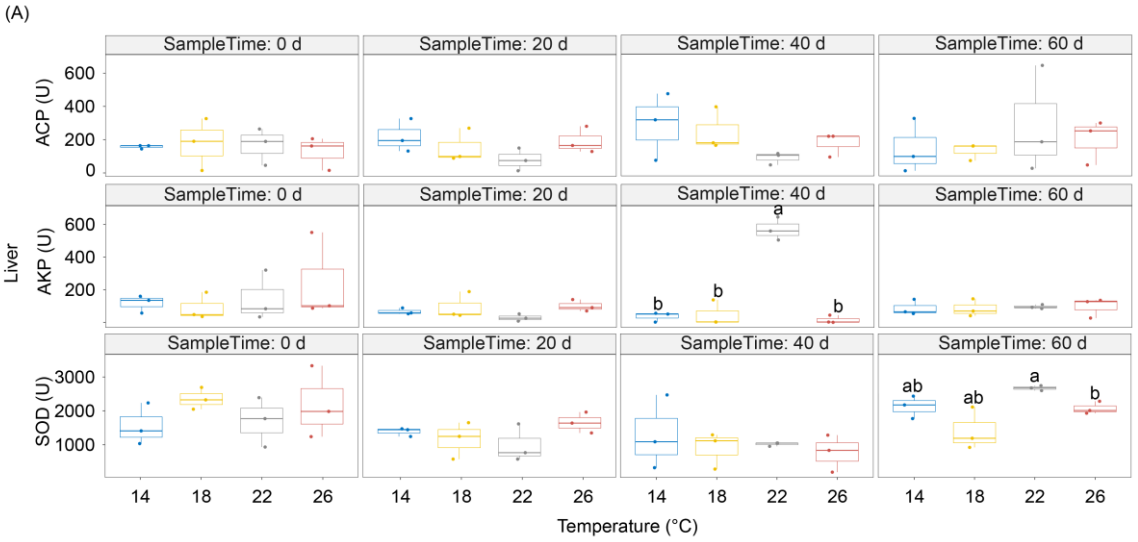

Figure 1. *Cont.*

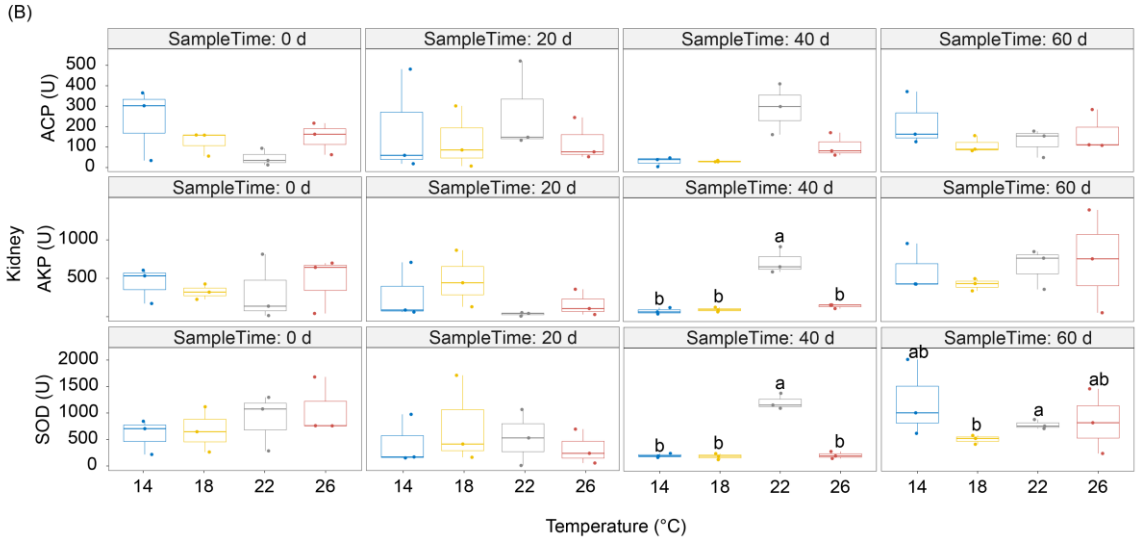

Figure 1. Enzymatic activities of ACP, AKP, and SOD in the liver (**A**); and kidney (**B**) of *Percocypris pingi* at different culture temperatures. The difference in the lower-case letters above the boxes indicates a significant difference between the two groups ($p < 0.05$).

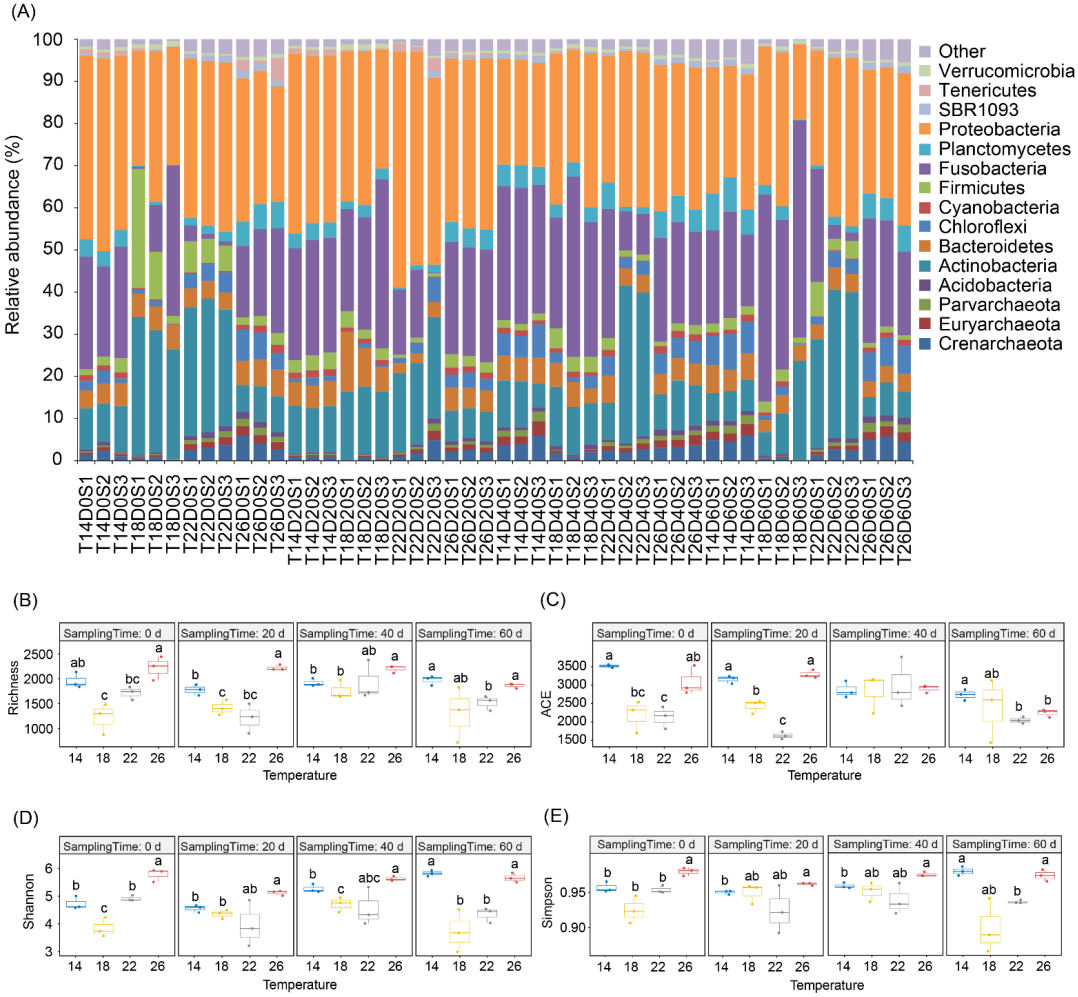

Figure 2. Dominant phylum compositions (**A**); and α-diversity indices (**B**–**E**) of *Percocypris pingi* gut microbiota at different culture temperatures. (**B**) Richness index; (**C**) ACE index; (**D**) Shannon index; and (**E**) Simpson index. Dots in the boxplots indicate values measured in samples. Different letters above the boxes indicate significant differences ($p < 0.05$).

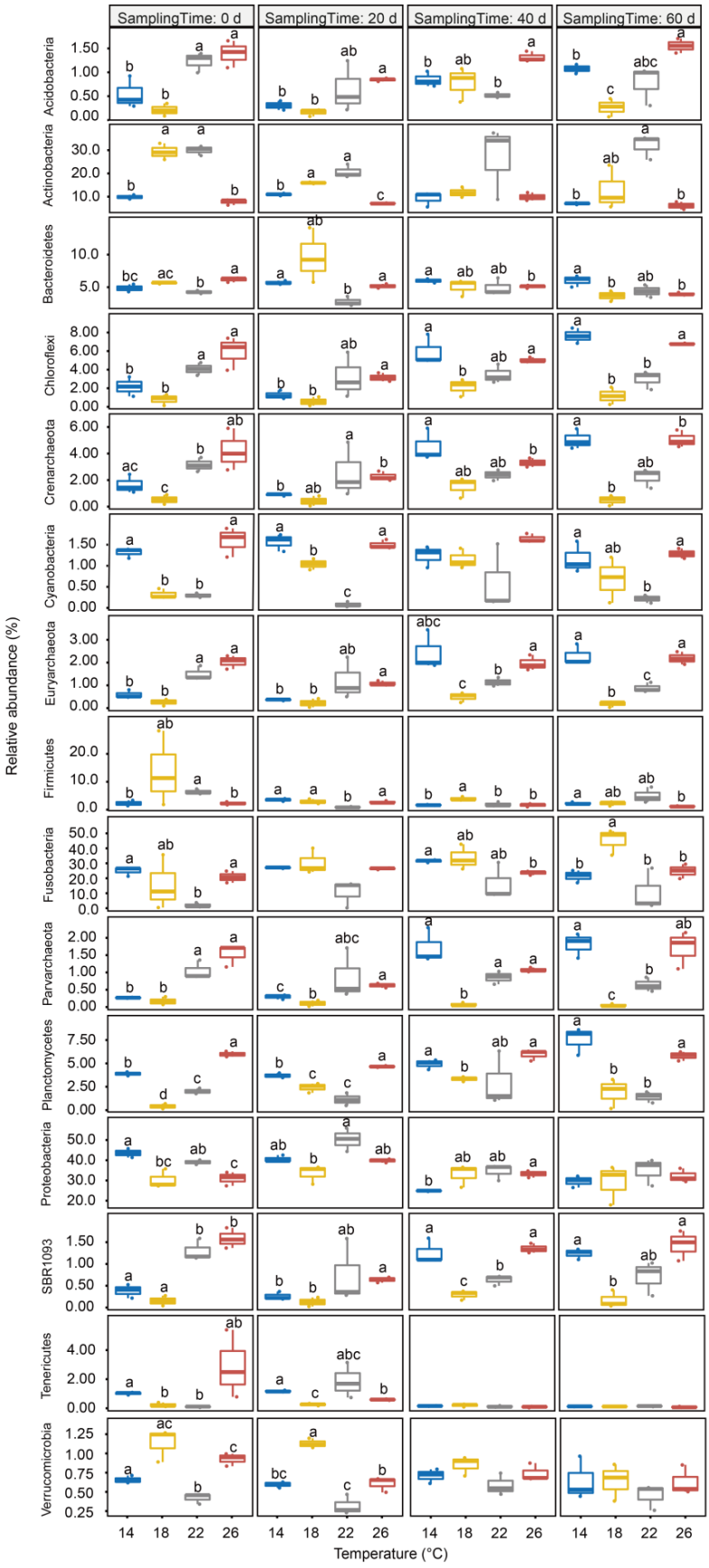

Figure 3. Relative abundance changes of commonly dominant phyla of *Percocypris pingi* gut microbiota at different culture temperatures. Dots in the boxplots indicate values measured in samples. Distinct letters above the boxes indicate significant differences ($p < 0.05$).

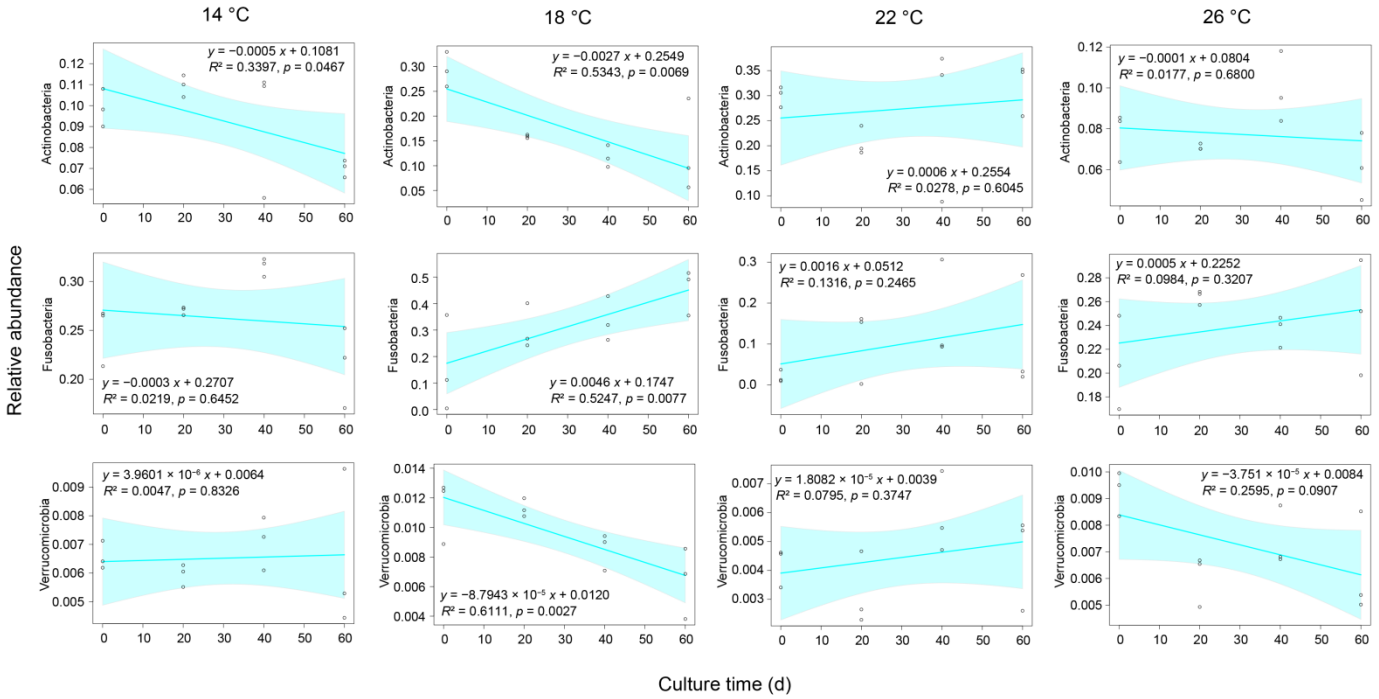

Figure 4. Correlation between the culture time and the relative abundances of *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia* in *Percocypris pingi* gut microbiota. Dots in the boxplots indicate values measured in samples.

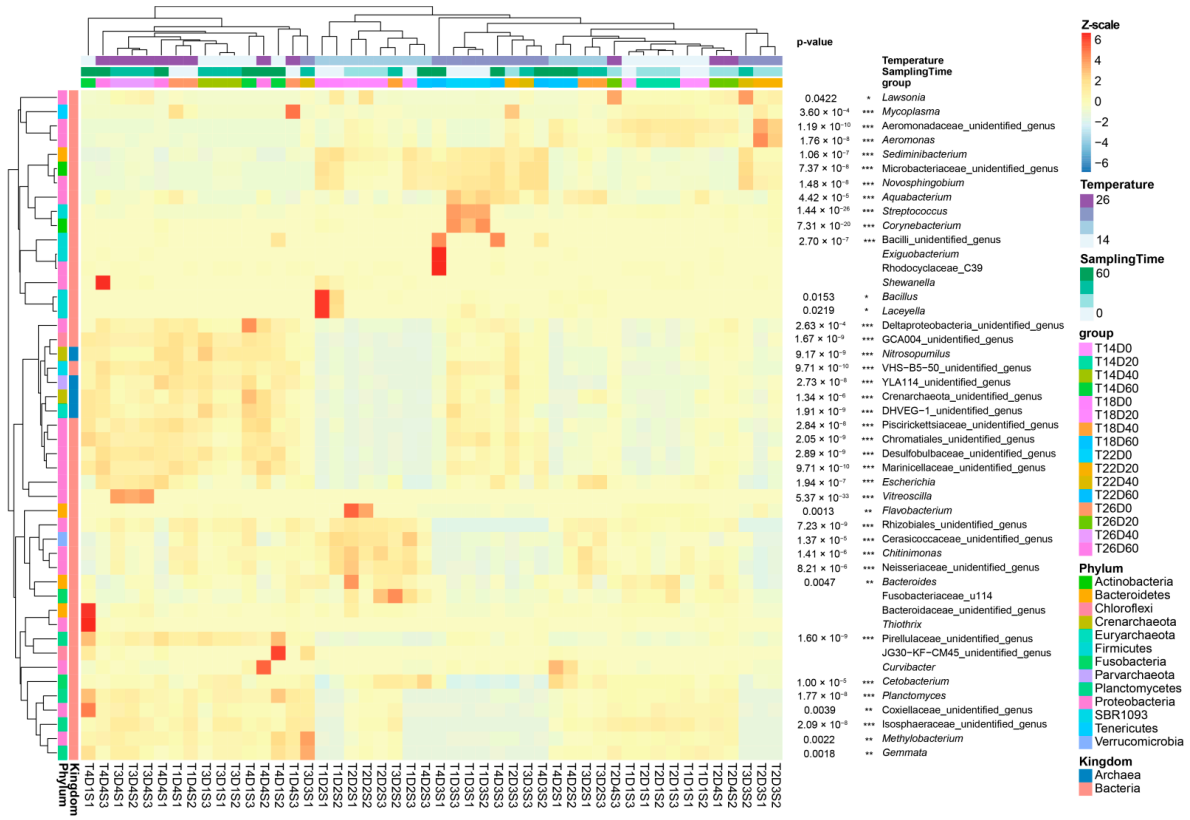

Figure 5. Heatmap profile of dominant genera of *Percocypris pingi* gut microbiota at different culture temperatures. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

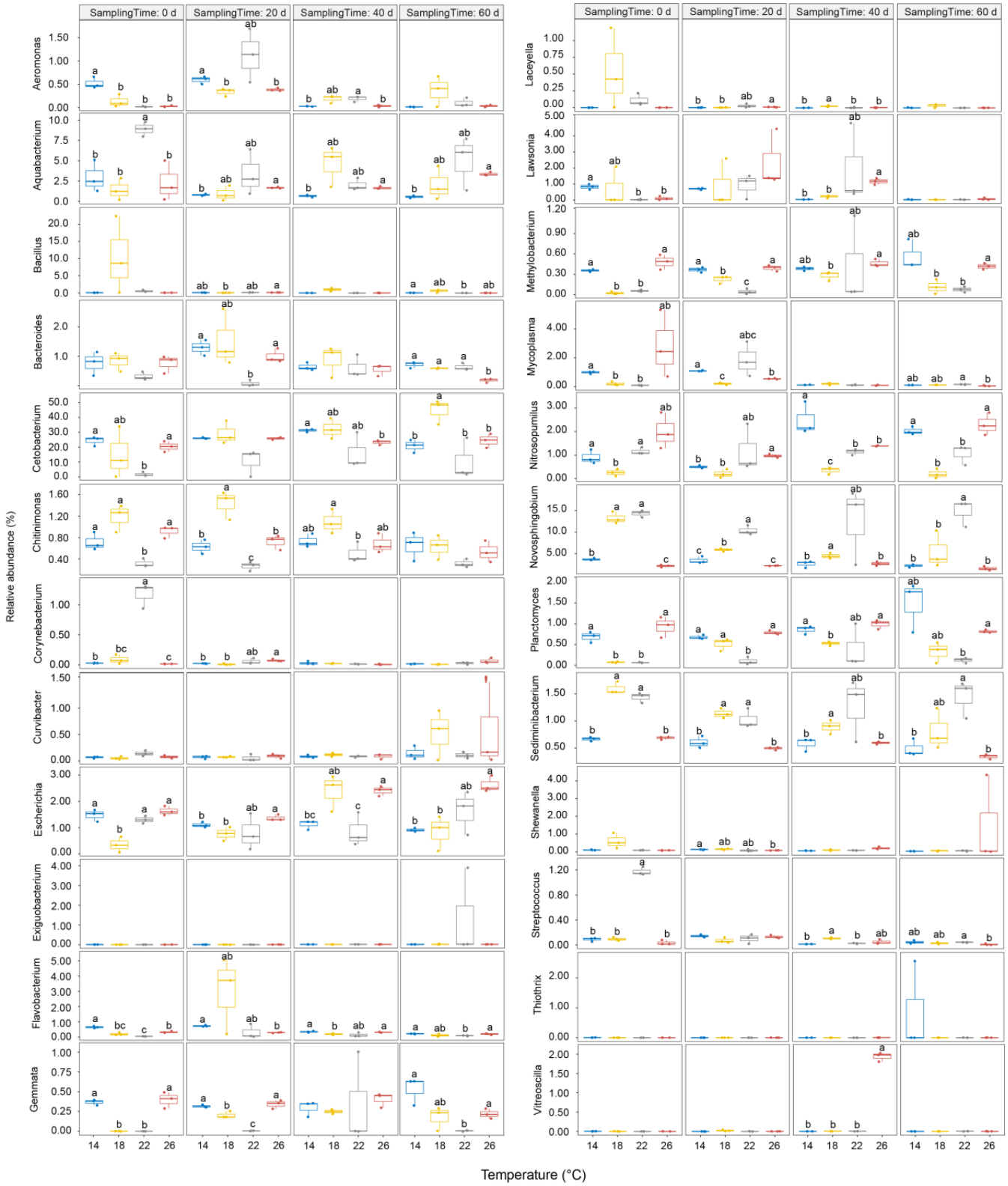

Figure 6. Relative abundance changes of commonly dominant genera of *Percocypris pingi* gut microbiota at different culture temperatures. Dots in the boxplots indicate values measured in samples. Distinct letters above the boxes indicate significant differences ($p < 0.05$).

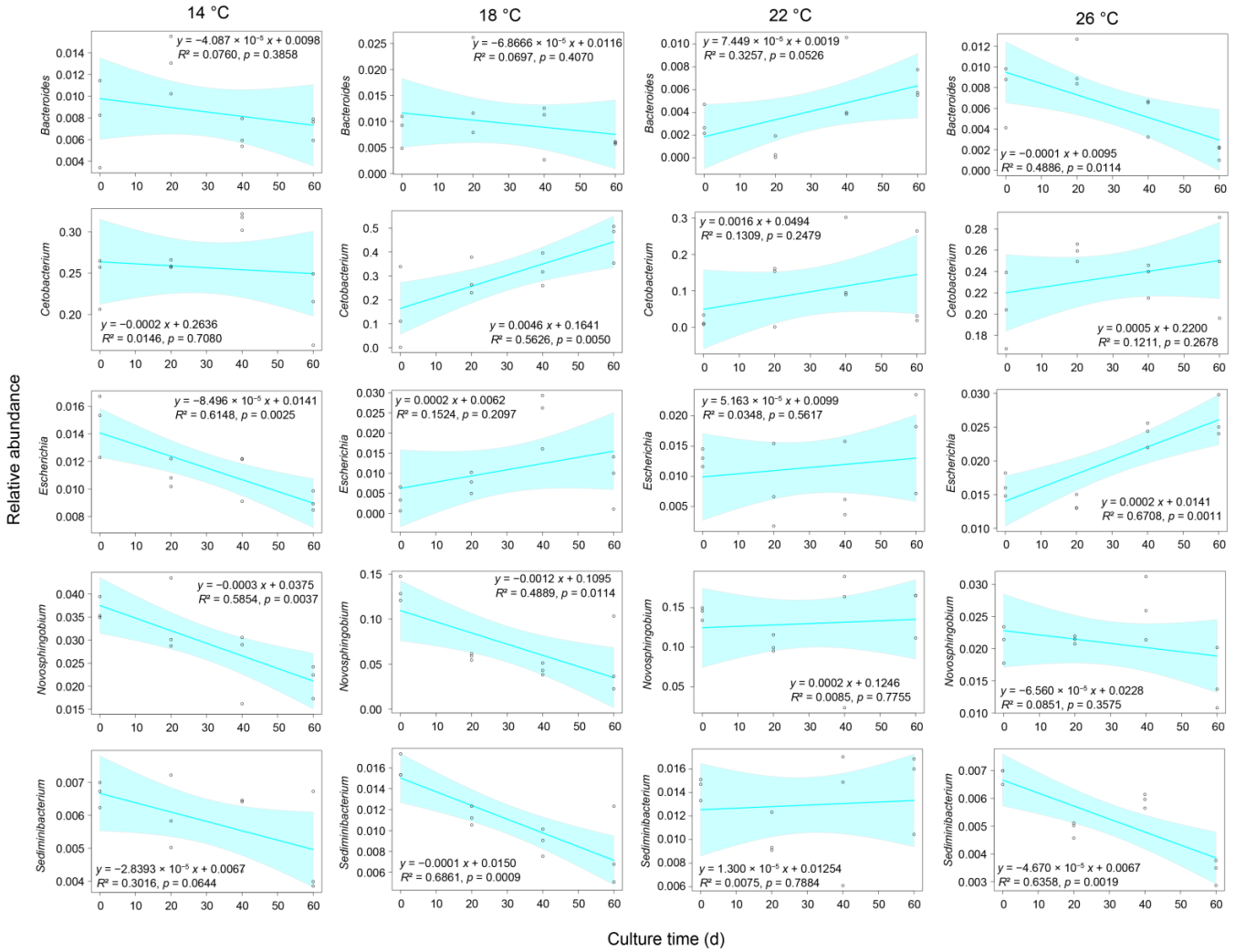

Figure 7. Correlation between the culture time and the relative abundances of *Bacteroides*, *Cetobacterium*, *Novosphingobium*, *Sediminibacterium*, and *Escherichia* in *Percocypris pingi* gut microbiota. Dots in the boxplots indicate values measured in samples.

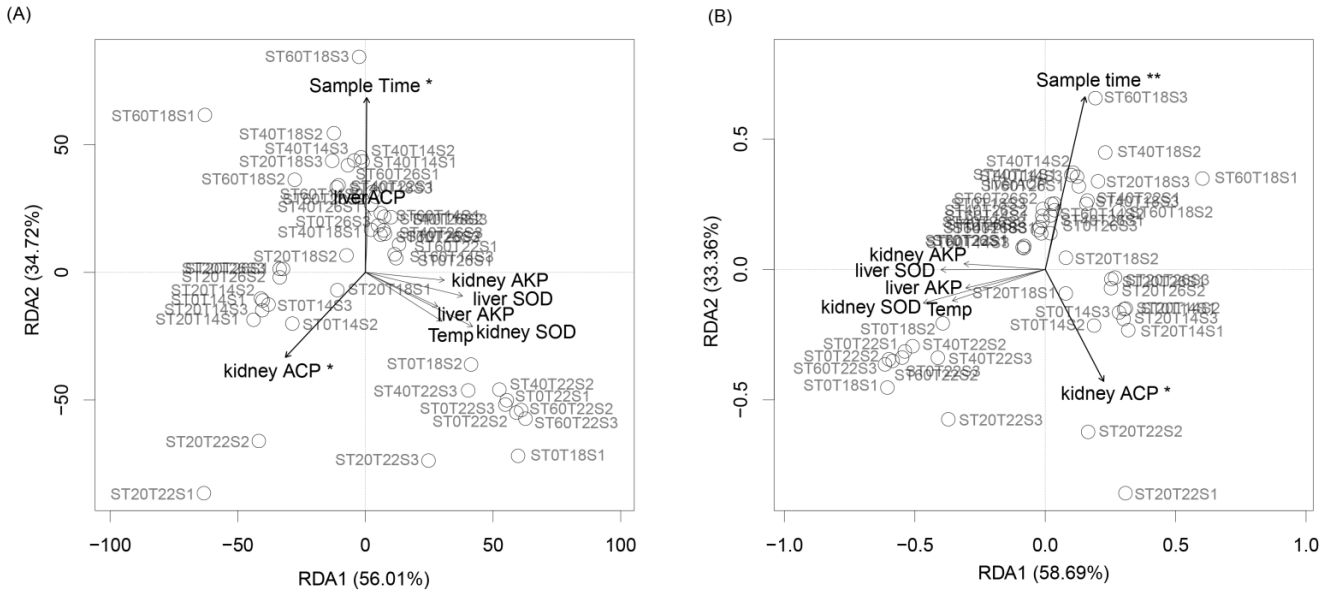

Figure 8. *Cont.*

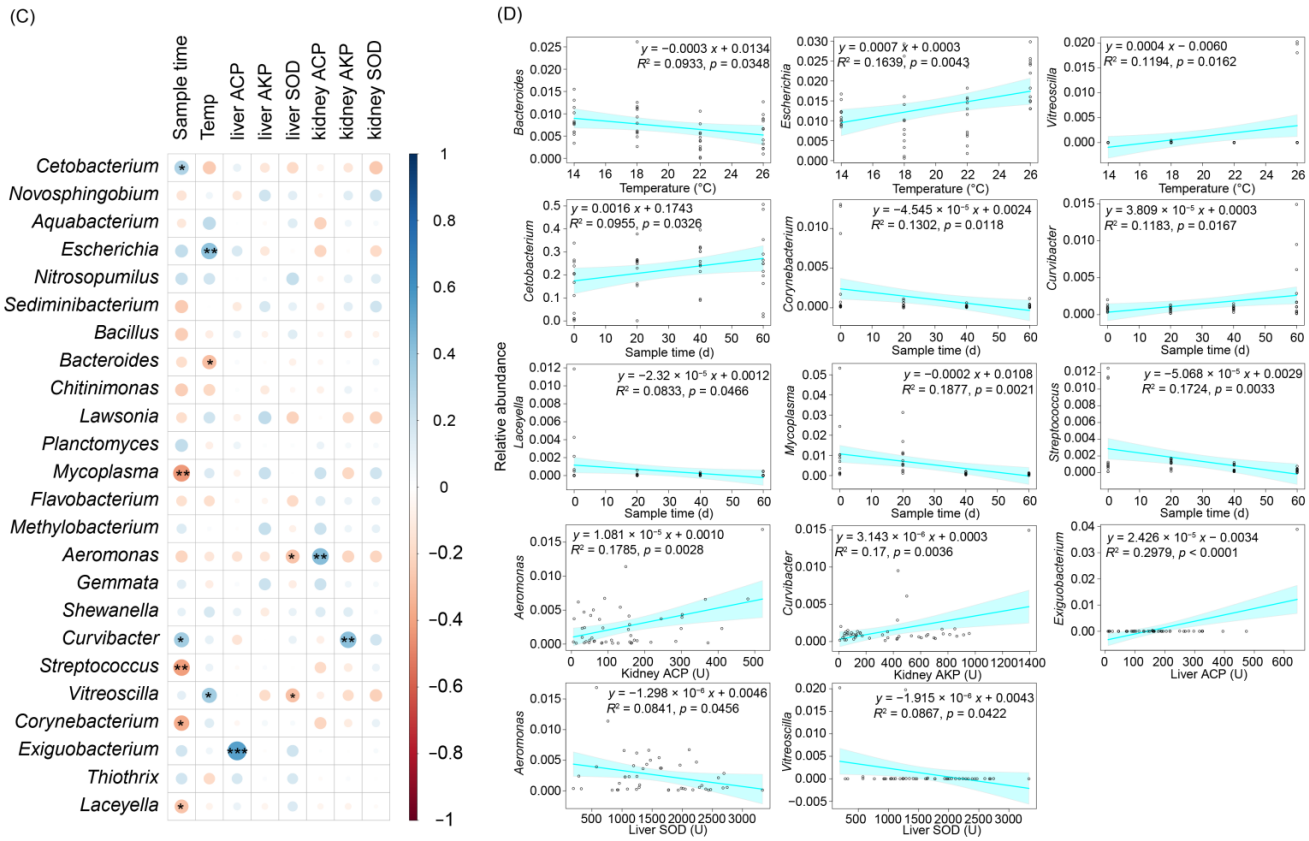

Figure 8. Correlation between gut microbiota compositions and activities of ACP, AKP, and SOD in the liver and kidney of *Percocypris pingi*: (**A**) RDA profile shows the correlation between operational taxonomic unit (out) compositions of *P. pingi* gut microbiota and internal- and external-physicochemical factors; (**B**) RDA profile shows the correlation between dominant genus compositions of *P. pingi* gut microbiota and internal- and external-physicochemical factors; (**C**) the bubble chart shows the correlation between the commonly dominant genera of *P. pingi* gut microbiota and internal- and external-physicochemical factors; and (**D**) significant correlation between the commonly dominant genera of *P. pingi* gut microbiota and internal- and external-physicochemical factors. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4. Discussion

With the continuous increase in global temperatures, the impact of temperature rise on aquatic ecosystems has attracted extensive attention [30–33]. The most noticeable ecological impact of global warming is a shift in species' range toward higher altitudes and latitudes, in agreement with their thermal preferences at biogeographical scales [34–36]. Daufresne et al. [31] reported a significant increase in the proportion of small-sized species and young age classes and a decrease in the size of fish in aquatic ecosystems, in agreement with Bergmann's, James', and temperature-size rules [37,38]. Moreover, the temperature-size rule predicts a higher growth rate but a lower final size at higher temperatures within the appropriate temperature range [39]. However, when the temperature exceeds the suitable growth temperature, the growth rates of ectotherms decrease with an increase in temperature [39]. In this study, our results showed that the growth rates of *P. pingi* at 18 and 22 °C were significantly higher than those at 14 and 26 °C (Table 1), although the optimum temperature of *P. pingi* was reported to range from 8–27 °C [6]. These results imply that the temperatures ranging from 8–14 °C and from 26–27 °C probably were not the optimum temperature for *P. pingi*, which were similar to results obtained in the report by Chen et al. [12].

The effect of temperature on the gut microbiota of vertebrates has attracted extensive attention because the gut microbiota plays multiple essential functions in hosts, in-

cluding digestion, immunity, and life history [19,40]. Bestion et al. [19] reported that a 2–3 °C warmer climate caused a 34% loss of gut microbiota diversity in common lizard (*Zootoca vivipara*) populations. Chen et al. [41] reported that the OTU number of the gut microbiota of *Rhinogobio cylindricus* collected in autumn was significantly higher than that collected in summer, but they could not exclude the influence of fish size on the results. However, our results did not show a significant impact of temperature changes on the composition and diversity of *P. pingi* gut microbiota when temperatures were within the optimum range. This may be because of the short experimental period. Therefore, longer experimental periods, including intergenerational experiments, are needed to study the impact of long-term temperature changes on fish gut microbiota.

Adaptation to changing temperatures involves adjustments of both the density and functional properties of fish mitochondria, thus affecting reactive oxygen species (ROS) generation and antioxidant defenses [42]. Low temperatures increase unsaturated fatty acids in membrane lipids, which increases the risk of lipid hydrogen peroxide formation and oxidative injury [43]. Yang et al. [44] investigated the effects of temperature on the activities of antioxidant enzymes in *Schizothorax prenanti* by raising the culture water temperature from 11 °C to the critical thermal maximum within 1 °C/h and sampled and analyzed the activities of antioxidant enzymes at 11, 16, 21, 26, and 31 °C. They found that at 21 °C, catalase activity was significantly lower than that at 11, 16, and 26 °C in the livers of *S. prenanti*, and SOD activities at 16 and 21 °C were significantly lower than those at 11 and 26 °C [44]. However, our results showed that only SOD activity at 22 °C was significantly higher than that at 26 °C in the livers of *P. pingi* collected on the 60th day. No significant difference in ACP activity in the liver and kidney of *P. pingi* among the different temperature groups was observed. Based on the existing data, the water temperature range upstream of the Yangtze River is between 6 and 25 °C [45–47]. Therefore, we speculate that climate warming over a short period does not threaten the survival of wild *P. pingi* individuals.

Although our results did not show the significant impact of temperature changes on the composition and diversity of *P. pingi* gut microbiota, it is still noteworthy that there were significant positive correlations between *Escherichia* and *Vitreoscilla* and temperature, and a significant negative correlation between *Bacteroides* and temperature. *Escherichia* is a common pathogen [48,49], the bacterial hemoglobin from *Vitreoscilla* can support the aerobic growth of *Escherichia coli* lacking terminal oxidases [50], and *Bacteroides* has potential as a probiotic [51,52]. The impact of changes in the relative abundance of these bacteria in the gut microbiota caused by temperature changes on *P. pingi* health requires further study.

5. Conclusions

In the experimental temperature range (14–26 °C), the environmental water temperature significantly affected the growth of *P. pingi* but did not affect the activities of the ACP, AKP, and SOD in the liver and kidney or the composition of gut microbiota. *P. pingi* grew fastest at 22 °C, and this information helps *P. pingi* culture. Moreover, our results imply that climate warming over a short period does not threaten the survival of wild *P. pingi*.

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Data Availability Statement: All DNA sequences were deposited in the NCBI Sequence Read Archive database with the accession number PRJNA893412.

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