GROWTH AND IMMUNE RESPONSE OF *PROCAMBARUS CLARKII* IN HIGH-TEMPERATURE AQUACULTURE ENVIRONMENT UNDER CLIMATE CHANGE

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Abstract. Global climate change has a significant impact on aquatic ecosystems, especially on aquaculture. Summer high temperature stress is the main environmental factor affecting its aquaculture efficiency and biological safety. In response to this issue, this study proposes a series of evaluation methods to determine the most suitable growth temperature and dissolved oxygen concentration, and optimize aquaculture conditions. In the experiment, the water temperature was controlled within the range of 5°C to 25°C, and the dissolved oxygen concentration was adjusted. The weight growth of *Procambarus clarkii* was observed, and specific growth rates and other indicators were used as evaluation criteria. At 25°C, the weight gain effect of *Procambarus clarkii* was particularly prominent, indicating that this temperature range was most suitable for its growth. The digestive enzyme activity test showed that the digestive enzyme activity reached its peak at 20°C, and the appropriate water temperature significantly increased the digestive enzyme activity. This study clarifies the importance of appropriate water temperature and light conditions for the growth and immune response of *Procambarus clarkii*, which helps to ensure the breeding efficiency and biological safety of *Procambarus clarkii.*
Keywords: *Procambarus clarkii, high-temperature stress, immune response, growth performance, dissolved oxygen, gut microbiota diversity*

Introduction

Climate change has had profound impacts on global aquatic ecosystems, particularly on aquaculture. *Procambarus clarkii* (PC), as an important economic freshwater crustacean aquatic organism, plays a crucial role in pond aquaculture and shrimp rice co culture in southern China (Blaha et al., 2022; Brown et al., 2022). However, summer high temperature stress has become the main environmental factor affecting its aquaculture efficiency and biological safety. Although acute experimental methods have been used to evaluate the response of aquatic organisms to high temperature stress, systematic research on the growth and immune response of PC under high temperature stress is still insufficient (Couch and Hayes, 2022; Deng et al., 2023). Therefore, more researchers are paying attention to and analyzing the effects of high-temperature aquaculture environment on the growth and immunity of PC under the background of climate change (Foysal et al., 2022).

Graham et al. (2023) proposed a strategy to develop crayfish and crayfish rice production in Chongzhou, Sichuan, in response to China's agricultural transformation and

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multiple food anxiety issues, with the aim of generating revenue. Crayfish rice was marketed as a high-quality and eco-friendly product, which not only responded to the country's biopolitical concerns about the "quality" of the population, but also met the general anxiety of middle-class consumers about food safety and environmental pollution (Graham et al., 2023). Hays et al. (2023) proposed a method to evaluate the invasive potential of the cricoid crayfish species by comparing their morphological characteristics among different subspecies and populations. There were significant differences in morphological characteristics among different subspecies and populations of the cricoid crayfish, indicating that certain subspecies and populations might have pre-adapted to the role of invaders (Hays et al., 2023). He et al. (2022) proposed using genetic and morphological analysis methods to investigate whether the PC exhibited multiple characteristics or contains multiple taxonomic units. Broadly speaking, Faxonius jeffersonii actually contained two non-sisters taxa and described a new species accordingly (He et al., 2022).

Krumpalova et al. (2022) proposed using comprehensive monitoring to determine the spatial distribution of the vulnerable species, PC, in response to external abnormalities and body deformities. More than one-third of the 223 crayfish sampled at 30 research sites exhibited abnormalities, physical damage, body deformities, or the presence of ectosymbionts. Lipták (2023) proposed a comprehensive survey of crayfish in response to the lack of data, unclear distribution, unknown habitat utilization, and population structure, and used it in conjunction with habitat assessment methods. Liu et al. (2024) proposed the use of High Hydrostatic Pressure (High Hydrostatic Pressure, HHP) treatment to improve the shelling effect and studied its impact on the quality of crayfish, in response to the difficulty of shelling crayfish. All HHP treatments reduced the difficulty of shell peeling for crayfish, increased meat yield, improved the texture and color of crayfish, and expanded the gap between shell and meat (Liu et al., 2024). Liu et al. (2022) proposed a method of using hemolymph glucose as a biomarker, which was validated through a combination of laboratory experiments and field data. The physical fitness and stress levels of wild PC responded differently to environmental conditions, with individuals under greater stress exhibiting higher blood sugar levels but similar physical fitness (Liu et al., 2022).

Although the above research covers multiple aspects of PC, including agricultural transformation, biodiversity, morphological variation, species classification, environmental health assessment, etc., there are still some shortcomings. Research studies specific regions or issues, lacking a comprehensive analysis of the overall situation. Secondly, some studies rely on morphological feature analysis, which may overlook in-depth exploration at the genetic level. In view of this, this study innovatively systematically analyzes the effects of high-temperature aquaculture environment on the growth and immune response of PC under the background of climate change. By precisely controlling the water temperature and dissolved oxygen concentration, combined with changes in light conditions, the effects of different environmental factors on the growth performance and immune status of PC are comprehensively evaluated. The deep mechanisms of changes in meat quality, immune enzyme activity, gene expression, and diversity of gut microbiota have been further explored, providing a scientific basis for optimizing the breeding conditions of PC. The purpose of this study is to clarify the physiological response mechanism of PC under high temperature stress, and provide effective strategies for addressing the impact of climate change on aquaculture.

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Materials and methods

Preliminary study on the main effects of high temperature aquaculture environment on PC

The PC, belonging to the Procambarus genus of the Procambarus family, is a well-known arthropod commonly known as crayfish or freshwater crayfish. Its adult body length is usually between 6-13 cm, with a robust body shape and a slightly flattened back and abdomen. The body structure is distinct, divided into the head, chest, and abdomen, and the whole body is covered by a shell (Luo et al., 2023; Magoulick et al., 2022). The body color of mature individuals often appears as dark red or deep red, while immature individuals may exhibit various colors such as light brown, reddish brown, and even blue in a few individuals. This species is native to northeastern Mexico and central southern United States, but its distribution range has significantly expanded since then, and now it is almost ubiquitous worldwide except for the Antarctica and Oceania (Morales Pedraza, 2023). In recent years, with the intensification of global climate change, the impact of high-temperature aquaculture environment on PC has become increasingly significant. High temperature not only affects the dissolved oxygen content in water, leading to a decrease in dissolved oxygen levels, but may also exceed the optimal growth temperature range of PC, seriously affecting its normal growth and posing a serious challenge to its aquaculture industry. The main impacts of high-temperature aquaculture environment on PC under climate change are shown in *Figure 1* (Mouser et al., 2022).

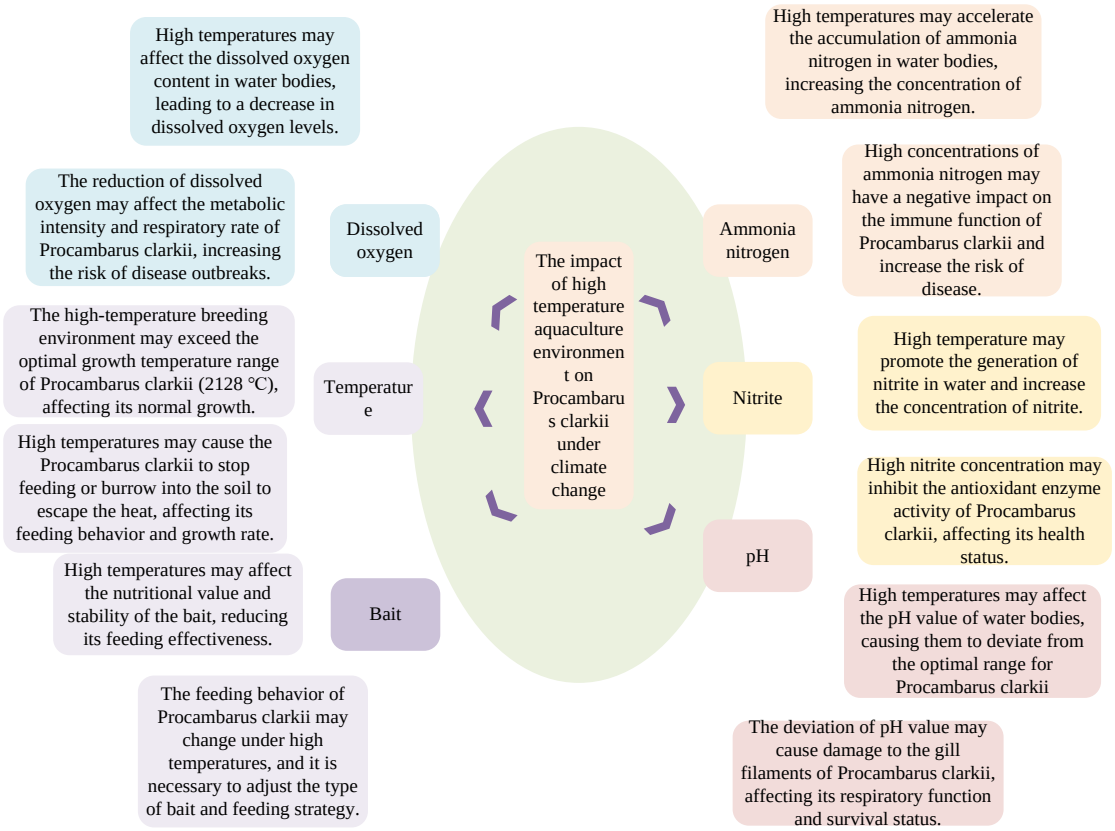

Figure 1. *The impact of high temperature aquaculture environment on PC under climate change*

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The impact of high-temperature aquaculture environment on PC is reflected in multiple aspects. Firstly, high temperatures may reduce the dissolved oxygen content in water, which in turn affects the metabolic intensity and respiratory rate of PC, increasing the likelihood of disease outbreaks (Palillo et al., 2022a, b). Secondly, if the aquaculture water temperature exceeds the optimal growth temperature range of PC (21℃ -28℃), it may have adverse effects on its normal growth and development, and even cause it to stop feeding or hide in the mud to escape high temperatures. In addition, under high temperature conditions, the nutritional value and stability of the bait may change, which not only reduces the effective utilization rate of the bait, but also requires breeders to adjust the type of bait and feeding strategy in a timely manner to adapt to the changes in the feeding behavior of PC (Pantaazis et al., 2022; Rataj and Váčný, 2022). High temperatures may also accelerate the accumulation of ammonia nitrogen in water bodies, increasing the concentration of ammonia nitrogen. High concentrations of ammonia nitrogen can damage the immune system of PC and increase the risk of disease. Similarly, high temperatures may also promote the generation of nitrite, leading to an increase in nitrite concentration and subsequently inhibiting the antioxidant enzyme activity of PC, affecting its overall health status. Finally, high temperatures may also cause changes in the pH value of the water, deviating from the optimal range for PC (6.5-7.5). Abnormal changes in pH value may damage its gill filament structure, thereby affecting its respiratory function and survival status (Shen et al., 2024; Sladkova et al., 2023).

Feeding environment and experimental instruments

In order to accurately control the feeding temperature and experimental environment of PC, a feeding box is specially designed for this study. The feeding box is separated by plastic boards into an isolation zone and a feeding zone, with a feed feeding zone located in the center of the feeding zone (Soares et al., 2022). Each feeding box is equipped with a fixed infrared camera automatic recording system to accurately capture and record the behavior of experimental animals during the experimental process. After the recording of feeding behavior is completed, the experimental animals are removed, and the water and feed are immediately replaced. New experimental animals are reintroduced to repeat the above observation process. To ensure the representativeness and accuracy of the experimental results, 40 experimental animals are placed in each feeding box for each temperature and pH condition. Accurate control of experimental temperature using graphene heating plates. After the experiment, the data collected through the video surveillance system is used for further analysis to explore the impact of different environmental factors on the PC, as shown in *Figure 2*.

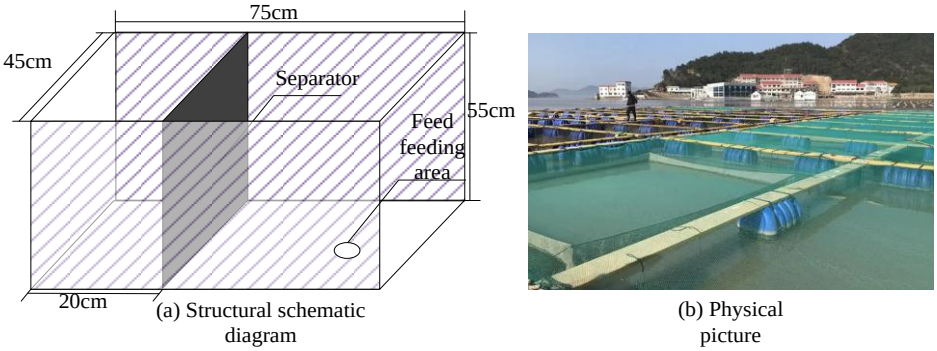

Figure 2. *Schematic diagram of feeding box structure*

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The basic dataset presented comes from two different water environments set up in the experiment: Taihu Lake and Dongting Lake. A series of key instruments and reagents are required for conducting experiments on the growth and immune response of PC. This includes YSI 55 dissolved oxygen analyzer, GL-20 water temperature gauge, HQ40d pH meter, etc., used to monitor water quality parameters; BX43 microscope is used to observe tissue structure; Allegra X-22R freeze centrifuge, Trizol reagent, and PrimeScriptTM RT reagent kit are used for RNA extraction and cDNA synthesis; NovaSeq 6000 high-throughput sequencer is used for gene expression analysis; Clarus 500 gas chromatograph and TSQ Quantum mass spectrometer are used for precise chemical analysis; And laboratory equipment such as Labconco freeze dryer and Eheim heating rod. These instruments and reagents provide a precise and efficient operating platform for the experiment, ensuring the accuracy and reliability of experimental data, as shown in *Table 1*.

Table 1. *Experimental instruments and reagents*

Experimental instrument/reagent name	Model	Manufacturer full name	Manufacturer headquarters location
Dissolved oxygen analyzer	Ysi55	Ysi incorporated	Yellow Springs, Ohio, USA
Water temperature gauge	Gl-20	Hach Company	Loveland, Colorado, USA
Ph meter	Hq40d	Hach Company	Loveland, Colorado, USA
Amtax compact	Quickcheme	Lachat Instruments	Milwaukee, Wisconsin, USA
Nitrite analyzer	Nitratest	Hach Company	Loveland, Colorado, USA
Microscope	Bx43	Olympus Corporation	Tokyo, Japan
Liquid nitrogen tank	Ln-80	Taylor-Wharton	Buffalo, New York, USA
Cryocentrifuge	Allegra x-22r	Beckman Coulter, Inc.	Brea, California, USA
Chloroform reagent	Chci3	Sigma-Aldrich Co. LLC	St. Louis, Missouri, USA
Ethanol reagent (75%)	-	Merck KGaA	Darmstadt, Germany
Trizol reagent	15596018	Invitrogen (Thermo Fisher Scientific)	Waltham, Massachusetts, USA
Primescripttm rt kit	Drr037	Takara Bio Inc.	Shiga, Japan
Gdna eraser	-	Takara Bio Inc.	Shiga, Japan
Electrophoresis equipment	Mini-proteano	Bio-Rad Laboratories, Inc.	Hercules, California, USA
High throughput sequencer	Novaseq 6000	Illumina, Inc.	San Diego, California, USA
Heating rod	Th-100	Eheim GmbH & Co. KG	Deizisau, Germany
Aeration system	As-20	Aeration Industries International, Inc.	Fort Worth, Texas, USA
Tissue homogenizer	Bullet blendere	Next Advance, Inc.	Troy, New York, USA
Freeze dryer	Freezone 4.5	Labconco Corporation	Kansas City, Missouri, USA
Liquid nitrogen freezer	Cryo 1	Planer Products, Inc.	Sun Prairie, Wisconsin, USA
Gas chromatograph	Clarus 500	PerkinElmer, Inc.	Waltham, Massachusetts, USA
Mass spectrometer	Tsq quantum	Thermo Fisher Scientific Inc.	Waltham, Massachusetts, USA
Amino acid analyzer	Sykam s433d	Elemental Instruments GmbH	Erfurt, Germany
Gc-ms	Trace 1300-tsq 9000	Thermo Fisher Scientific Inc.	Waltham, Massachusetts, USA

The water temperature control range is set at 5℃ to 25℃, and a gradient is set every 5℃, which is accurately controlled by the graphene heating plate. Dissolved oxygen concentrations were adjusted from 10 mg/L to 35 mg/L and monitored using a YSI 55 dissolved oxygen analyzer. The pH value was maintained within the PC optimal range of 6.5-7.5 and tested regularly using a HQ40d pH meter. Light conditions were set to all black, natural and specific light to study the effects of different light on PC growth.

Using an infrared camera automatic recording system to continuously monitor the eating behavior of a PC. And advanced artificial intelligence recognition algorithms are

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introduced to process the video data, accurately identifying each individual's eating actions, such as mouth opening and closing, food intake, etc., in order to determine their eating behavior patterns. At the same time, by using video analysis software to track the consumption of feed in the feeding area, combined with the recognition results of individual eating behavior, the amount of feed consumed by each individual can be accurately calculated. This method not only improves the accuracy and efficiency of data collection, but also provides strong support for in-depth research on the relationship between PC's feeding behavior and environmental factors.

Experimental method

In order to fully evaluate the growth and immune response of PC in high-temperature aquaculture environments under climate change, a series of experiments are designed in this study, and the overall process of the plan is as follows (Tamulyonis et al., 2023). The first step is experimental preparation, including setting different water temperatures and dissolved oxygen concentrations, materials and equipment required for the experiment, such as crayfish, water quality monitoring equipment, breeding containers, etc. By separately controlling variables, the effects of water temperature and dissolved oxygen concentration on the growth and immune response of *Procambarus clarkii* can be more accurately evaluated. This method can clearly reveal the specific role of each environmental factor in promoting or inhibiting the physiological functions of *Procambarus clarkii*. The second step is to set the breeding conditions, with a water temperature range of 0℃-25℃, and the dissolved oxygen concentration is adjusted from 10 mg/L-35 mg/L. The experimental sites are respectively established in a certain water area of the Taihu Lake and Dongting Lake. The third step is to evaluate the growth status, observe and record the weight growth and growth rate of PC; A microscope is used to observe tissue sections of crayfish raised at different temperatures and evaluate changes in meat quality; The changes in body length, large claw length, abdominal muscle weight, pancreatic coefficient, and hepatopancreatic coefficient of PC at different temperatures are recorded.

In the study, the following specific methods were used to measure growth indicators such as body length, paw length, and muscle weight of *Procambarus clarkii* (PC): Body length measurement: using a precision scale or vernier caliper, measure from the tip of the PC's head to the end of its tail, ensuring that the PC is in a straight state during measurement to obtain accurate body length data. Measurement of Large Claw Length: Select the front claws of the PC (usually the larger pair) and use a vernier caliper to measure the straight-line distance from the tip to the base of the claws, ensuring that the maximum length of the claws is measured. Muscle weight measurement: After dissecting the PC, carefully separate the abdominal muscle tissue to avoid attaching other non muscle tissues. Weigh the separated abdominal muscles using a precision balance and record their weight. Other indicators: Pancreatic coefficient and hepatopancreatic coefficient are calculated by measuring the weight of pancreas and hepatopancreas, and dividing by the overall weight of PC. These indicators help evaluate the development of visceral organs in PC. All measurements were conducted under different temperature conditions set in the experiment and repeated multiple times to ensure the accuracy and reliability of the data. Through these detailed measurements, researchers are able to systematically evaluate the specific effects of high-temperature aquaculture environments on the growth performance of PCs.

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The fourth step is to conduct an assessment of the impact of light conditions: divide the PC into three groups and raise them under all black, natural light, and specific light conditions, respectively; The initial body weight, initial total length, final body weight, final total length, weight gain rate, specific growth rate, body length growth rate, and survival rate of each group are recorded and compared. The fifth step is immune response analysis: the PC is randomly assigned to three predetermined breeding environments: all black environment, control environment, and high temperature environment; Immune indicators such as MDA enzyme content, SOD, LSZ, AKP, and CAT specific activity are measured in each group. The sixth step is to conduct digestive enzyme activity testing: the activities of lipase, amylase, and protease in PC are tested under different water temperature conditions. The seventh step is the detection of non-specific immune indicators. Under continuous high temperature stress, the activities of hemocyanin (HC), Acid Phosphatase (ACP), Alkaline Phosphatase (AKP), and Glutamic Oxaloacetic Transaminase (AST) in PC are monitored. The eighth step is to deeply analyze the expression level changes of three key immune related genes AIP8, TNF, and Toll in *Procambarus clarkii* before and after heat stress. Using high-throughput sequencing techniques such as NovaSeq 6000, RNA extraction and cDNA synthesis were performed on PC samples, followed by precise detection of the expression levels of these genes through real-time fluorescence quantitative PCR (qPCR). By comparing the differences in gene expression before and after heat stress, the impact of high temperature environment on the regulation of PC immune genes is revealed. The ninth step is the analysis of gut microbiota diversity, and high-throughput sequencing technology is used to analyze the diversity of gut microbiota in PC before and after heat stress. The tenth step is data analysis and statistics, which involves conducting statistical analysis on the collected data to determine the effects of different conditions on the growth and immunity of PC; Multiple comparative analysis is conducted to evaluate the significant differences in growth and immune indicators between groups. The overall process of the experiment is shown in *Figure 3*.

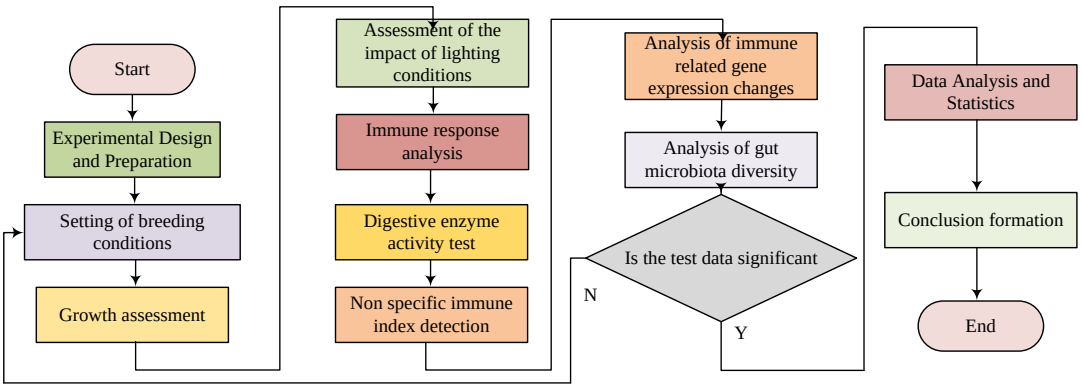

Figure 3. *Overall experimental plan process design*

When conducting analysis of immune related gene expression changes, a series of steps are required and various experimental instruments are used. The following is the specific process. Step 1: A tissue homogenizer is used to take an appropriate amount of liver, pancreas, and gill tissues from PC for subsequent determination of the relative expression of messenger ribonucleic acid (mRNA) of the genes to be tested in the two

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tissues. Step 2: RNA is extracted, and a Cryocentrifuge (Allegra X-22R, Beckman Coulter) is used to thaw the frozen sample on ice and homogenize it. Step 3: Chloroform is added and centrifuged. 200 μL of chloroform reagent (CHCI3, Sigma Aldrich) is added to every 1 mL of Trizol lysed sample. After vigorous shaking for 30 s, it is left to stand at room temperature for 5 minutes. A Cryocentrifuge is used to centrifuge for 10 min at 4℃ and 12,000 r/min.

Step 4: The upper liquid is aspirated and added with isopropanol. An equal volume of pre-cooled isopropanol (ethanol reagent (75%), Merck KGaA) is added, mixed evenly, and left to stand at -20℃ for 20 min. Then, a Cryocentrifuge is used again and centrifuged at 4℃ and 12,000 r/min for 10 min. This step is repeated twice. After centrifugation, ethanol is absorbed and the lid is opened to air dry for 3 min. Step 5: RNA is dissolved and detected by electrophoresis, and an appropriate amount of DEPC water is used to dissolve RNA. The electrophoresis equipment (Mini PROTEAN, Bio Rad) is used to detect the quality of RNA by electrophoresis. Purification of mRNA and synthesis of cDNA are performed, and cDNA is stored at -20℃ for future use. Step 7: Conduct non ultra-high temperature pressure testing and sample collection. The water temperature gauge (GL-20, Hach) is used to monitor water temperature. After adapting to the environment, collect samples as controls. Then, increase the water temperature to 25℃ at a rate of 1.0℃/h and keep it constant for 48 h. During this period, samples were collected for low and high temperature stress at 4 h, 8 h, 12 h, 16 h, 20 h, 24 h, and 28 h without u. Step 8: The sample after coercion is processed and stored; during the coercion period, samples are taken at set time points, with 3 replicates set for each time point. Liver, pancreas, intestine, and gill tissues of 5 shrimp are collected from each replicate. The experimental steps are shown in *Figure 4*.

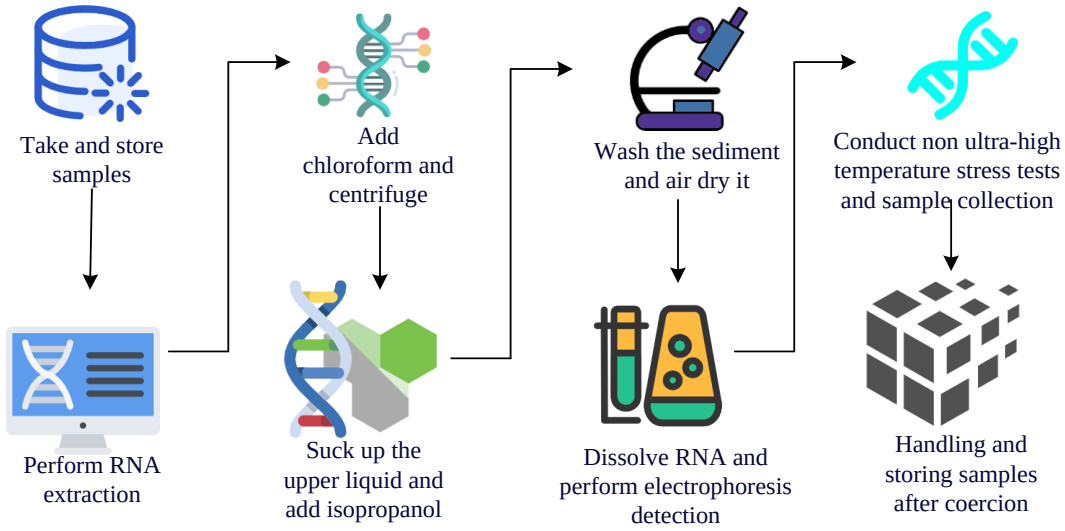

Figure 4. *Main steps of analyzing changes in immune related gene expression*

The experiment selected healthy, sexually mature, and age-matched individuals with a weight range of 10-20 grams to ensure the consistency and comparability of the experimental results. All experimental animals were from the same batch of aquaculture ponds to avoid the influence of genetic background differences on the experimental results. Under each temperature and pH condition, 40 PCs were placed in each feeding

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box, which were randomly assigned to eliminate potential interference from individual differences on the experimental results. By strictly controlling the source, age, gender, and weight range of the experimental animals, this study ensured the accuracy and reproducibility of the experimental results. In the video monitoring system, in order to accurately determine the eating behavior and feed consumption of each Procambarus clarii individual, we installed high-resolution infrared cameras above the feeding area and set fixed shooting angles and focal lengths to ensure clear capture of each individual's eating process. Through video playback and analysis software, we can record in detail the feeding time, frequency, and amount of feed consumed per meal for each PC. In addition, by adding harmless tracers or markers to the feed, we can further track the consumption of feed, thereby more accurately evaluating the feeding efficiency and growth status of each PC. These measures ensure the accuracy and reliability of experimental data, providing a solid foundation for subsequent analysis.

The research mainly includes the following seven experiments. Experiment 1: Determination of the optimal growth temperature and dissolved oxygen concentration by controlling the water temperature (5℃-25℃) and dissolved oxygen concentration (10 mg/L-35 mg/L), observing the weight gain of PC, and determining the optimal growth temperature and dissolved oxygen concentration. Experiment 2: The effect of light conditions on PC growth. PCs were divided into three groups and cultured under all black, natural light, and specific light conditions, respectively. Their growth indicators were recorded, and the effects of different light conditions on PC growth were compared. Experiment 3: The effect of high temperature on PC immune response. PC was randomly assigned to a completely black environment, a control environment, and a high temperature environment. MDA enzyme content, SOD, LSZ, AKP, and CAT specific activities were detected to analyze the effect of high temperature on PC immune response. Experiment 4: The effect of high temperature on the activity of PC digestive enzymes. Under different water temperature conditions, the activities of lipase, amylase, and protease in PC were detected to analyze the effect of high temperature on the activity of PC digestive enzymes. Experiment 5: The effect of high temperature on non-specific immune indicators of PC. Under continuous high temperature stress, the activities of hemocyanin (HC), acid phosphatase (ACP), alkaline phosphatase (AKP), and glutamate oxaloacetate transaminase (AST) in PC hemolymph were detected to analyze the effect of high temperature on non-specific immune indicators of PC. Experiment 6: The effect of high temperature on the expression of PC immune related genes. The relative expression levels of AIP8, TNF, and Toll genes in PC liver, pancreas, and gill tissues before and after high temperature stress were detected by qPCR to analyze the effect of high temperature on the expression of PC immune related genes. Experiment 7: The Effect of High Temperature on the Diversity of PC Intestinal Microbiota. Through high-throughput sequencing technology, the diversity and composition of PC Intestinal Microbiota before and after high temperature stress were analyzed, and the effect of high temperature on PC Intestinal Microbiota was explored.

Determine the optimal growth temperature and dissolved oxygen concentration of PC through Experiment 1, providing basic conditions for subsequent experiments. Then, through Experiment 2, the effect of light conditions on PC growth was studied to further optimize the breeding conditions. Next, through experiments three and four, analyze the effects of high temperature on PC immune response and digestive enzyme activity, and explore the impact of high temperature stress on PC physiological functions.

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Furthermore, through experiments five and six, the effects of high temperature on non-specific immune indicators and immune related gene expression in PC were examined, and the impact of high temperature stress on the PC immune system was further studied. Finally, through Experiment 7, the impact of high temperature on the microbiota diversity of PC gut was analyzed, and the changes in the composition of PC gut microbiota under high temperature stress were explored.

Results

Growth of PC in high-temperature aquaculture environment under climate change

The impact of high-temperature aquaculture environment on the growth of PC was investigated, and the most suitable growth temperature and dissolved oxygen concentration were determined to optimize aquaculture conditions (Uckun, 2022). By controlling the water temperature within the range of 5℃ to 25℃ and adjusting the dissolved oxygen concentration from 10 mg/L to 35 mg/L, the weight growth of PC is observed. The study conducted the same experiment in a certain water area of the Taihu Lake Lake and Dongting Lake respectively. With weight gain as the evaluation index, the growth of body weight can be evaluated by specific growth rate (SGR). The experimental results are shown in *Figure 5*.

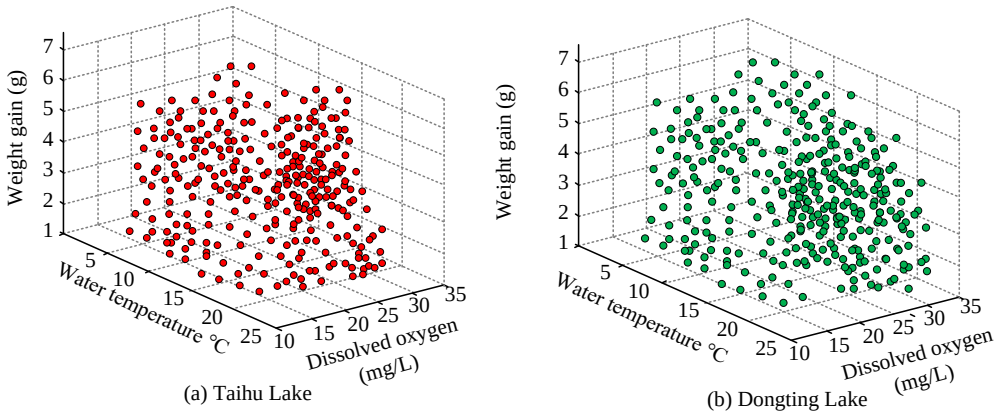

Figure 5. *Effects of different water temperatures and dissolved oxygen content on the weight gain of PC*

In *Figure 5*, when the feeding temperature gradually increases from the low temperature zone (below 10℃) to the range of 20℃ to 30℃, the weight gain of PC reached its peak, indicating the optimal growth state. At around 25℃, its weight gain effect was particularly prominent, indicating that this temperature range was most suitable for its growth. Meanwhile, the concentration of dissolved oxygen was also a key factor affecting growth. When the dissolved oxygen concentration was around 20 mg/L, the weight gain of PC reached its maximum value, and then the weight gain trend slowed down or even slightly decreased with further increase of dissolved oxygen. Overall, the results obtained from Taihu Lake and Dongting Lake were not significantly different. In order to study the growth of PC in high-temperature aquaculture environments under climate change, this study observed the meat quality of PC raised at 10, 15, 20, and 25 degrees Celsius through microscopic staining, as shown in *Figure 6*.

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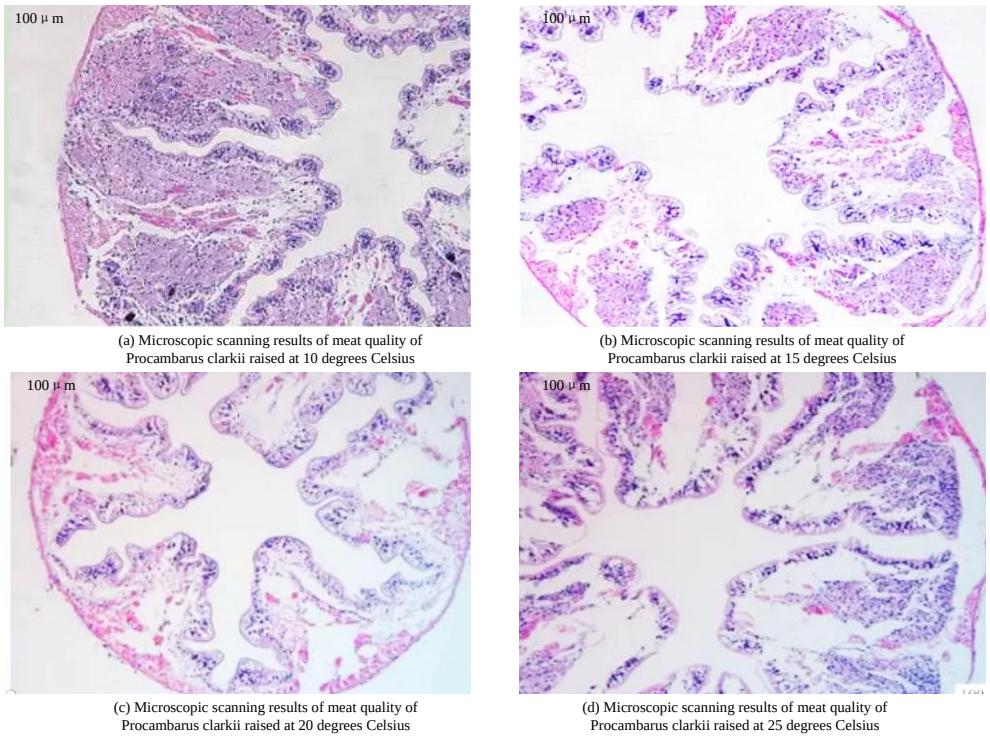

(a) Microscopic scanning results of meat quality of Procambarus clarkii raised at 10 degrees Celsius

(b) Microscopic scanning results of meat quality of Procambarus clarkii raised at 15 degrees Celsius

(c) Microscopic scanning results of meat quality of Procambarus clarkii raised at 20 degrees Celsius

(d) Microscopic scanning results of meat quality of Procambarus clarkii raised at 25 degrees Celsius

Figure 6. *Microscopic images of tissue sections of PC raised at different temperatures*

In *Figure 6*, as the temperature increases, the morphology and density of shrimp meat cells undergo significant changes. At low temperatures, such as 10℃, the arrangement of shrimp meat cells is extremely tight, with cell gaps of only about 0.1 microns, which may be due to the good contraction and compaction of muscle fibers in low-temperature environments. When the temperature rises to 15℃, the intercellular space slightly increases to about 0.15 microns, but still maintains a relatively tight state. However, at higher temperatures, such as 20℃, shrimp meat cells become significantly looser, with cell gaps increasing to about 0.3 microns, which may be due to the relaxation of muscle fibers caused by the high temperature environment. When further heated to 25℃, the intercellular space continued to expand to about 0.4 microns, and the arrangement of shrimp meat cells became looser. In addition, as the temperature increases, the color of shrimp meat also undergoes a certain degree of change. At low temperatures, such as 10℃ and 15℃, shrimp meat appears dark purple with a rich color. At higher temperatures, such as 20℃, the color of shrimp meat begins to lighten, appearing light purple. When the temperature further rises to 25℃, the color of the shrimp meat completely turns light pink, which may be due to the high temperature environment accelerating the decomposition of pigments in the shrimp body, resulting in a lighter color of the shrimp meat. Overall, temperature has a significant impact on the meat quality of *Procambarus clarkii*. Low temperature environments (such as 10 ℃ and 15℃) are beneficial for maintaining the density and deep purple color of shrimp meat, while high temperature environments (such as 20℃ and 25℃) may cause shrimp meat to become loose and lighter in color. To further verify this conclusion, this study set different temperatures in 24 different aquaculture areas and raised them for one cycle. The body length and large claw length of the PC in 24 different aquaculture areas were used as morphological evaluation indicators, and the results are shown in *Figure 7*.

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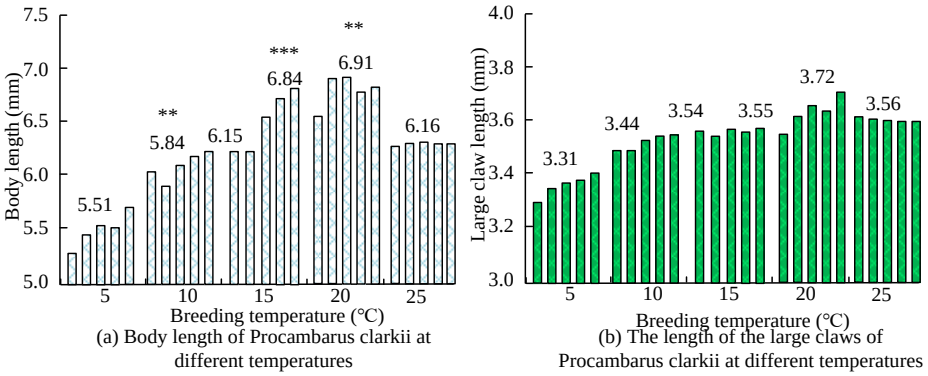

Figure 7. *Effects of different temperatures on morphological indicators of PC*

Figure 7 revealed the changes in body length and large claw length of PC at different breeding temperatures. In *Figure 7a*, in terms of body length, as the breeding temperature increased, the body length of PC generally showed an upward trend. Among them, at 20℃, the average body length reached 6.91 mm, which was the highest among all temperatures. In *Figure 7b*, as the breeding temperature increased, the length of the large claws of PC also gradually increased. At 24℃, the average length of the large claws reached 3.72 mm, which was also the highest set of data. Therefore, within a certain range, increasing the breeding temperature could help promote the growth and development of PC, especially with a more significant impact on its body length and large claw length. The changes in abdominal muscle weight, pancreatic coefficient, and hepatopancreatic coefficient of PC at different breeding temperatures are shown in *Figure 8*.

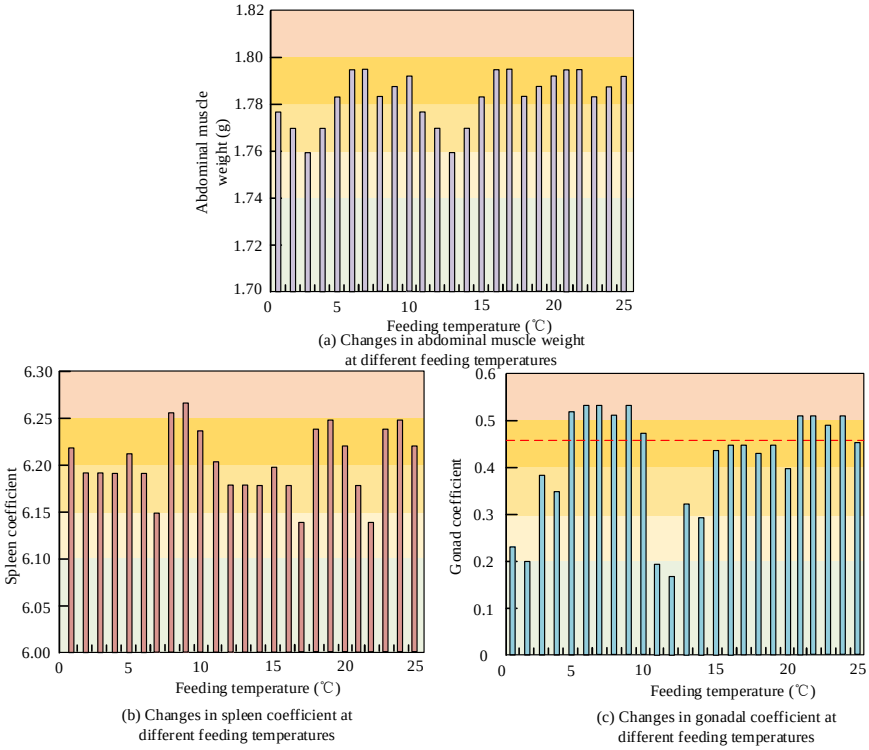

Figure 8. *Changes in procrayfish at different reproductive temperatures*

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In *Figure 8a*, as the breeding temperature increased, the abdominal muscle weight of PC generally showed an upward trend. Among them, the average abdominal muscle weight reached 1.80 g between 12℃ and 20℃, which was the highest among all temperatures. In *Figure 8b*, the pancreatic coefficient of PC first decreased and then increased, with the lowest point occurring at around 14℃ and a value of 6.15. With the increase of breeding temperature, the hepatopancreatic coefficient of PC was gradually increasing. At 10℃, the average liver pancreas coefficient reached 0.5. Therefore, within a certain range, increasing the breeding temperature could help promote the growth and development of PC, especially with the most significant impact on its abdominal muscle weight. Light is one of the key environmental factors that affect the growth and behavior of aquatic organisms. Evaluating different lighting conditions is crucial for understanding the impact of high temperatures on the growth performance and survival rate of PC in climate change, as light can directly affect their metabolism, feeding activities, and health status, thereby affecting aquaculture efficiency and economic benefits. To determine the most suitable lighting environment, this study divided the PC into three groups and recorded and compared their initial body weight, initial total length, final body weight, final total length, weight gain rate, specific growth rate, body length growth rate, and survival rate. Through multiple comparative analysis, the significant impact of lighting conditions on the above growth indicators was evaluated. The experimental results are shown in *Table 2*.

Table 2. *Effects of different light conditions on the growth and survival of Procambarus clarkii*

Growth indexes	Group		
	All black team	**Natural group**	**Light group**
First body weight (g)	4.66 ± 0.41	4.54 ± 0.15	5.12 ± 0.21
Initial full-length length (cm)	3.34 ± 0.64ab	3.13 ± 0.64b	4.24 ± 0.27a
Last body weight (g)	10.67 ± 1.33b	13.46 ± 0.44a	13.83 ± 0.94gb
Last full-length length (cm)	8.78 ± 0.41	10.63 ± 0.34a	9.77 ± 0.86b
Weight gain rate of (%)	129.23 ± 13.52b	208.31 ± 13.41a	140.22 ± 11.71b
Specific growth rate of (%)	1.14 ± 0.12b	1.78 ± 0.33a	1.45 ± 0.66b
Body-length growth rate of (%)	136.66 ± 26.78b	218.66 ± 14.84a	135.13 ± 17.12b
Survivability (%)	66.67 ± 6.11a	56.10 ± 4.00b	53.86 ± 6.11b

The data in the table are expressed as mean ± standard error. Different letters (a, b) in the same column indicate significant differences ($P < 0.05$) between each other through multiple tests

In *Table 2*, the experimental data were statistically tested to assess the effect of different light conditions on the growth index of *Procambarus clarkii*. Statistical tests were performed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc multiple comparison tests to determine significant differences between groups ($P < 0.05$). Different light conditions had significant effects on PC growth and survival. Under natural lighting conditions, the final body weight (13.46 ± 0.44 g) and weight gain rate (208.31 ± 13.41%) of PC were the highest, and significantly higher than the all black group and the light group. This indicated that natural lighting conditions were more conducive to the weight gain of PC. In terms of body length growth, the natural group also showed the best performance, with the highest total length (10.63 ± 0.34 cm) and body length growth rate (218.66 ± 14.84%), and

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significant differences compared to the other two groups. This indicated that natural lighting conditions also had a positive promoting effect on the body length growth of PC. However, in terms of survival rate, the all black group showed the highest survival rate (66.67 ± 6.11%), significantly higher than the natural group and the light group. This might be due to the fact that the all black environment provided a more comfortable living environment for the PC, thereby increasing its survival rate. Therefore, natural lighting conditions were most favorable for the growth of PC, while a completely black environment was more conducive to its survival.

Immune response of PC in high-temperature aquaculture environment under climate change

To investigate the effects of different breeding temperature conditions on the immune enzyme system of PC, and how these conditions affect its immune ability and health status, a series of controlled experiments were conducted in this study. The experiment randomly assigned PC to three predetermined breeding environments: a completely black environment to simulate no light conditions, a control environment to simulate normal natural light conditions, and a high-temperature environment to simulate the impact of temperature rise on breeding. By measuring immune indicators such as MDA enzyme content, SOD, LSZ, AKP, and CAT specific activity in each group, the effects of different temperature conditions on the immune response of PC were analyzed. The experimental results are shown in *Table 3*.

Table 3. *Effects of different breeding temperatures on the activity of immune enzymes in PC*

Immune indexes	Group		
	All black team	**Control group**	**High temperature group**
Mda enzyme content (nmol/mgprot)	4.65 ± 1.32b	5.67 ± 1.52²	6.78 ± 1.18a
Sod specific activity (u/ml)	73.23 ± 11.64b	102.46 ± 10.18²	91.23 ± 5.22ab
Lsz specific activity (u/ml)	23.24 ± 1.69°	44.29 ± 2.23	35.84 ± 2.31b
Akp specific activity (guinness units/ml)	10.21 ± 1.87	12.86 ± 0.16	12.81 ± 1.22
Cat specific activity (u/ml)	0.53 ± 0.28	0.89 ± 0.13	0.42 ± 0.11

The data in the table are expressed as mean ± standard error. Different letters (a, b) in the same column indicate significant differences ($P < 0.05$) between each other through multiple tests

In *Table 3*, the MDA enzyme content in the high-temperature group (6.78 ± 1.18 nmol/mgprot) was significantly higher than that in the all black group (4.65 ± 1.32 nmol/mgprot) and the control group (5.67 ± 1.52 nmol/mgprot), indicating that high temperature may increase lipid peroxidation levels. Secondly, the specific activity of SOD was higher in both the high-temperature group (91.23 ± 5.22 U/mL) and the control group (102.46 ± 10.18 U/mL) than in the all black group (73.23 ± 11.64 U/mL), but higher in the control group, indicating that normal light conditions may be more favorable for the activity of SOD enzyme. The specific activity of LSZ was highest in the control group (44.29 ± 2.23 U/mL), significantly higher than that in the high-temperature group (35.84 ± 2.31 U/mL) and the all black group (23.24 ± 1.69 U/mL), indicating that normal light may enhance lysozyme activity. There was no significant difference in the specific activity of AKP and CAT among the

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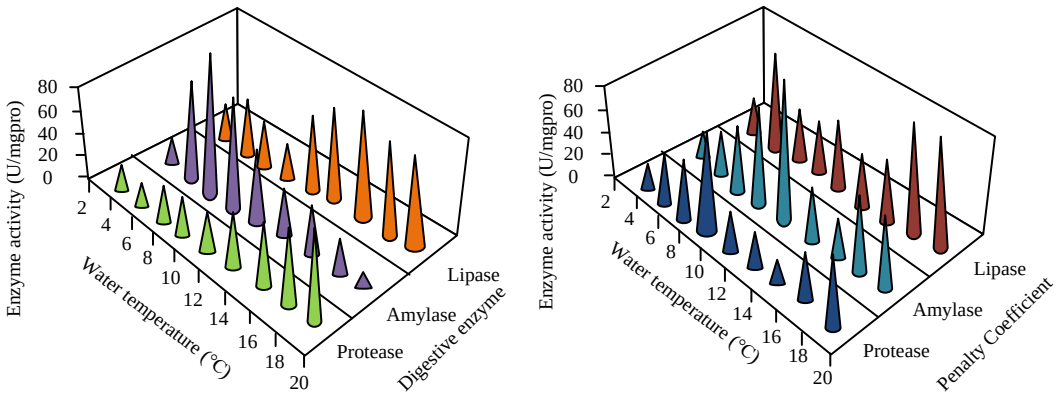
groups. Therefore, normal light and appropriate temperature may be more beneficial for the immune ability and health status of PC. Although the all black group was slightly higher than the high-temperature group, the overall trend indicated that normal breeding temperature was more conducive to maintaining CAT specific activity. The effect of sustained high temperature stress on the digestive enzyme activity of PC is shown in *Figure 9*.



(a) The effect of different water temperatures on the activity of digestive enzymes in Procambarus clarkii under subtropical climate environment

(b) The effect of different water temperatures on the activity of digestive enzymes in Procambarus clarkii under temperate oceanic climate environment

Figure 9. *Effects of continuous high temperature stress on digestive enzyme activity in PC*

In *Figure 9a*, in a subtropical climate, as the water temperature increases from 2°C to 20°C, the activities of lipase, amylase, and protease in PC first increased and then decreased, reaching their peak at 20°C at 80 U/mgpro, 60 U/mgpro, and 40 U/mgpro, respectively, before gradually decreasing. This indicated that suitable water temperature could significantly enhance the activity of digestive enzymes and promote the digestive ability of PC. This fluctuation may be due to the inhibition of enzyme molecule activity at lower temperatures, and as the temperature increases, enzyme activity gradually increases. However, when the temperature exceeds a certain range (such as 20°C), excessively high temperatures may begin to damage the structure of enzyme molecules, leading to a decrease in enzyme activity. Therefore, there exists an optimal temperature (20°C) at which enzyme activity reaches its maximum and then decreases as the temperature continues to rise. This indicates that changes in enzyme activity are closely related to environmental temperature. In *Figure 9b*, under temperate oceanic climate, although the trend of enzyme activity changes was similar, the overall activity level seemed to be higher, which might be related to the smaller fluctuations in water temperature and more stable environment in this climate. Similarly, at 20°C, the activities of various digestive enzymes also reached their maximum values, but the specific values were similar to those in subtropical climates, indicating that this temperature was the optimal temperature point for digestive enzyme activity in PC. At suitable water temperatures (such as 20°C), digestive enzyme activity was highest, which was beneficial for the digestion and survival of PC. The changes in non-specific immune indicators of hemolymph after continuous high temperature stress are shown in *Figure 10*.

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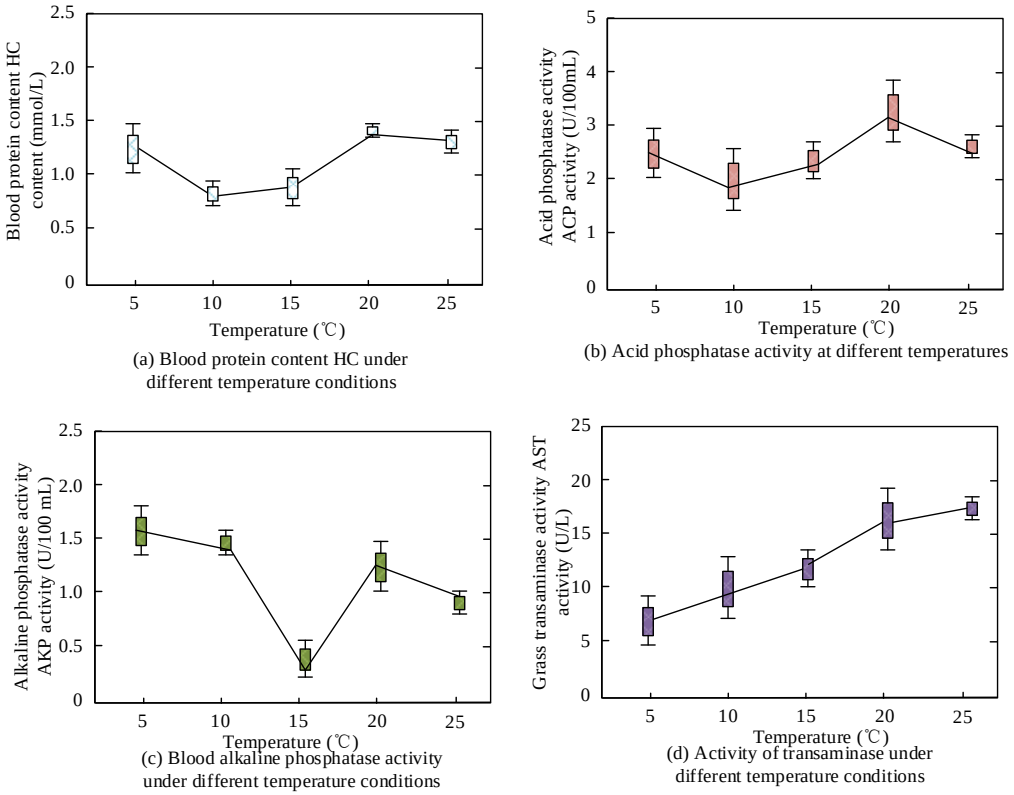

Figure 10. *Effects of continuous temperature stress on immune indicators of PC*

In *Figure 10,* significant changes were observed in the non-specific immune indicators of the hemolymph of PC under sustained high temperature stress. When the water temperature rose from the appropriate 24℃ to 19℃, 22℃, and 25℃, the content of HC generally decreased, especially outside the 22℃ group. The HC content in each high-temperature group was significantly lower than that in the control group ($P < 0.05$). This indicated that high temperature stress affected the synthesis or stability of HC in PC. Meanwhile, the activity of ACP significantly decreased with increasing temperature, indicating that the non-specific immune function of PC was inhibited at high temperatures. On the contrary, the activity of aspartate aminotransferase (AST) gradually and significantly increased with temperature, reaching its highest value at 25℃ ($P < 0.05$), reflecting the exacerbation of liver cell damage and metabolic stress under high temperature stress. As a result, sustained high temperature stress reduced the non-specific immune function of PC and may exacerbate liver cell damage and metabolic stress. The changes in immune related gene expression before and after heat stress are shown in *Figure 11*.

In *Figure 11a*, before heat stress, the relative expression level of AIP8 gene was relatively low, basically below 1, and the fluctuation was not significant. After heat stress, the relative expression level of AIP8 gene significantly increased, especially after 23 h, the expression level rapidly increased and reached a peak of about 3.5. In *Figure 11b*, the relative expression level of TNF gene was also low before heat stress, but after heat stress, its expression level rapidly increased, especially after 24 h, reaching a peak of about 2.0. In *Figure 11c*, the relative expression level of Toll gene was also relatively low before heat stress, but after heat stress, its expression level

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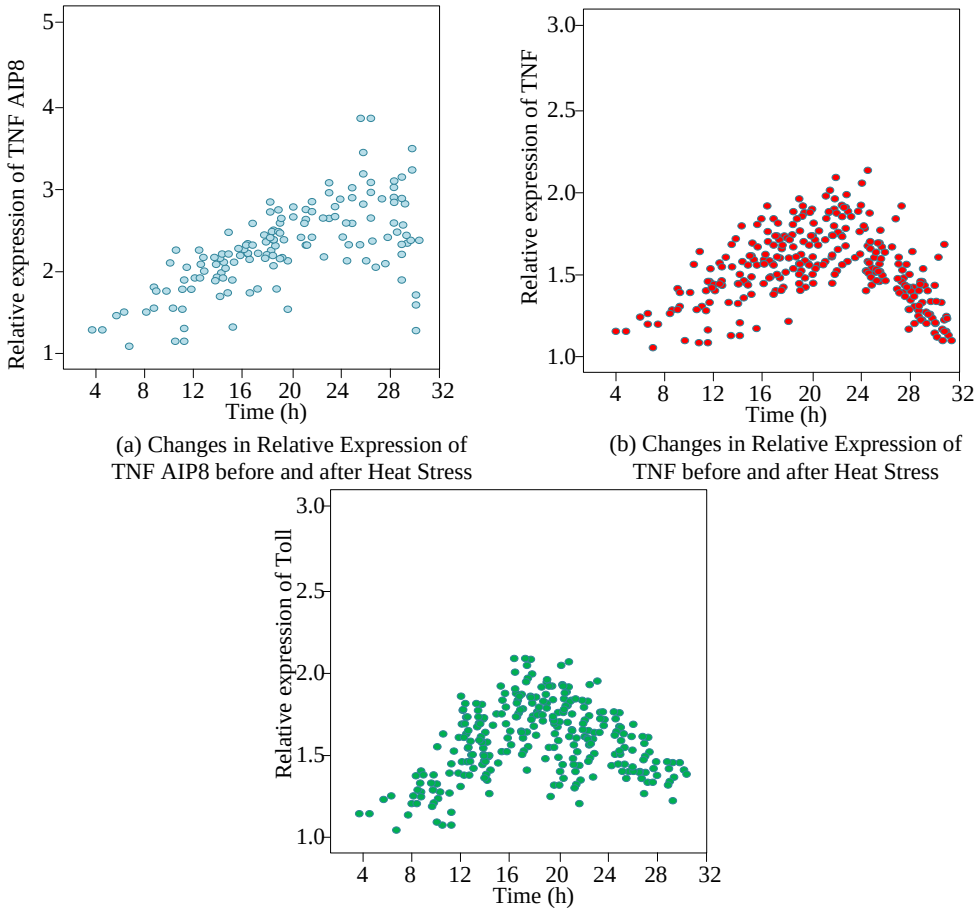
increased, especially after 16 h, reaching a peak of about 2.3. Overall, these three immune related genes showed a significant increase in expression levels after heat stress, especially AIP8 and TNF genes. This may be due to heat stress exacerbating the inflammatory response in the body of PC, thereby promoting the expression of these immune related genes. The changes in gut microbiota diversity of PC before and after heat stress are shown in *Figure 12*.



(a) Changes in Relative Expression of TNF AIP8 before and after Heat Stress

(b) Changes in Relative Expression of TNF before and after Heat Stress

(c) Changes in Relative Expression of Toll before and after Heat Stress

Figure 11. *Changes in immune related gene expression before and after heat stress*

In *Figure 12a*, the red column represented the AG group and the blue column represented the NG group. As the Number of Reads Sampled increased, the Sobs index on OTU level of both groups showed an increasing trend, but the AG group had a faster growth rate, ultimately higher than the NG group. In *Figure 12b*, as the OTU Rank increased, the OTU Abundance (log2) of both groups showed a downward trend, but the AG group had a slower decline rate and ultimately higher than the NG group. The flattening of the abundance level curve also indicated a high degree of uniformity in community composition, and the distribution of OTUs in the community was relatively balanced. In addition, the study also found that there were 422 OTUs unique to the NG group and 1366 OTUs unique to the AG group. Therefore, heat stress significantly increased the number of unique OTUs, while shared OTUs showed insensitivity to heat stress, further emphasizing the profound impact of heat stress on gut microbiota

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composition and diversity. To investigate the effects of high temperature stress on the dominant species in the gut microbiota of *Procambarus clarkii*, and to reveal the structural changes and potential ecological functions of the gut microbiota under high temperature conditions. By analyzing the abundance changes of dominant species in the gut microbiota before and after high temperature stress, the potential impact of high temperature on PC digestion, immunity, and overall health is evaluated, providing scientific basis for optimizing the high-temperature breeding environment. The experiment set up a control group and a high-temperature stress group. The control group was subjected to standard breeding conditions to ensure that the microbiota was in a stress-free baseline state, and the average abundance percentage of each dominant species in the gut microbiota was recorded. The high-temperature stress group simulates a high-temperature environment and observes the changes in the abundance of these dominant species under high-temperature conditions. Using 16S rRNA high-throughput sequencing technology to analyze the composition of gut microbiota and calculate the relative abundance of each dominant species. Then compare the average abundance of dominant species between the control group and the high-temperature stress group, calculate the rate of change, and the results are shown in *Table 4*.

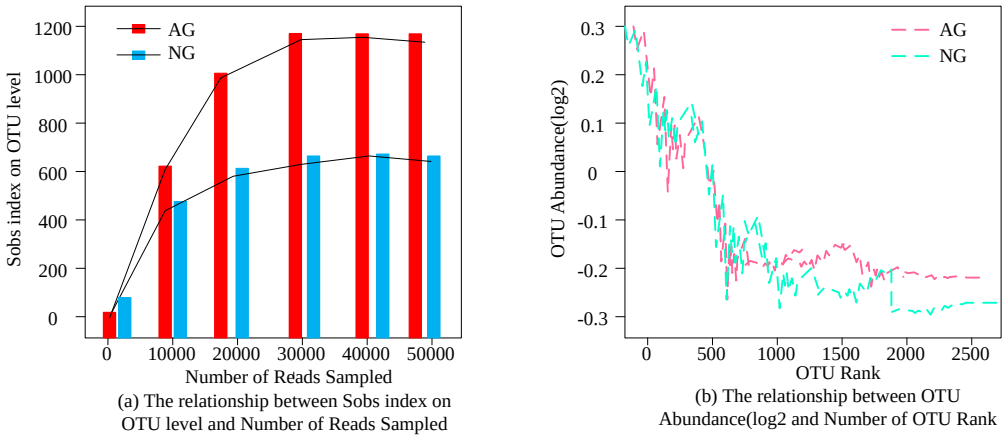

***Figure 12**. Diversity of gut microbiota in PC before and after heat stress*

***Table 4.** Experimental results of changes of the dominant species in the CP gut microbiota under high temperature stress*

Dominant species	Average abundance of control group (%)	Average abundance of high temperature stress group (%)	Change rate (%)	P value
Lactobacillus acidophilus	25.34	18.45	-26.87	<0.001
Bacillus subtilis	12.78	9.56	-25.23	<0.001
Escherichia coli	8.9	11.23	26.18	<0.001
Pseudomonas aeruginosa	5.67	4.32	-23.81	<0.01
Clostridium perfringens	3.45	2.12	-38.55	<0.001
Streptococcus thermophilus	7.89	6.54	-17.06	<0.05
Bifidobacterium longum	10.23	7.98	-22.01	<0.001
Enterococcus faecalis	4.56	3.21	-30.04	<0.01
Vibrio cholerae	2.34	1.56	-33.33	<0.05
Bacteroides fragilis	6.78	5.43	-19.94	<0.01

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The analysis of the experimental data in *Table 4* clearly shows the effect of high temperature stress on the abundance of dominant species in the gut microbiota of *Procambarus clarkii*. Under high temperature stress conditions, the abundance of most dominant species showed a significant decrease. For example, the abundance of Lactobacillus acidophilus decreased from 25.34% in the control group to 18.45% in the high-temperature stress group, with a high rate of change of -26.87%, and a P-value less than 0.001, indicating that this decrease is highly statistically significant. Similarly, Bacillus subtilis, Pseudomonas aeruginosa, Clostridium perfringens, Streptococcus thermophilus, Bifidobacterium longum, Enterococcus faecalis. The abundance of Vibrio cholerae decreased by 25.23%, 23.81%, 38.55%, 17.06%, 22.01%, 30.04%, and 33.33%, respectively, indicating a significant negative impact of high temperature stress on the gut microbiota structure of PC. The mechanisms that lead to these results may involve multiple aspects. High temperatures may disrupt the balance of the gut microenvironment, leading to inhibition of the growth of beneficial bacteria such as Lactobacillus acidophilus and Bacillus subtilis. Meanwhile, high temperatures may also promote the proliferation of certain pathogenic bacteria such as Escherichia coli, whose abundance increased by 26.18% in the high-temperature stress group, which may further exacerbate the imbalance of the gut microbiota. In addition, high temperatures may also affect the digestion and immune system of PC, leading to a decrease in its ability to regulate the microbial community, thereby causing changes in the structure of the microbial community. These changes may not only affect the health status of PC, but also have adverse effects on breeding efficiency and product quality. Therefore, optimizing the high-temperature breeding environment and maintaining the balance of gut microbiota are of great significance for the healthy breeding of PC.

Discussion

The study systematically controlled water temperature and dissolved oxygen concentration, combined with different light conditions, to investigate the effects of high temperature stress on the growth and immune response of *Procambarus clarkii*. The experimental results showed that high temperature stress significantly altered the abundance of dominant species in the gut microbiota of PC, providing a new perspective for understanding the physiological and ecological adaptation mechanisms of PC in high temperature environments. Under high temperature stress conditions, the abundance of dominant species in most gut microbiota significantly decreases, such as beneficial bacteria like Lactobacillus acidophilus and Bacillus subtilis. This may indicate that high temperature disrupts the balance of the gut microenvironment and inhibits the growth of beneficial bacteria. On the contrary, the abundance of certain pathogenic bacteria such as Escherichia coli has increased, which may further exacerbate the imbalance of the gut microbiota and pose a potential threat to the health of PC.

In addition to changes in gut microbiota, high temperature stress also has a significant impact on the growth performance and immune response of PC. Research has shown that at suitable water temperatures (such as 25℃), the weight gain effect of PC is most significant, and the weight gain of PC reaches its maximum value when the dissolved oxygen concentration is 20 mg/L. However, high temperature stress significantly reduced the immune enzyme activity of PC, such as the specific activity of SOD and LSZ, which decreased in the high temperature stress group, indicating that high temperature may inhibit the immune function of PC.

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In addition, high temperature stress also affects the digestive enzyme activity of PC. Although the digestive enzyme activity reaches its peak at suitable water temperatures (such as 20°C), high temperature may cause damage to the enzyme molecular structure, thereby reducing enzyme activity. This change not only affects the digestive ability of PC, but may also have adverse effects on its overall health.

In summary, high temperature stress has a significant negative impact on the growth, immune, and digestive functions of PC. These findings emphasize the importance of optimizing high-temperature aquaculture environments and maintaining gut microbiota balance. Future research should further explore the adaptation mechanism of PC under high temperature stress, and how to alleviate the adverse effects of high temperature stress by adjusting breeding conditions, thereby improving the breeding efficiency and biosafety of PC.

Conclusion

This study delves into the impact of high-temperature aquaculture environments on the growth and immune response of PC under climate change. At 25°C, the weight gain effect of PC was most significant, with a specific growth rate reaching its maximum, which was consistent with the suitable water temperature promoting its metabolism and feeding behavior. When the dissolved oxygen concentration was 20 mg/L, the growth of PC reached its peak, emphasizing the importance of sufficient dissolved oxygen for its normal physiological activities. In addition, under natural light conditions, the PC exhibited the highest final body weight and weight gain rate, indicating the positive effect of natural light on its growth and development. High temperature stress had a negative impact on the immune response of PC (Wang et al., 2024). The increase in MDA enzyme content and the decrease in SOD, LSZ, AKP, and CAT specific activities in completely black and high-temperature environments revealed that these environmental conditions might inhibit or interfere with the immune function of PC. The results of the digestive enzyme activity test showed that the digestive enzyme activity reached its peak at 20°C, which might be related to the enhanced metabolic activity at an appropriate temperature. The monitoring of non-specific immune indicators also confirmed the decrease in hemoglobin content and ACP and AKP activity under high temperature stress, while the increase in AST activity suggested possible damage to liver cells (Yeh et al., 2021). The analysis of immune related gene expression change revealed an upward trend in the expression levels of AIP8, TNF, and Toll genes after heat stress, especially AIP8 and TNF genes, which may be related to the exacerbation of inflammation in the body. The analysis of gut microbiota diversity further emphasized the significant impact of heat stress on the composition of gut microbiota in PC. Heat stress significantly increased the number of unique OTUs, while shared OTUs showed insensitivity to heat stress (Yuan et al., 2022; Miles et al., 2024). In summary, the decrease in immune enzyme activity and changes in immune related gene expression under high temperature stress, as well as the significant impact on gut microbiota diversity, all indicate the potential negative effects of environmental stress on the physiological functions of PC. However, this study has not yet covered the adaptive changes in behavior and physiology of PC under long-term environmental stress. Future research needs to focus on these adaptive changes and their potential impact on aquaculture management strategies.

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