Influences of oxygen and temperature interaction on the antibacterial activity, antioxidant activity, serum biochemical indices, blood indices and growth performance of crucian carp (#72877)

Second revision

Guidance from your Editor

Please submit by **4 Oct 2022** for the benefit of the authors (and your token reward) .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Custom checks
Make sure you include the custom checks shown below, in your review.

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files

Download and review all files from the materials page.

1 Tracked changes manuscript(s)
1 Rebuttal letter(s)
5 Figure file(s)
1 Table file(s)
1 Raw data file(s)
1 Other file(s)

Custom checks

Vertebrate animal usage checks

- Have you checked the authors ethical approval statement?
- Were the experiments necessary and ethical?
- Have you checked our animal research policies?

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Influences of oxygen and temperature interaction on the antibacterial activity, antioxidant activity, serum biochemical indices, blood indices and growth performance of crucian carp

Bin Wang [1] , **Hanping Mao** [Corresp., 1] , **Jian Zhao** [2] , **Yong Liu** [1] , **Yafei Wang** [1] , **Xiaoxue Du** [1]

[1] School of Agricultural Engineering, Jiangsu University, Jiangsu, Zhenjiang, China

[2] College of Biosystems Engineering and Food Science, Zhejiang University, Zhejiang, Hangzhou, China

Corresponding Author: Hanping Mao
Email address: maohpujs@163.com

In aquaculture, fish welfare is attached increasing importance to. Oxygen and temperature are the main factors affecting the welfare of crucian carp, and there are few studies on the interaction of the two on crucian carp. Therefore, this study investigated the effects of different temperature (18℃, 24℃, 30℃) and oxygen concentration (2.1 mgL $^{-1}$, 5.4 mgL $^{-1}$, 9.3 MgL $^{-1}$) on serum antibacterial activity, antioxidant activity, hematological parameters and growth performance of crucian carp. The results showed that the antibacterial properties of hypoxia 18°C (L18) and high oxygen 24°C (H24) were the best. The activities of catalase, glutathione peroxidase and total superoxide dismutase were the highest at 24°C under both mesoxia and hyperoxia conditions. In addition, the contents of glucose and total protein increased first and then decreased with the change of temperature, and the triglyceride was the lowest at 30℃. Blood parameters at 24°C were within normal limits. Besides, the growth rate of crucian carp was the worst under hypoxia treatment at 30℃ (L30). The study showed that high temperature impairs the antibacterial ability, antioxidant capacity and growth performance of crucian carp, and high oxygen can alleviate these adverse reactions. This provides a theoretical basis for subsequent aquaculture research.

Article

Influences of oxygen and temperature interaction on the antibacterial activity, antioxidant activity, serum biochemical indices, blood indices and growth performance of crucian carp

Bin Wang[1], Hanping Mao[1,*], Jian Zhao[2], Yong Liu[1], Yafei Wang[1] and Xiaoxue Du[1]

[1] School of Agricultural Engineering, Jiangsu University, Zhenjiang 212013, China; 2112016004@stmail.ujs.edu.cn (B.W.); 2111816007@stmail.ujs.edu.cn (Y.L.); 2111916018@stmail.ujs.edu.cn (Y.W.); 2111916004@stmail.ujs.edu.cn (X.D.);

[2] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, 310000, People's Republic of China. zhaojzju@zju.edu.cn(J.Z.);

Corresponding Author:
Hanping Mao[1,*]
School of Agricultural Engineering, Jiangsu University, Zhenjiang 212013, China;
Email address: maohpujs@163.com.

Abstract

In aquaculture, fish welfare is attached increasing importance to. Oxygen and temperature are the main factors affecting the welfare of crucian carp, and there are few studies on the interaction of the two on crucian carp. Therefore, this study investigated the effects of different temperature (18℃, 24℃, 30℃) and oxygen concentration (2.1 mgL^{-1}, 5.4 mgL^{-1}, 9.3 mgL^{-1}) on serum antibacterial activity, antioxidant activity, hematological parameters and growth performance of crucian carp. The results showed that the antibacterial properties of hypoxia 18°C (L18) and high oxygen 24°C (H24) were the best. The activities of catalase (P=0.0003, F=40.68), glutathione peroxidase (P=0.0008, F=29.06) and total superoxide dismutase (P=0.0398, F=5.785) were the highest at 24°C under both mesoxia and hyperoxia conditions. In addition, the contents of glucose (P=0.0071, F=12.63) and total protein (P=0.0128, F=9.813) increased first and then decreased with the change of temperature, and the triglyceride (P=0.035, F=6.17) was the lowest at 30°C. Blood parameters at 24°C were within normal limits. Besides, the growth rate of crucian carp was the worst under hypoxia treatment at 30°C (L30). The study showed that high temperature impairs the antibacterial ability, antioxidant capacity and growth performance of crucian carp, and high oxygen can alleviate these adverse reactions. This provides a theoretical basis for subsequent aquaculture research.

Keywords: Environmental factors; Growth performance; Physiology; Biochemistry

1.Introduction

In many European countries, the survival pressure of crucian carp has been affected due to the invasion of exotic species such as carp and goldfish[1]. Therefore, crucian carp is considered as one of the main threatened species[2]. While it is growing rapidly in size, it suffers from many environmental challenges. Fish growth is related to temperature and oxygen[3,4,5].

Temperature plays an important role in the physiological state and ecosystem function of fish. Therefore, the appropriate temperature condition is of great significance for the management of aquaculture[6-10]. Enzyme activity, metabolic rate, and immunoglobulin levels in fish can be affected by sudden changes in temperature, thereby affecting fish survival[11-13]. Fish have their optimum temperature range. When the limits of fish tolerance are exceeded by temperature, the fish growth can be inhibited, followed by decreased antibacterial ability, disruption of physiological balance, and even leading to death[1,14]. Oxygen is an indispensable and pivotal substance for every aquatic animal to maintain metabolic activity[15], and it is also one of the crucial limiting factors in aquaculture production[16,17]. Growth parameters of fish are not only reduced by hypoxic conditions[18,19], furthermore, hematological parameters and apoptotic indices of fish are also affected[20]. In addition, fish can be subjected to a certain degree of oxidative damage by hypoxia. The growth of fish and the serum biochemical indicators are significantly changed, which lead to the influence on the material metabolism and immune system of the fish body[21].

There is a negative correlation between the temperature of the water environment and the oxygen content[4]. Some fish may exhibit agitation or avoidance behavior until the water temperature reaches a specific tolerance temperature. At the same time, the functional activities of the organs and tissues in the fish will be changed with the temperature transform, so that the fish body can maintain a balanced state as much as possible in the high temperature[22]. Under hypoxic conditions, fish may move to an environment with sufficient oxygen. In addition, fish can resist hypoxia by adjusting their behavior, such as increasing the oxygen transmission capacity of the operculum[23]. The metabolic rate of fish is increased by high temperature[24], equally, the growth rate of fish will be reduced by low oxygen conditions[25]. Therefore, to some extent, fish heat stress is relieved by higher oxygen concentrations. The effects of temperature and oxygen alone on crucian carp have been extensively studied and discussed, however, few experts have studied the effect of the interaction of the two factors on crucian carp.

Therefore, the interaction effects of 3 temperatures (18℃, 24℃ and 30℃) and 3 oxygen levels (2.1mgL^{-1}, 5.4mgL^{-1} and 9.3mgL^{-1}) have been set in the study, and the changes in the antibacterial properties, antioxidant capacity, serum biochemistry, blood parameters and growth parameters of crucian carp have been explored in the nine combinations. The research will provide a theoretical basis for aquaculture environmental control system.

2.Materials & Methods

The maintenance, handling and experiments conducted on fish during this study were carried out in strict accordance with the guidelines of the Association for the Study of Animal Behaviour Use of Zhejiang University（no. ZJU20190073）.

2.1 Fish Collection and Maintenance

All crucian carp used in this study were provided by Xiong Feng Co. Ltd. (Guangdong, China). The experimental fish were male. Before the experiment, 200 flexible and responsive crucian carp were put into five 400L circulating water tanks for a week. Temperature controllers were used to keep the water temperature at 17±0.5℃. The photoperiod was 12:12h (light/dark), and dissolved oxygen was kept at 6.7±0.1mgL^{-1}. pH was 7.1±0.1, and nitrite was 0-0.12mg/L, and ammonia nitrogen was 0-0.12mg/L. Our water quality parameters are determined by water quality detection device which is from Shanghai Lanchang Automation Technology Co., LTD. Fish were fed commercial pellets (protein 35%, fat 5%, ash 15%, and water 12.5%) twice daily (8:30 and 17:30). Commercial pellets was from Shandong Binzhou Ruixing Biological Technology Co., LTD. The feeding amount per day was set to 5% of the body weight of the shoal. Debris and manure were cleaned and replaced by 60% fresh water daily.

2.2 Trial design

The experimental crucian carp (initial body weight 109.82±2.16g) which were choosed from the circylating water tanks were randomly distributed in 9 circulating water tanks, 21 in each tank, and the size of these water tanks are 150mm×100mm×100mm, and reared for one week before the experiment. A two-factor, three-level experiment was designed, including temperatures of 18℃, 24℃, and 30℃, and oxygen concentrations of 2.1mgL^{-1}, 5.4mgL^{-1}, and 9.3mgL^{-1} with three replicates for each treatment. Each tank was divided into three feeding spaces of the same size by two partitions, and there were seven experimental crucian carp in each space. Temperature controller were employed to gradually change the water temperature from 17℃ to 18℃, 24℃, and 30℃ at a rate of 1℃ per hour. Temperature controllers were from Guangdong Foshan Lvban Electronic Commerce Co., LTD. In this experiment, hyperoxia conditions were created by increasing the oxygen content, and hypoxic conditions were created by increasing the nitrogen content. Results were analyzed in each trial at 18℃ and hypoxia as a control group. The schematic diagram of the oxygen regulation system is shown in Figure 1, including an oxygenator, a nitrogen container, a water tank, and a portable dissolved oxygen meter. The trial runs from December 19, 2021 to January 19, 2022, for a total of 30 days.

2.3 Antibacterial experiment

The proliferation ability of bacterial isolates in crucian carp serum was determined by 96-well plate. Specifically, freshwater bacteria (*Aeromonas*) were utilized to determine the antibacterial capacity in serum samples. *Aeromonas* generally grew normally on agar plates at 25°C. First of all, fresh single colonies were picked and diluted in 5mL of nutrient broth, it was then incubated for 12 hours in an orbital incubator at 28°C with the speed set at 150-200rpm. Then, serum antibacterial ability was tested[26]. Simultaneous serum samples and bacterial suspensions were serially diluted with PBS twice. Furthermore, 100μL of bacterial diluent and a same volume of crucian carp serum were added into a 96-well plate. 100μL of crucian carp serum and 100μL of nutrient solution were added to the negative wells. Instead, 100μL of bacterial suspension and 100μL of nutrient broth were added to positive wells. This experiment was repeated three times. The 96-well plates were incubated at 28°C. Afterwards, the OD value at 600nm was measured by the microplate reader, with an interval of 1 hour, and a total of 12 hours was measured. The determination of bacterial viability was determined by the ratio between the number of viable bacteria and positive control bacteria, and the results were showed as a percentage (100%).

2.4 Sampling procedures

The experimental crucian carp were starved 24 hours before sampling, and three experimental fish were randomly selected from each independent area. Each fish was individually sampled and stored. All sampling was performed after MS-222 anesthesia. Blood was drawn from the caudal vessel with heparinized syringe, and one part of which was used for the measurement of blood parameters, the other part was centrifuged at 1350×g for 10min, and the serum was obtained after coagulation at 4℃ for 5h. The serum was stored at -20℃ for antibacterial, antioxidant and biochemical tests.

2.5 Physiological indicators

In the three spaces divided by each experimental water tank, 3 crucian carp were randomly selected for physiological and biochemical tests, and the results were expressed as mean±SD. Finally, these data were sorted and analyzed. Total superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were measured with a microplate reader using SOD Assay Kit (WST-1 method), CAT Visible Light Kit and GSH-PX Assay Kit respectively. Total superoxide dismutase activity was determined by the hydroxylamine method at 550nm[27]. Catalase activity was measured at 405nm by the ammonium molybdate method[28]. Glutathione peroxidase activity was measured colorimetrically at 412nm[29]. The kit was purchased from Nanjing Jiancheng Bioengineering Institute. Blood biochemical parameters (Glucose, Triglycerides, and Total protein) were determined with a Cobas C-311 automatic biochemical analyzer and commercial assay kits. Hematocrit (HCT), mean hemoglobin content (MCH) and hemoglobin (HGB) were detected with a BM830 automatic hematology analyzer.

2.6 Growth performance

$$\text{Specific growth rate (SGR)} = 100\%\times(\ln W_2 - \ln W_1), \quad (1)$$

$$\text{Relative growth rate (WGR)} = 100\%\times(W_2 - W_1)/W_1, \quad (2)$$

$$\text{Survival rate (SR)} = 100\%\times(N_2/N_1), \quad (3)$$

W_1: the initial weight of fish, W_2: the final weight of fish, N_2: the final number of fish, N_1: the initial number of fish.

2.7 Statistical Analyses

Data analysis was performed using GraphPad Prism 5.0 software (GraphPad Software Inc., USA). Differences between groups were calculated by multiple comparison test by Student's t-test. $P<0.05$ indicate a statistical significance. Results were expressed as the mean±*SD* (n=9).

3. Results

3.1 Antibacterial experiment

The growth of bacteria in the serum of crucian carp under nine treatments were analyzed. *Aeromonas* is a general pathogen of aquatic animals. Figure 2 showed that the relative growth rates of *Aeromonas* was decreased in all 9 treatment groups over time. Under the same oxygen condition, the relative growth rate of *Aeromonas* was increased with the increase of temperature. Relative growth rate of *Aeromonas* was slowed down with the increase of oxygen concentration. Among them, the relative growth rate of H24 and H18 treatments were low, and the antibacterial effect of serum at this time was better. The relative growth rate of L30 and L18 treatments were higher, and the serum antibacterial effect was poor at this time. The antibacterial effect of other treatment groups were between the best and the worst.

3.2 Serum antioxidant test

To explore the antioxidant capacity of crucian carp, the activities of three antioxidant enzymes were measured in the serum of nine treatment groups. As shown in Figure 3(a-c), the activities of CAT, GPx and SOD in serum of crucian carp were significantly enhanced with the increase of oxygen concentration (*P*=0.0014, *F*=23.7) under the same temperature conditions. Compared with 18°C, the serum CAT activity at 24°C and 30°C were decreased by 15.24% and 36.67% under hypoxia condition. The serum CAT activity were increased by 38.40% and 55.20% at 24°C and 30°C when exposed to medium oxygen. When exposed to high oxygen, serum CAT activity were increased by 41.41% and 53.20% at 24°C and 30°C, as shown in Figure 3(a). Figure 3(b) shows that under hypoxia, the serum GPx activity of Crucian carp under 24°C and 30°C were decreased by 4.76% and 21.43%, respectively, compared with 18°C. Under medium oxygen condition, the serum GPx activity of crucian carp under 24°C and 30°C were increased by 11.85% and 24.44%, respectively, compared with 18°C. Under high oxygen condition, compared with 18°C, the serum GPx activity of crucian carp under 24°C and 30°C were increased by 16.46% and 25.32%, respectively. As shown in Figure 3(c), under hypoxia condition, the serum SOD activity of Crucian carp was increased by 11.86% and was decreased by 9.32% under 24°C and 30°C compared with 18°C. Under medium oxygen condition, the serum SOD activity of crucian carp were increased by 16.32% and 10.10% under 24°C and 30°C compared with 18°C. Under high oxygen condition, the SOD activity of crucian carp serum was increased by 5.37% and was decreased by 4.19% under 24°C and 30°C compared with 18°C.

3.3 Serum biochemical experiment

It can be seen from Figure 4(a) that with the increase of oxygen concentration, glucose levels were increased significantly under the three temperature conditions (*P*=0.029, *F*=6.767). At the same oxygen concentration, the glucose level of crucian carp were increased first and then decreased with the increase of temperature, and reached the highest at 24°C (*P*=0.0071, *F*=12.63). Figure 4(b) shows the change of total protein content in crucian carp serum. Under the same oxygen concentration, total protein content at 24°C was higher than that at 18°C and 30°C (*P*=0.0324, *F*=6.408). Total protein content under hypoxia was lower than that under medium oxygen and hyperoxia conditions (*P*=0.0033, *F*=17.23). Changes in serum triglyceride content of crucian carp were shown in Figure 4(c). At the same temperature, with the increase of oxygen concentration, hyperoxia condition was higher than medium oxygen and hypoxic condition (*P*=0.0025, *F*=19.05). When oxygen concentration was the same, triglyceride content was decreased with increasing temperature (*P*=0.0128, *F*=9.813).

3.4 Hematological analysis

The differences in blood HCT, MCH and HGB levels of crucian carp were shown in Figure 5. As shown in Figure 5(a), HCT at 24°C was significantly higher than that at 18°C and 30°C (*P*=0.0421, *F*=5.622). At the same temperature, the HCT of hypoxia was higher than that of hyperoxia and moderate oxygen (*P*=0.0107, *F*=10.63). Figure 5(b) shows that under the same oxygen concentration, MCH at 30°C was significantly higher than that at 18°C (*P*=0.0122, *F*=10.04), slightly higher than 24°C. Under the same temperature condition, MCH under hypoxia condition was significantly higher than that under hyperoxia and medium oxygen condition (*P*=0.0126, *F*=9.897). As shown in Figure 5(c), at the same temperature, HGB under hypoxic condition was higher than that under medium oxygen and hyperoxia conditions (*P*<0.0001, *F*=106.7). At the same oxygen concentration, HGB at 18°C was slightly lower than that at 24°C and 30°C (*P*<0.0001, *F*=74.01).

3.5 Growth performance

The FW, SGR, WGR and SR of crucian carp after different treatments were shown in Table 1. The FW of crucian carp was different between different treatments of temperature and oxygen concentration. Under hypoxic conditions, the FW of crucian carp in L30 was lower than that in L18 and L24 (P=0.0002, F=45.15). Under medium oxygen conditions, the FW of M30 crucian carp was lower than that of M18 and M24 (P=0.0013, F=24.26). Under hyperoxia condition, the FW of H30 crucian carp was significantly lower than that of H18 and H24 treatments (P=0.0219, F=7.731). In addition, under the same temperature conditions, the FW of crucian carp under hyperoxia was significantly higher than that under hypoxia (P=0.0262, F=7.098). According to the analysis results, H24 (136.71±2.51g) and L30 (105.86±2.96g) were the highest and lowest values of the FW. When the temperature is the same, the SGR of crucian carp under hyperoxia was significantly higher than that under mesoxia and hypoxia (P=0.001, F=27.09). When the oxygen concentration was same, the SGR of crucian carp reached the highest at 24℃and the lowest at 21℃ (P=0.0043, F=15.39). The SGR of crucian carp under H24 and M24 treatments were the highest, which were 0.68% and 0.63%, respectively. When the oxygen concentration was same, the WGR of crucian carp under 18°C was lower than that at 24°C and 30°C. When the temperature was same, the WGR of crucian carp under hyperoxia was significantly higher than that under medium oxygen and hypoxia (P<0.0001, F=94.56). Crucian carp under H24 and M24 had the highest WGR. The SR of crucian carp was affected by temperature. When the oxygen concentration was same, the SR was relatively high at 18°C and 24°C, and the SR was relatively low at 30°C. The SR was most affected by oxygen concentration, and the SR of H24 and H18 was 100%, respectively. The SR under L30 was the lowest, which was 71.43%.

4. Discussion

Fish serum is considered to be one of the commonly used methods to assess the physiological state and health status of fish [30]. It contains a variety of immune molecules, such as immune proteins, lysozyme, etc., with high antibacterial properties [31]. In the range of 18-30 °C, when the oxygen concentration was the same, the enzyme activity gradually increased with the increase of temperature, and the antibacterial activity of crucian carp serum gradually increased. However, the bacteriostatic effect of low temperature is better than that of moderate temperature and high temperature, because the optimal growth temperature of Aeromonas is 22℃-28℃[32]. At the same temperature, with the increase of oxygen concentration, the relative growth rate of Aeromonas decreases, the reason is that the amount of oxygen increases, the respiration and metabolic rate of crucian carp increase, the immune system in the fish works, so that the antibacterial ability is enhanced, Therefore, H18 and H24 have the strongest antibacterial properties.

The growth performance and metabolism of fish are affected not only by temperature and oxygen, but also by the antioxidant defense system of fish [33]. When the temperature and oxygen content change, excessive oxygen free radicals will be generated in the environment, and macromolecules such as proteins and lipids in the fish will be destroyed, thereby affecting the health of the fish [34-36]. However, this oxygen radical can be eliminated by the antioxidant defense system of fish, including CAT, GPx and SOD [37]. Under anoxic conditions, the activities of CAT and GPx in crucian carp were slightly weakened at 18℃-30℃, the SOD activity was slightly increased at 24℃, and decreased slightly at 30℃. This means that the crucian carp is in relatively good condition at this time. The activities of these two enzymes were significantly enhanced under mesoxia and hyperoxia conditions. At the same temperature, the activities of the three antioxidant enzymes were the highest in hyperoxia, followed by mesoxia, and lowest in hypoxia. This indicates that the antioxidant system of crucian carp can be activated by hyperoxia and mesoxia conditions. However, the enzyme activity is extremely low under hypoxia conditions, and the antioxidant capacity is the weakest [38]. Furthermore, the heat stress experienced by crucian carp can be resisted by hyperoxia conditions.

Glucose is the main substance for cell and tissue metabolism and physiological activities. Glucose levels in fish are affected by temperature and oxygen levels [39]. Low glucose concentrations can affect growth rate and appetite function in fish [40]. The results showed that the L27-treated crucian carp had the lowest glucose concentration, which resulted in decreased appetite and slow growth of crucian carp. The main function of serum proteins is to repair damaged tissues and participate in the regulation of plasma osmotic pressure [41]. Under the same oxygen concentration, the total protein activity increased at 24°C and 30°C, indicating that the body tissue of crucian carp may have been damaged when the water temperature increased [42]. In addition, total protein content was lower under hypoxic conditions than under meso- and hyperoxia conditions. Therefore, high concentration of oxygen can reduce the degree of tissue damage and accelerate tissue recovery in crucian carp. Oxidized triglycerides are an energy source for fish metabolism [43]. Compared with 18℃ and 24℃, the survival rate of crucian carp at 30℃ was the lowest, indicating that crucian carp needs to consume a lot of triglycerides under high temperature conditions. At the same temperature, the reduction of triglyceride in crucian carp under hyperoxia conditions was smaller than that under mesoxia and hypoxia conditions. This suggests that hyperoxia helps to alleviate the high temperature-induced triglyceride consumption in crucian carp.

Studies have shown that hematocrit is one of the important blood parameters that reflect the health of fish. At the same temperature, the hematocrit under hypoxic conditions is higher than that under mesoxia and hyperoxia conditions [44]. This is because when the oxygen concentration is low, the hemoglobin in the body increases, the oxygen transport capacity also increases, and the body can maintain normal physiological functions [45]. When the oxygen concentration is too high, although the oxygen carrying capacity is strong, the blood viscosity increases, the blood flow resistance increases, and the hematocrit decreases to a certain extent. Under the condition of constant oxygen conditions, the body temperature increases and the metabolic capacity increases. At this time, the content of hemoglobin increases, the hematocrit increases, and the physiological function of the body can be regulated to a certain extent through this cycle [46]. Conversely, when the body temperature is low, the utilization of oxygen may be affected, the synthesis of hemoglobin is reduced, and the hematocrit is also reduced. The experimental results are consistent with this conclusion.

The growth and health of fish can be directly reflected in their growth performance and survival rate. The test results are shown in Table 1. The survival rate of crucian carp under hyperoxia conditions was significantly higher than that under mesoxia and hypoxia conditions. Crucian carp growth index SGR and WGR were the highest at 24℃, followed by 30℃, and the lowest at 18℃. This is similar to the results of previous studies, that is, the optimal growth temperature of crucian carp is 25°C [47]. When the ambient temperature was the same, the growth of crucian carp under hyperoxia conditions was higher than that under mesoxia and hypoxia conditions. Therefore, the growth of crucian carp may be inhibited by hypoxia [48, 49]. It was also reported that the growth rate of crucian carp showed a trend of increasing first and then decreasing within a certain range [50]. The results showed that the growth rate of H24 crucian carp was the highest, and the growth rate of L30 crucian carp was the lowest. In addition, when the temperature was set to 30°C, the growth rate under the hyperoxia condition was higher than that under the mesoxia and hypoxic conditions, suggesting that hyperoxia could protect crucian carp from the adverse effects of high temperature to a certain extent.

5.Conclusions

Crucian carp had better growth performance under hyperoxia (9.3mgL^{-1}) than under medium oxygen (5.4mgL^{-1}) and hypoxia (2.1mgL^{-1}). High temperature and hypoxia stress will reduce the antibacterial properties of crucian carp, and various physiological indicators will be disordered. However, these adverse effects can be mitigated to some extent by increasing the oxygen concentration. Therefore, according to the changing trends of various parameters in the 9 treatment groups, the results show that the optimal growth environment for crucian carp is H24 treatment, that is, in the state of high oxygen, the water temperature is kept at 24℃.

The sample size of this study is not sufficient, and the conclusions may not be accurate enough. The sample size will be increased for further verification.

Acknowledgements

The authors would like to thank the Key Laboratory of Agricultural Engineering in Jiangsu University for supporting the experimental conditions of the research.

References

1. Bin Wang.; Guoxin Ma.; YongLiu.; YafeiWang.; XiaoxueDu.; QiangShi.; HanpingMao. Effects of Different Temperatures on the Antibacterial, Immune and Growth Performance of Crucian Carp Epidermal Mucus. *Fishes* **2021**, *6*, 66. DOI 10.3390/fishes6040066.
2. Missinhoun Dagoudo.; JunQiang.; Jin-Wen Bao.; Yi-Fan Tao.; Hao-Jun Zhu.; Ezra Mutebi Tumukunde.; Tlou Kevin Ngoepe.; Pao Xu. Effects of acute hypoxia stress on hemato-biochemical parameters, oxidative resistance ability, and immune responses of hybrid yellow catfish (Pelteobagrus fulvidraco×*P.*vachelli) juveniles. *Aquaculture International* **2021**, *29*, 2181-2196. DOI 10.1007/s10499-021-00742-1.
3. JosephTetreault.; RachelFogle.; ToddGuerdat. Towards a Capture and Reuse Model for Aquaculture Effluent as a Hydroponic Nutrient Solution Using Aerobic Microbial Reactors. *Horticulturae* **2021**, *7*, 334. DOI 10.3390/horticulturae7100334.
4. Xuyang Jiang.; Shuanglin Dong.; Rongxin Liu.; Ming Huang.; Kang Dong.; Jian Ge.; Qinfeng Gao.; Yangen Zhou. Effects of temperature, dissolved oxygen, and their interaction on the growth performance and condition of rainbow trout (Oncorhynchus mykiss). *Journal of Thermal Biology* **2021**, *98*, 102928. DOI 10.1016/j.jtherbio.2021.102928.
5. Lilian F. Nitz.; Lucas Pellegrin.; Daniel S.B. Pint.; Lucas C. Malte.; Carlos E. Copatti.; Luciano Garcia. Secondary stress responses to hypoxia and re-oxygenation at different temperatures in pacu (Piaractus mesopotamicus) juveniles. *Aquaculture Research* **2020**, *51*, 4471-4481. DOI 10.1111/are.14792.
6. Lindmark, Mt.; Ohlberger, J.; Gardmark, A. Optimum growth temperature declines with body size within fish species. *Freshwater Biology* **2021**, *00*, 1-9. DOI 10.1111/gcb.16067.
7. J. M. Burt, S. G. Hinch, D. A. Patterson. The importance of parentage in assessing temperature effects on fifish early life history: a review of the experimental literature. *Fish Biol Fisheries* **2011** *21*:377–406 DOI 10.1007/s11160-010-9179-1.
8. EOINJ. O'GORMAN[1], OLAFURP.OLAFSSON[2], BENO^ITO . L.DEMAR S[3], NIKOLAI FRIBERG[4], GUÐNI GUÐBERGSSON[5], ELISABETR . HANNESDOTTIR[2,5], MICHELLEC. JACKSON[6], LISELOTTE S. JOHANSSON[7], ORLAB. MCLAUGHLIN[1,8], JONS. OLAFSSON[5], GUY WOODWARD[1] and GISLIM.GISLASON[2]. Temperature effects on fish production across a natural.thermal gradient Global Change Biology. **2016** DOI: 10.1111/gcb.13233.
9. Edison Barbieri, Aline Maria Zigiotto de Medeiros & Marcelo Barbosa Henriques. Oxygen consumption and ammonia excretion of juvenile pink shrimp (Farfantepenaeus paulensis) in culture: temperature effects. *Marine and Freshwater Behaviour and Physiology*. **2016** 49:1, 19-25, DOI: 10.1080/10236244.2015.1108057.
10. Edison Barbieri & Ana Cristina Vigliar Bondioli. Acute toxicity of ammonia in Pacu fish (Piaractus mesopotamicus, Holmberg, 1887) at different temperatures levels. *Aquaculture Research*, **2013**, 1–7 DOI:10.1111/are.12203.
11. Neeraj Kumar. Dietary riboflavin enhances immunity and anti-oxidative status against arsenic and high temperature in Pangasianodon hypophthalmus. *Aquaculture* **2021**, *533*, 736209. DOI 10.1016/j.aquaculture.2020.736209.
12. Yining Chen.; Enguang Liu.; Caijuan Li.; Chenglong Pan.; Xuqian Zhao.; Yunfeng Wang.; Qufei Ling. Effects of heat stress on histopathology, antioxidant enzymes, and transcriptomic profiles in gills of pikeperch Sander lucioperca. Aquaculture **2021**, *534*, 736277. DOI 10.1016/j.aquaculture.2020.736277.
13. Fawzy I. Magouz.; Asem A. Amer.; Alaa Faisal.; Hani Sewilam.; Salama M. Aboelenin.; Mahmoud A.O. Dawood. The effects of dietary oregano essential oil on the growth performance, intestinal health, immune, and antioxidative responses of Nile tilapia under acute heat stress. *Aquaculture* **2021**, *548*, 737632. DOI 10.1016/j.aquaculture.2021.737632.
14. João Neves.; Eva Giacomello.; Gui M. Menezes.; Jorge Fontes.; Susanne E. Tanner. Temperature-Driven Growth Variation in a Deep-Sea Fish: The Case of Pagellus bogaraveo (Brünnich, 1768) in the Azores Archipelago. ORIGINAL RESEARCH 2021, 703820. DOI 10.3389/fmars.2021.703820.

15. Morten Omholt Alver.; Martin Føre.; Jo Arve Alfredsen. Predicting oxygen levels in Atlantic salmon (Salmo salar) sea cages. *Aquaculture* **2021**, *548*, 737720. DOI 10.1016/j.aquaculture.2021.737720.
16. Farzaneh Vakili.; Zahra Roosta.; Seyed Hossein Hoseinifar.; Arash Akbarzadeh. Effects of thermal stress and hypoxia on skin mucus immune and stress responses in blue gourami (Trichogaster trichopterus) cultured in intensive recirculation aquaculture system and semi-intensive systems. *Aquaculture Research* **2021**, *52*, 6581-6590. DOI 10.1111/are.15529.
17. Hong-Bo Lv.; Ying-ying Ma.; Chun-Ting Hu.; Qiu-Yu Lin.; Jun-jia-yu Yue.; Li-Qiao Chen.; Mei-Ling Zhang.; Zhen-Yu Du.; Fang Qiao. The individual and combined effects of hypoxia and high-fat diet feeding on nutrient composition and flesh quality in Nile tilapia (Oreochromis niloticus). Food Chemistry 2021, 343, 128479. DOI 10.1016/j.foodchem.2020.128479.
18. Mohamed El-S. Salem.; Heba Mohamed Abdel-Ghany.; Hebatollah M. Almisherfi. Role of dietary Laurencia obtusa in enhancing growth, blood indices, and hypoxia resistance of red tilapia (Oreochromis niloticus x O. mossambicus). *Journal of Applied Phycology* **2021**, *33*, 2617–2628. DOI 10.1007/s10811-021-02484-2.
19. Claudio A. Álvarez.; Ismael Jerez-Cepa.; Claudia B. Cárcamo.; Pedro Toledo.; Héctor Flores.; Katherina Brokordt. Growth performance, physiological responses to hypoxia and flesh quality of Chilean croaker (Cilus gilberti) stocked at different densities. *Aquaculture* **2020**, *525*, 735316. DOI 10.1016/j.aquaculture.2020.735316.
20. Jinghua Li.; Kai Huang.; Lida Huang.; Yanyu Hua.; Kai Yu.; Ting Liu. Effects of dissolved oxygen on the growth performance, haematological parameters, antioxidant responses and apoptosis of juvenile GIFT (Oreochromis niloticus). *Aquaculture Research* **2020**, *51*, 3079–3090. DOI 10.1111/are.14684.
21. Brittney G. Borowiec.; Graham R. Scott. Rapid and reversible modulation of blood haemoglobin content during diel cycles of hypoxia in killifish (Fundulus heteroclitus). *Comparative Biochemistry and Physiology, Part A* **2021**, *261*, 111054. DOI 10.1016/j.cbpa.2021.111054.
22. Daiani Kochhann.; Carolina G. Sarmento.; Jomara C. de Oliveira.; Helder L. Queiroz.; Adalberto L. Val.; Lauren J. Chapman. Take time to look at the fish: Behavioral response to acute thermal challenge in two Amazonian cichlids. *J. Exp. Zool* **2021**, *335*, 735-744. DOI 10.1002/jez.2541.
23. Neal J.Dawson.; Caroline Millet.; Colin Selman.; Neil B. Metcalfe. Measurement of mitochondrial respiration in permeabilized fish gills. Journal of Experimental Biology **2022**, *4*, 223. DOI 10.1242/jeb.216762.
24. Tingting Zhao.; Aijun Ma.; Shuangshuang Yang.; Zhihui Huang. Integrated metabolome and transcriptome analyses revealing the effects of thermal stress on lipid metabolism in juvenile turbot Scophthalmus maximus. *Journal of Thermal Biology* **2021**, *99*, 102937. DOI 10.1016/j.jtherbio.2021.102937.
25. Conall McNicholl.; François Oberhaensli.; Angus Taylor.; Isak Holmerin.; Peter W. Swarzenski.; Marc Metian. Deoxygenation reduces growth rates and increases assimilation of essential trace metals in gilthead seabream (Sparus aurata). *Environmental Pollution* **2021**, *288*, 117786. DOI 10.1016/j.envpol.2021.117786.
26. Miao Dong.; Yimin Liang.; Rajkumar Ramalingam.; Sze Wing Tang.; Wei Shen.; Rui Ye.; Singaram Gopalakrishnan.; Doris Wai Ting Au.; Yun Wah Lam. Proteomic characterization of the interactions between fish serum proteins and waterborne bacteria reveals the suppression of anti-oxidative defense as a serum-mediated antimicrobial mechanism. *Fish & Shellfish Immunology* **2017**, *62*, 96-106. DOI 10.1016/j.fsi.2017.01.013.
27. Na Li.; Hua-Qiong Huang.; Gen-Sheng Zhang.; Wei Cui. Effect of 5- AZn-2 '-deoxycytidine on proliferation of human lung adenocarcinoma cell line A549 in vitro. *Asian Pacific Journal of Tropical Medicine* **2013**, 982-985. DOI 10.1016/S1995-7645(13)60176-5.
28. Milad Adel.; Amir Hossein Omidi.; Mahmoud A.O. Dawood.; Behnaz Karimi.; Seyed Pezhman Hosseini Shekarabi. Dietary Gracilaria persica mediated the growth performance, fillet colouration, and immune response of Persian sturgeon (Acipenser persicus). *Aquaculture* **2021**, *530*, 735950. DOI 10.1016/j.aquaculture.2020.735950.
29. Cuifeng Jiang.; Cong Zhang.; Juan Song.; Xiaojie Ji.; Wei Wang. Cytidine-gold nanoclusters as peroxidase mimetic for colorimetric detection of glutathione (GSH), glutathione disulfifide (GSSG) and glutathione reductase (GR). *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **2021**, *250*, 119316. DOI 10.1016/j.saa.2020.119316.
30. Małgorzata Witeska.; Elzbieta Kondera.; Katarzyna Ługowska.; Bartosz Bojarski. Hematological methods in fish – Not only for beginners. *Aquaculture* **2022**, *547*,737498. DOI 10.1016/j.aquaculture.2021.737498.
31. Fatma Ahmed.; , Faiza M. Soliman.; Mohamed A. Adly.; Hamdy A. M. Soliman.; Mansour El-Matbouli.; Mona Saleh. Dietary Chitosan Nanoparticles: Potential Role in Modulation of Rainbow Trout (Oncorhynchus mykiss) Antibacterial Defense and Intestinal Immunity against Enteric Redmouth Disease. *Mar. Drugs* **2021**, *19*, 72. DOI 10.3390/md19020072.
32. Miguel L. Grilo.; Ana Pereira.; Carla Sousa-Santos.; Joana I. Robalo.; Manuela Oliveira. Climatic Alterations Influence Bacterial Growth, Biofilm Production and Antimicrobial Resistance Profiles in Aeromonas spp. *Antibiotics* **2021**, *10*, 1008. DOI 10.3390/antibiotics10081008.
33. Daniel J. Ritchie.; Christopher R. Friesen. Invited review: Thermal effects on oxidative stress in vertebrate ectotherms. *Comparative Biochemistry and Physiology, Part A* **2022**, *263*, 111082. DOI 10.1016/j.cbpa.2021.111082.
34. Bing Liu.; Youling L. Xiong.; Jiang Jiang.; Dongyou Yu.; Gang Lin. Cellular antioxidant mechanism of selenium-enriched yeast diets in the protection of meat quality of heat-stressed hens. *Food Bioscience* **2021**, *39*, 100798. DOI 10.1016/j.fbio.2020.100798.
35. Ponma Kuzhiyil Aswathy.; Chokki Veetil Priyatha.; John Nikhil.; Kumari Chidambaran Chitra. Triclosan at environmental concentration alters the hepatic antioxidant defence system in the fish, Anabas testudineus (Bloch, 1792). *Aquaculture Research*. **2021**, *52*, 6641–6655. DOI 10.1111/are.15534.
36. Matheus D. Baldissera.; Carine F. Souza.; Carla Cristina Zeppenfeld.; Maiara C. Velho.; Bruna Klein.; Lorenzo B. Abbad.; Aline F. Ourique.; Roger Wagner.; Aleksandro S. Da Silva.; Bernardo Baldisserotto. Dietary supplementation with

nerolidol nanospheres improves growth, antioxidant status and fillet fatty acid profiles in Nile tilapia: Benefits of nanotechnology for fish health and meat quality. *Aquaculture* **2020**, *516*, 734635. DOI 10.1016/j.aquaculture.2019.734635.

37. Matheus D. Baldissera.; Carine F. Souza.; Sharine N. Descovi.; Tiago G. Petrolli.; Aleksandro S. da Silva.; Bernardo Baldisserotto. A caffeine-supplemented diet modulates oxidative stress markers and prevents oxidative damage in the livers of Nile tilapia (Oreochromis niloticus) exposed to hypoxia. *Fish Physiol Biochem* **2019**, *45*, 1041–1049. DOI 10.1007/s10695-019-00616-7.
38. Kai Yang.; Qixue Fan.; Lei Zhang.; Bo Li.; Yinai Gao.; Kewei Zeng.; Qingyun Wang.; Sihua Zhu.; Gang Fang. Effect of dissolved oxygen levels on growth performance, energy budget and antioxidant responses of yellow catfish, Pelteobagrus fulvidraco (Richardson). *Aquaculture Research* **2015**, *46*, 2025–2033. DOI 10.1111/are.12359.
39. Danilo da Costa Barroso.; Vera Maria Fonseca Almeida-Val.; Adalberto Luis Val. Temperature and food availability alters the physiology and aerobic capacity of tambaqui (Colossoma macropomum). *Comparative Biochemistry and Physiology, Part A* **2020**, *245*, 110704. DOI 10.1016/j.cbpa.2020.110704.
40. Zhilu Zhang.; Ke Lu.; Xu-Fang Liang.; Jiao Li.; Shulin Tang.; Yanpeng Zhang.; Wenjing Cai.; Qiaoqiao Xiao.; Qiwei Zhang. Effects of early low temperature exposure on the growth, glycolipid metabolism and growth hormone (gh) gene methylation in the late stage of Chinese perch (Siniperca chuatsi). *Comparative Biochemistry and Physiology, Part B* **2022**, *259*, 110705. DOI 10.1016/j.cbpb.2021.110705.
41. Muhammad A.B. Siddik.; Md Javed Foysal.; Ravi Fotedar.; David S. Francis.; Sanjay K. Gupta . Probiotic yeast Saccharomyces cerevisiae coupled with Lactobacillus casei modulates physiological performance and promotes gut microbiota in juvenile barramundi, Lates calcarifer. *Aquaculture* **2022**, *546*, 737346. DOI 10.1016/j.aquaculture.2021.737346.
42. Ying-Ying Wang.; Yu Zhou.; Huang-Cui Fu.; He-Zhong Huang.; Ze Li.; Rui-Ming Jin.; Xiao-Zhe Fu.;Ning-Qiu Li. Transcriptomic and proteomic analyses of the immune mechanism in pathogenetic and resistant mandarin fish (Siniperca chuatsi) infected with ISKNV. *Aquaculture* **2021**, *545*, 737198. DOI 10.1016/j.aquaculture.2021.737198.
43. Shannon G. Moorhead.; Austin J. Gallagher.; Liza Merly.; Neil Hammerschlag. Variation of body condition and plasma energy substrates with life stage, sex, and season in wild-sampled nurse sharks Ginglymostoma cirratum. *J Fish Biol* **2021**, 98, 680–693. DOI 10.1111/jfb.14612.
44. Daniel F. Gomez Isaza.; Rebecca L. Cramp.; Craig E. Franklin. Exposure to Nitrate Increases Susceptibility to Hypoxia in Fish. *Physiological and Biochemical Zoology* **2021**, *94*, 124–142. DOI 10.1086/713252.
45. Brittney G. Borowiec.; Graham R. Scott. Rapid and reversible modulation of blood haemoglobin content during diel cycles of hypoxia in killifish (Fundulus heteroclitus). *Comparative Biochemistry and Physiology, Part A* **2021**, *261*, 111054. DOI 10.1016/j.cbpa.2021.111054.
46. Gail D. Schwieterman.; Jodie L. Rummer.; Ian A. Bouyoucos.; Peter G. Bushnell.; Richard W. Brill. A lack of red blood cell swelling in five elasmobranch fishes following air exposure and exhaustive exercise. *Comparative Biochemistry and Physiology, Part A* **2021**, *258*, 110978. DOI 10.1016/j.cbpa.2021.110978.
47. Justyna Sikorska.; Elżbieta Kondera.; Rafał Kamiński.; Katarzyna Ługowska.; Małgorzata Witeska.; Jacek Wolnicki. Effect of four rearing water temperatures on some performance parameters of larval and juvenile crucian carp, Carassius carassius, under controlled conditions. *Aquaculture Research* **2018**, *49*, 3874–3880. DOI 10.1111/are.13855.
48. Anne Beemelmanns.; Fábio S. Zanuzzo.; Xi Xue.; Rebeccah M. Sandrelli.;Matthew L. Rise.; A. Kurt Gamperl. The transcriptomic responses of Atlantic salmon (Salmo salar) to high temperature stress alone, and in combination with moderate hypoxia. *BMC Genomics* **2021**, *22*, 261. DOI 10.1186/s12864-021-07464-x.
49. Truong Quoc Phu.; Bui Thi Bich Hang.; Dang Diem Tuong.; Vila-Gispert Anna.; Toyoji Kaneko.; Nguyen Thanh Phuong.; Do Thi Thanh Huong. Effects of size and nitrite exposure on respiration, oxygen partitioning, and growth of obligate air-breathing fsh Channa striata. *Fisheries Science* **2022**, *88*, 149–159. DOI 10.1007/s12562-021-01562-1.
50. Dariusz P. Fey.; Martyna Greszkiewicz. Effects of temperature on somatic growth, otolith growth, and uncoupling in the otolith to fish size relationship of larval northern pike, Esox lucius L. *Fisheries Research* **2021**, *236*, 105843. DOI 10.1016/j.fishres.2020.105843.

Figure 1

Schematic diagram of oxygen regulation system

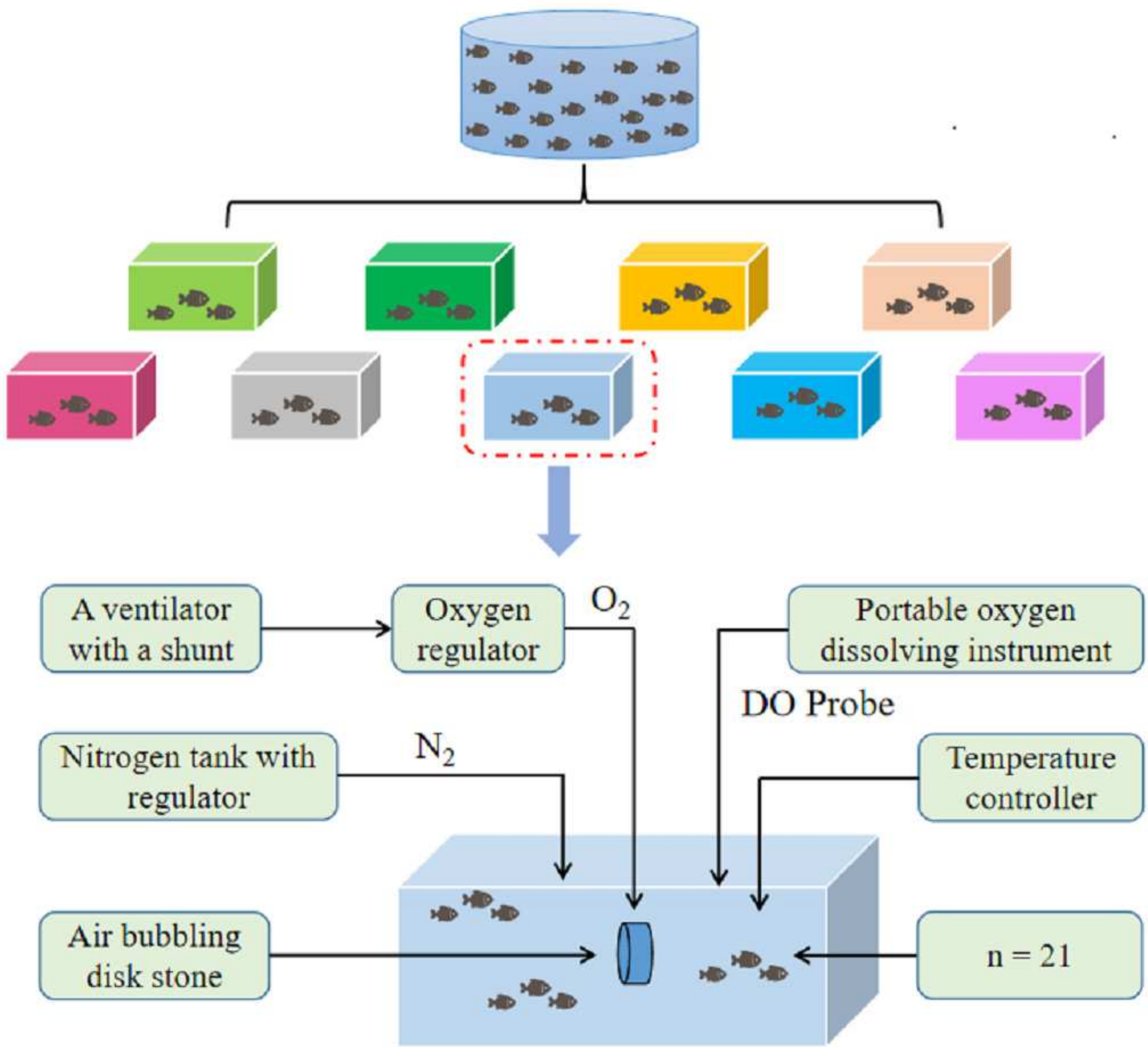

Figure 2

The growth rates of Aeromonas in crucian carp serum were compared by Student's t-test under the interaction conditions of three temperatures (18℃, 24℃ and 30℃) and three oxygen levels (2.1mgL $^{-1}$, 5.4mgL $^{-1}$ and 9.3mgL $^{-1}$).

Values are expressed as mean ± SD of triplicate pooled data for each treatment. Means of different letters in the same row were significantly different (*P* < 0.05).

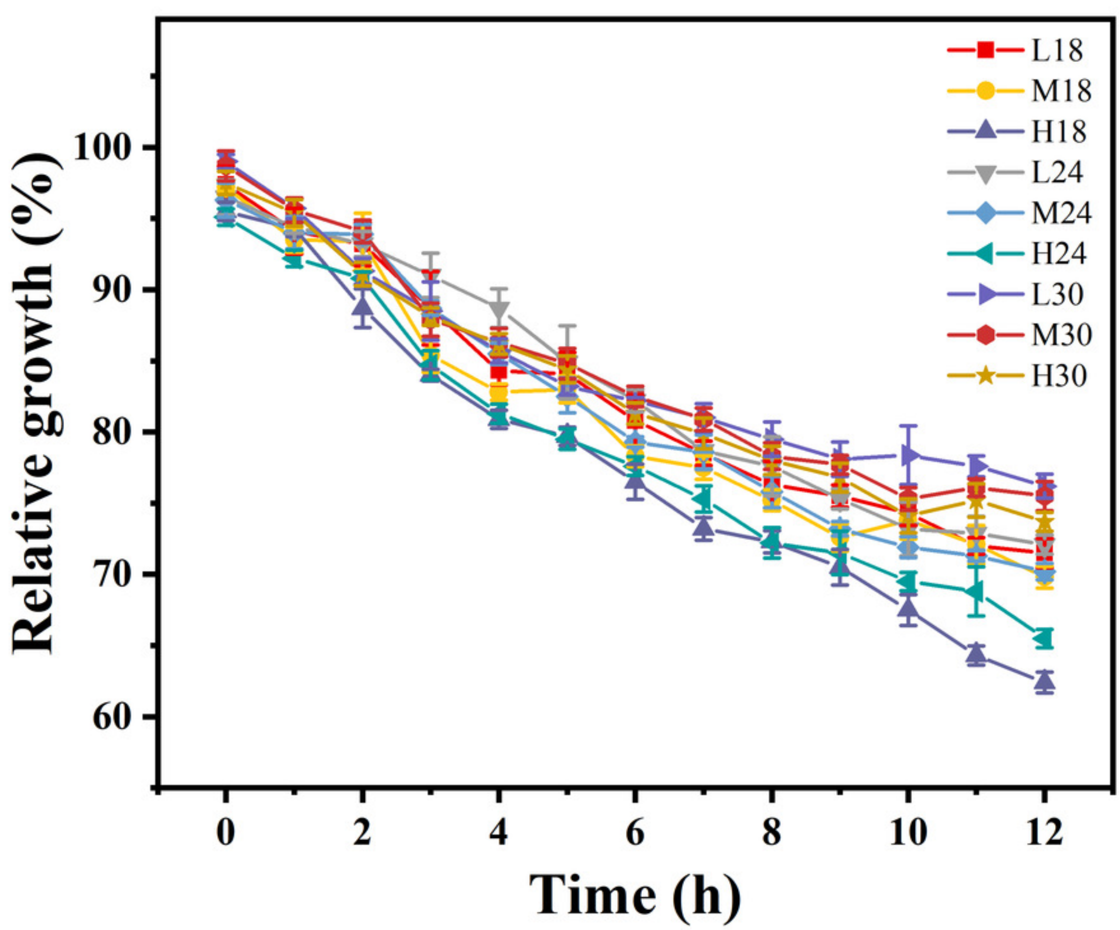

Figure 3

The Student’s t-test was used to detect the changes of serum antioxidant enzyme activities of crucian carp at different temperatures and oxygen levels. (n = 9)

Capital letters represent significant differences under different oxygen conditions at the same temperature ($P<0.05$). Lowercase letters represent significant differences under the same oxygen and different temperature conditions ($P<0.05$). Values are expressed as mean ± SD of triplicate pooled data for each treatment.L represents hypoxic conditions; M represents medium oxygen conditions; H represents hyperoxic conditions.Figure a represents CAT, figure b represents GPx, and figure c represents SOD.

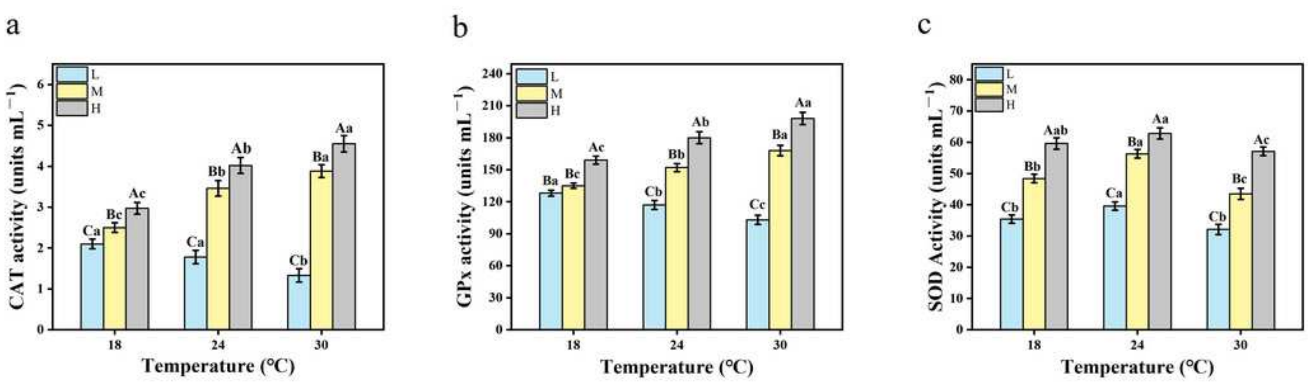

Figure 4

The Student's t-test was used to detect the changes of serum biochemical parameters of crucian carp at different temperatures and oxygen levels. (n = 9)

Capital letters represent significant differences under different oxygen conditions at the same temperature (P<0.05). Lowercase letters represent significant differences under the same oxygen and different temperature conditions (P<0.05). Values are expressed as mean ± SD of triplicate pooled data for each treatment.L represents hypoxic conditions; M represents medium oxygen conditions; H represents hyperoxic conditions. TP: Total protein, TG: Triglycerides. Figure a represents Glucose, figure b represents TP, and figure c represents TG.

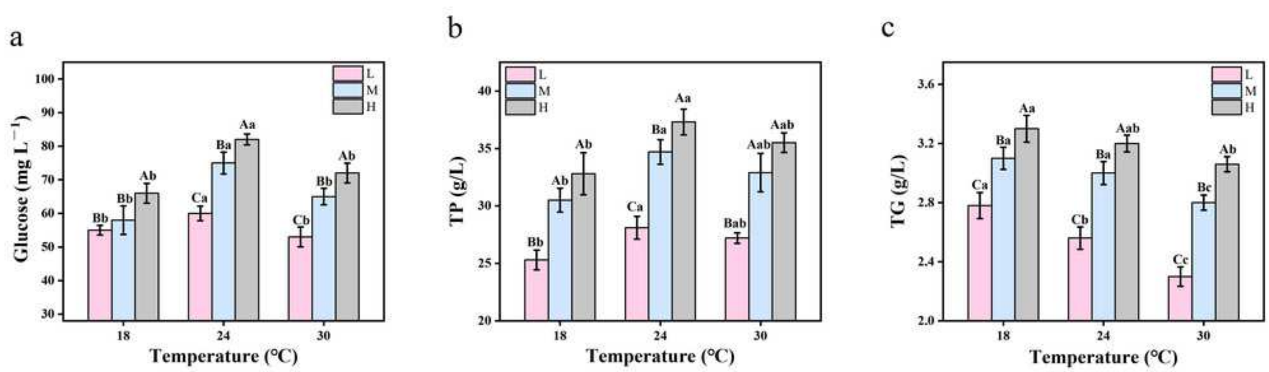

Figure 5

The Student’s t-test was used to detect the changes of hematological variables in crucian carp at different temperatures and oxygen levels. (n = 9)

Different capital letters indicate significant differences (P<0.05) under different oxygen conditions at the same temperature. Different lowercase letters indicate significant differences in the same oxygen at different temperatures (P<0.05). Values are expressed as mean ± SD of triplicate pooled data for each treatment. L represents hypoxic conditions; M represents medium oxygen conditions; H represents hyperoxic conditions. HCT: Hematocrit, MCH: Mean hemoglobin content, HGB: Hemoglobin. Figure a represents HCT, figure b represents MCH, and figure c represents HGB.

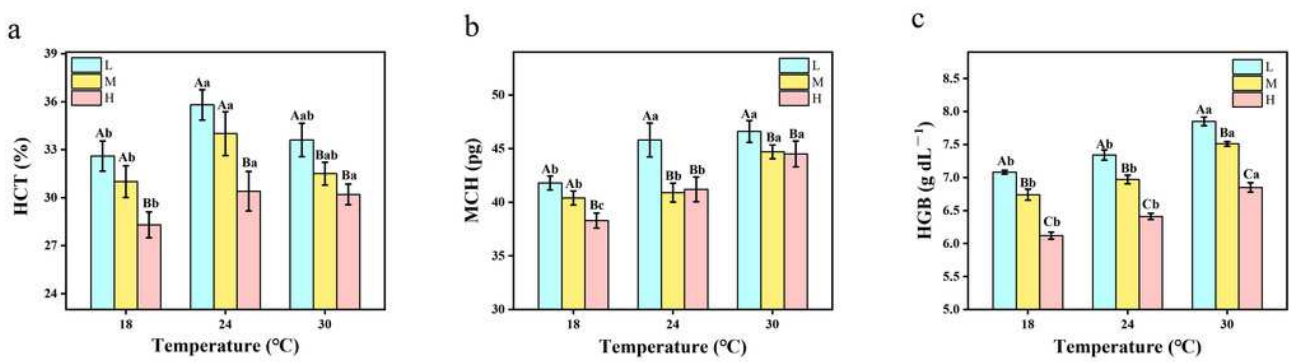

Table 1(on next page)

Summary of the Student's t-test results of Growth performance of crucian carp under different interaction treatments.

Values are expressed as mean ± SD of triplicate pooled data for each treatment. Means in the same column with different letters are significantly different ($P < 0.05$).

Treatment	IW (g)	FW (g)	SGR (%)	WGR (%)	SR (%)
L18	112.05±2.13	116.10±2.22Bb	0.12±0.04Cb	3.62±1.13Cb	80.95±6.73
M18	112.43±2.48	124.10±2.81Bb	0.33±0.08Bb	10.39±2.69Bb	95.24±6.73
H18	112.86±2.23	134.60±3.10Ab	0.59±0.08Ab	19.25±2.95Ab	100.00±0.00
L24	109.20±3.10	125.05±3.20Ca	0.45±0.13Ca	14.61±4.58Ca	76.19±6.73
M24	110.62±2.72	133.52±3.81Ba	0.63±0.10Ba	20.74±3.76Ba	95.24±6.73
H24	111.48±2.97	136.71±2.51Aa	0.68±0.13Aa	22.72±4.71Aa	100.00±0.00
L30	104.20±2.91	105.86±2.96Cc	0.05±0.02Cc	1.59±0.77Cc	71.43±0.00
M30	105.43±2.84	114.40±2.75Bc	0.27±0.07Bc	8.52±2.34Bc	90.48±6.73
H30	109.90±3.01	127.38±2.44Ac	0.49±0.09Ac	15.90±3.43Ac	95.24±6.73