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

The well-being of fish used in aquaculture is of great interest. Oxygen and temperature are the main factors affecting the welfare of the crucian carp; however, there are few studies on the combined effects of these on the species. Therefore, this study investigated the impact of different temperatures (18 ℃, 24 ℃, 30 ℃) and oxygen concentrations (2.1 mgL^{-1}, 5.4 mgL^{-1}, 9.3 mgL^{-1}) on serum antibacterial activity, antioxidant activity, hematological parameters and growth performance of the crucian carp. The results showed that there were greater antibacterial properties under conditions of hypoxia at 18 °C (L18) and hyperoxia at 24 °C (H24). The activities of catalase, glutathione peroxidase and total superoxide dismutase were the highest at 24 °C under hypoxia and hyperoxia. In addition, the contents of glucose and total protein first increased and then decreased with the change of temperature; triglycerides were the lowest at 30 ℃. The blood parameters of the carp were within a normal range at 24 °C; however, the growth rate was at its lowest under hypoxia treatment at 30 ℃ (L30). This study showed that high temperature impairs the antibacterial ability, antioxidant capacity and growth performance of the crucian carp, and high oxygen levels can alleviate these adverse reactions. This research provides a theoretical basis for subsequent aquaculture studies.

1 *Article*

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8 Bin Wang[1], Hanping Mao[1,*], Jian Zhao[2], Yong Liu[1], Yafei Wang[1] and Xiaoxue Du[1]
9 [1] School of Agricultural Engineering, Jiangsu University, Zhenjiang 212013, China;
10 2112016004@stmail.ujs.edu.cn (B.W.); 2111816007@stmail.ujs.edu.cn (Y.L.);
11 2111916018@stmail.ujs.edu.cn (Y.W.); 2111916004@stmail.ujs.edu.cn (X.D.);
12 [2] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou,
13 310000, People's Republic of China.
14 zhaojzju@zju.edu.cn(J.Z.);

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

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21 Abstract
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Introduction

In many European countries, the survival of the crucian carp has been affected by the invasion of exotic species such as silver carp and goldfish [1] and it is considered to be a threatened species [2]. The species grows rapidly in size, however, it suffers from many environmental challenges since its growth is related to temperature and oxygen conditions [3,4,5].

Temperature plays a key role in the physiological state and ecosystem function of fish, and maintaining the appropriate temperature is important for aquaculture management [6-10]. Enzyme activity, metabolic rate, and immunoglobulin levels can be affected by sudden changes in temperature, thereby affecting fish survival [11-13]. Temperature conditions that are not ideal can inhibit growth, decrease antibacterial ability, disrupt the physiological balance, and may even cause death [1,14]. Oxygen is an indispensable element for every aquatic animal to maintain metabolic activity [15], and it is also one of the crucial limiting factors in aquaculture production [16,17]. The growth parameters, hematological parameters, and apoptotic indices of fish are reduced by hypoxic conditions [18,19, 20]. Fish can be subjected to a certain degree of oxidative damage by hypoxia, which impacts growth and serum biochemical indicators, subsequently affecting the metabolism and immune system of the fish [21].

There is a negative correlation between the temperature of the water and the oxygen content [4] and some fish may exhibit agitation or avoidance behavior until the water temperature becomes tolerable. The functional activities of the organs and tissues in the fish are altered with the temperature variations so that the fish body can maintain a balanced state as much as possible under higher temperatures [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 increases with higher temperatures [24], and the growth rate of fish can be reduced by low oxygen conditions [25]. Studies have also shown that hyperoxia conditions can alleviate the heat stress response of fish to a certain extent. The effects of temperature and oxygen alone on the crucian carp have been extensively studied and discussed, however, few experts have studied the effect of the interaction of the two factors on the crucian carp.

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

Materials & Methods

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 active crucian carp were put into five 400 L circulating water tanks for one 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±0.1 mgL^{-1}. pH was 7.1±0.1, nitrite was 0-0.12 mg/L, and ammonia nitrogen was 0-0.12 mg/L. The water quality parameters were determined by a water quality detection device (Shanghai Lanchang Automation Technology Co., Ltd., Shanghai, China). Fish were fed commercial pellets (protein 35%, fat 5%, ash 15%, and water 12.5%) twice daily (8:30 and

83 17:30) (Shandong Binzhou Ruixing Biological Technology Co., Ltd., Shandong, China). Fish
84 were fed 5% of the body weight of the shoal. Debris and manure were cleaned and the water was
85 replaced daily with 60% fresh water.
86 *Trial design*
87 The experimental crucian carp (initial body weight 109.82±2.16 g) chosen from the
88 circulating water tanks were randomly distributed in nine circulating water tanks, with 21 fish in
89 each tank. The size of the tanks were 150 cm × 100 cm × 100 cm. The fish were reared for one
90 week before the experiment. A two-factor, three-level experiment was designed, including
91 temperatures of 18 ℃, 24 ℃, and 30 ℃, and oxygen concentrations of 2.1 mgL^{-1}, 5.4 mgL^{-1}, and
92 9.3 mgL^{-1} with three replicates for each treatment. Each tank was divided into three feeding
93 spaces of the same size using two partitions, and there were seven experimental crucian carp in
94 each space. A temperature controller (Guangdong Foshan Lvban Electronic Commerce Co., Ltd.,
95 Guangdong, China) was used to gradually change the water temperature from 17 ℃ to 18℃, 24
96 ℃, and 30 ℃ at a rate of 1 ℃ per hour. In this experiment, hyperoxia conditions were created by
97 increasing the oxygen content, and hypoxic conditions were created by increasing the nitrogen
98 content. Results were analyzed in each trial at 18 ℃ and the hypoxia group was used as a control
99 group. The schematic diagram of the oxygen regulation system is shown in Figure 1, and
100 includes an oxygenator, a nitrogen container, a water tank, and a portable dissolved oxygen
101 meter. The trials ran from December 19, 2021 to January 19, 2022, for a total of 30 days.

102 *Antibacterial experiment*
103 The proliferation ability of bacterial isolates in crucian carp serum was determined using a
104 96-well plate. Specifically, freshwater bacteria (*Aeromonas*) were utilized to determine the
105 antibacterial capacity in serum samples. *Aeromonas* generally grew normally on agar plates at 25
106 ℃. Fresh single colonies were picked and diluted in 5 mL of nutrient broth and incubated for 12
107 hours in an orbital incubator at 28 ℃ with the speed set at 150-200 rpm. Then, the serum
108 antibacterial ability was tested [26]. Simultaneous serum samples and bacterial suspensions were
109 serially diluted with PBS, twice. Furthermore, 100 μL of bacterial diluent and the same volume
110 of crucian carp serum were added into a 96-well plate. A total of 100 μL of crucian carp serum
111 and 100 μL of nutrient solution were added to the negative wells. A total of 100 μL of bacterial
112 suspension and 100 μL of nutrient broth were added to the positive wells. This experiment was
113 repeated three times. The 96-well plates were incubated at 28 ℃. Then, the OD value at 600 nm
114 was measured by a microplate reader with intervals of 1 hour, for a total of 12 hours measured.
115 The determination of bacterial viability was determined by the ratio between the number of
116 viable bacteria and positive control bacteria, and the results were showed as a percentage
117 (100%).

118 *Sampling procedures*
119 Food was withheld from the experimental crucian carp for 24 hours before sampling, and
120 three experimental fish were randomly selected from each independent area. Each fish was
121 individually sampled and stored. All sampling was performed after MS-222 anesthesia. Blood
122 was drawn from the caudal vessel using a heparinized syringe. One part of the sample was used
123 for the measurement of blood parameters, and the other part was centrifuged at 1,350 × g for 10
124 min, and the serum was obtained after coagulation at 4 ℃ for 5 h. The serum was stored at -20 ℃
125 for antibacterial, antioxidant, and biochemical tests.

126 *Physiological indicators*

127   Three crucian carp were randomly selected from each experimental water tank for
128   physiological and biochemical tests, and the results were expressed as mean ± SD. The data were
129   sorted and analyzed. The total superoxide dismutase (SOD), catalase (CAT), and glutathione
130   peroxidase (GPx) activities were measured with a microplate reader using a SOD assay kit
131   (WST-1 method), a CAT visible light kit, and a GSH-PX assay kit, respectively. The total
132   superoxide dismutase activity was determined by the hydroxylamine method at 550 nm [27].
133   Catalase activity was measured at 405 nm by the ammonium molybdate method [28].
134   Glutathione peroxidase activity was measured colorimetrically at 412 nm [29]. The kit was
135   purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Blood
136   biochemical parameters (glucose, triglycerides, and total protein) were determined with a Cobas
137   C-311 automatic biochemical analyzer and commercial assay kits. Hematocrit (HCT), mean
138   hemoglobin content (MCH), and hemoglobin (HGB) were detected with a BM830 automatic
139   hematology analyzer.

140   *Growth performance*

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

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

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

141   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
142   of fish.

143   *Statistical analyses*

144   Data analysis was performed using GraphPad Prism 5.0 software (GraphPad Software Inc.,
145   USA). Differences between groups were calculated by multiple comparison test by a two-way
146   ANOVA. $P < 0.05$ indicate a statistical significance. Results were expressed as the mean ± SD (n
147   = 9).

148   **Results**

149   *Antibacterial experiment*
150   We analyzed bacterial growth in the serum sampled from the crucian carp under nine
151   treatments. *Aeromonas* is a general pathogen of aquatic animals. Figure 2 shows a decrease in
152   the relative growth rates of *Aeromonas* in all nine treatment groups over time. Under the same
153   oxygen conditions, the relative growth rate of *Aeromonas* increased with a rise in temperature.
154   The relative growth rate of *Aeromonas* slowed with increased oxygen concentrations. The
155   relative growth rate of the H24 and H18 treatments were low, and the antibacterial effect of
156   serum improved. The relative growth rate of the L30 and L18 treatments were higher, and the
157   serum antibacterial effect was poorer.
158   *Serum antioxidant test*
159   To explore the antioxidant capacity of the crucian carp, the activities of three antioxidant
160   enzymes were measured in the serum sampled from nine treatment groups. As shown in Figure
161   3A-C, the activities of CAT, GPx, and SOD in the serum of the crucian carp were significantly
162   enhanced with the increase of oxygen concentration ($P < 0.0001$) under the same temperature
163   conditions. Compared with 18 ℃, the serum CAT activity at 24 ℃ and 30 ℃ decreased by
164   15.24% and 36.67% under hypoxic conditions. The serum CAT activity increased by 38.40%
165   and 55.20% at 24 ℃ and 30 ℃ when exposed to medium levels of oxygen. When exposed to
166   high oxygen, serum CAT activity increased by 41.41% and 53.20% at 24 ℃ and 30 ℃, as shown

167 in Figure 3A. Figure 3B shows that under hypoxia, the serum GPx activity of the crucian carp
168 under 24 ℃ and 30 ℃ decreased by 4.76% and 21.43%, respectively, compared with 18 ℃.
169 Under medium oxygen conditions, the serum GPx activity of the crucian carp at 24 ℃ and 30 ℃
170 increased by 11.85% and 24.44%, respectively, compared with 18℃. Under high oxygen
171 conditions, the serum GPx activity at 24 ℃ and 30 ℃ increased by 16.46% and 25.32%,
172 respectively, compared with 18 ℃. Figure 3C shows that under hypoxic condition, the serum
173 SOD activity of the crucian carp increased by 11.86% and decreased by 9.32% at 24 ℃ and 30
174 ℃, compared with 18 ℃. Under medium oxygen conditions, the serum SOD activity increased
175 by 16.32% and 10.10% at 24 ℃ and 30 ℃, compared with 18 ℃. Under high oxygen conditions,
176 the SOD activity of the serum increased by 5.37% and decreased by 4.19% at 24 ℃ and 30 ℃,
177 compared with 18 ℃.

178 *Serum biochemical experiment*

179   Figure 4A shows that with the increase of oxygen concentration, glucose levels increased
180 significantly under the three temperature conditions. At the same oxygen concentration, the
181 glucose level of the crucian carp increased and then decreased with the increase of temperature,
182 reaching the highest levels at 24 ℃ ($P < 0.05$). Figure 4B shows the change of total protein
183 content in crucian carp serum. Under the same oxygen concentrations, the total protein content at
184 24 ℃ was higher than that at 18 ℃ and 30 ℃. The total protein content under hypoxic conditions
185 was lower than that under medium oxygen and hyperoxia conditions ($P < 0.0001$). Changes in
186 the serum triglyceride content of crucian carp are shown in Figure 4C. At the same temperature,
187 with the increase of oxygen concentration, hyperoxia produced greater triglyceride contents than
188 medium oxygen and hypoxic conditions. When oxygen concentrations were the same, the
189 triglyceride content decreased with increasing temperatures ($P < 0.05$).

190 *Hematological analysis*

191   The differences in blood HCT, MCH and HGB levels are shown in Figure 5. As shown in
192 Figure 5A, HCT at 24 ℃ was significantly higher than that at 18 ℃ and 30 ℃ . At the same
193 temperature, the HCT of hypoxia was higher than that of hyperoxia and moderate oxygen ($P <$
194 0.01). Figure 5B shows that under the same oxygen concentration, MCH at 30 ℃ was
195 significantly higher than that at 18 ℃, and slightly higher than at 24 ℃. Under the same
196 temperature conditions, MCH under hypoxic conditions was significantly higher than that under
197 hyperoxia and medium oxygen condition ($P < 0.05$). As shown in Figure 5C, at the same
198 temperature, HGB under hypoxic conditions was higher than that under medium oxygen and
199 hyperoxia conditions. At the same oxygen concentration, HGB at 18 ℃ was slightly lower than
200 that at 24 ℃ and 30 ℃ ($P < 0.0001$).

201 *Growth performance*

202   The FW, SGR, WGR, and SR of crucian carp after different treatments are shown in Table
203 1. The FW of crucian carp was different between different treatments of temperature and oxygen
204 concentrations. Under hypoxic conditions, the FW of crucian carp in L30 was lower than that in
205 L18 and L24 ($P < 0.01$). Under medium oxygen conditions, the FW of the M30 crucian carp was
206 lower than that of M18 and M24. Under hyperoxia conditions, the FW of the H30 crucian carp
207 was significantly lower than that of the H18 and H24 treatments ($P < 0.05$). In addition, under
208 the same temperature conditions, the FW of crucian carp under hyperoxia was significantly
209 higher than that under hypoxia. According to the analysis results, H24 (136.71 ± 2.51 g) and L30
210 (105.86 ± 2.96 g) were the highest and lowest values of the FW. When the temperature remained

211 the same, the SGR of the crucian carp under hyperoxia was significantly higher than that under
212 mesoxia and hypoxia ($P < 0.01$). At static oxygen levels, the SGR of the crucian carp reached the
213 highest at 24 ℃ and the lowest at 21℃ ($P < 0.01$). The SGR of the crucian carp in the H24 and
214 M24 treatments were the highest, at 0.68% and 0.63%, respectively. When the oxygen
215 concentration was same, the WGR of crucian carp under 18 ℃ was lower than that at 24 ℃ and
216 30 ℃. When the temperature was same, the WGR of crucian carp under hyperoxia was
217 significantly higher than that under medium oxygen and hypoxia ($P < 0.0001$). The carp in H24
218 and M24 had the highest WGR. The SR of crucian carp was affected by temperature. When the
219 oxygen concentration was same, the SR was relatively high at 18 ℃ and 24 ℃, and the SR was
220 relatively low at 30 ℃. The SR was most affected by oxygen concentration, and the SR of H24
221 and H18 was 100%, respectively. The SR under L30 was the lowest, at 71.43%.

Discussion

223   Fish serum is considered to be one of the most commonly used methods to assess the
224 physiological state and health status of fish [30]. It contains a variety of immune molecules, such
225 as immune proteins, lysozyme, etc., with high antibacterial properties [31]. In the range of 18-30
226 ℃, when the oxygen concentration was the same, the enzyme activity gradually increased with
227 the increase of temperature. The antibacterial activity of the crucian carp serum also gradually
228 increased. However, the bacteriostatic effect of low temperature was better than that of moderate
229 and high temperatures, because the optimal growth temperature of *Aeromonas* is 22℃-28℃ [32].
230 At a static temperature, an increased oxygen concentration caused the relative growth rate of
231 *Aeromonas* to decrease. When the amount of oxygen increased, the respiration and metabolic
232 rate of the crucian carp increased and the immune system of the fish worked more efficiently, so
233 that the antibacterial ability was enhanced. Therefore, H18 and H24 were found to have the
234 strongest antibacterial properties.
235   The growth performance and metabolism of fish are affected not only by temperature and
236 oxygen, but also by their antioxidant defense system [33]. When the temperature and oxygen
237 content change, excessive oxygen free radicals are generated in the environment, and
238 macromolecules such as proteins and lipids in the fish are destroyed, thereby affecting their
239 health [34-36]. However, this oxygen radical can be eliminated by the antioxidant defense
240 system of fish, including CAT, GPx, and SOD [37]. Under anoxic conditions, the activities of
241 CAT and GPx in the crucian carp were slightly weakened at 18 ℃ - 30 ℃, the SOD activity was
242 slightly increased at 24 ℃, and decreased slightly at 30 ℃. This means that the crucian carp was
243 in relatively good condition. The activities of these two enzymes were significantly enhanced
244 under mesoxia and hyperoxia conditions. At the same temperature, the activities of the three
245 antioxidant enzymes were the highest in hyperoxia, followed by mesoxia, and lowest in hypoxia.
246 This indicates that the antioxidant system of crucian carp can be activated by conditions of
247 hyperoxia and mesoxia. However, the enzyme activity is extremely low under hypoxic
248 conditions, and the antioxidant capacity is the weakest [38]. The heat stress experienced by
249 crucian carp may also improve under hypoxic conditions.
250   Glucose is the main substance for cell and tissue metabolism and physiological activities.
251 Glucose levels in fish are affected by temperature and oxygen levels [39]. Low glucose
252 concentrations can affect the growth rate and appetite of fish [40]. The results revealed that the
253 L27-treated crucian carp had the lowest glucose concentrations, which resulted in decreased
254 appetite and slower growth. The main function of serum proteins is to repair damaged tissues and
255 participate in the regulation of plasma osmotic pressure [41]. Under the same oxygen
256 concentrations, the total protein activity increased at 24 °C and 30 °C, indicating that the body
257 tissue of crucian carp may have been damaged when the water temperature increased [42]. In
258 addition, the total protein content was lower under hypoxic conditions than under meso- and

259 hyperoxia conditions. Therefore, high concentration of oxygen may reduce the degree of tissue
260 damage and accelerate tissue recovery in crucian carp. Oxidized triglycerides are an energy
261 source for fish metabolism [43]. Compared with 18 ℃ and 24 ℃, the survival rate of crucian carp
262 at 30 ℃ was the lowest, indicating that crucian carp needs to consume a lot of triglycerides under
263 high temperature conditions. At the same temperature, there was less of a reduction of
264 triglycerides under hyperoxia conditions than that under mesoxia and hypoxia conditions. This
265 suggests that hyperoxia helps to alleviate the high temperature-induced triglyceride consumption
266 in crucian carp.
267   Studies have shown that hematocrit is one of the important blood parameters that reflect the
268 health of fish. At the same temperature, the hematocrit under hypoxic conditions was higher than
269 that under mesoxia and hyperoxia conditions [44]. Low oxygen concentrations cause the
270 hemoglobin and oxygen transport capacity in the body to increase and the body is able to
271 maintain normal physiological functions [45]. When the oxygen concentration is too high,
272 although the oxygen carrying capacity is strong, the blood viscosity increases, the blood flow
273 resistance increases, and the hematocrit decreases. Under the condition of constant oxygen
274 conditions, the body temperature increases and the metabolic capacity increases. At this time, the
275 content of hemoglobin increases, the hematocrit increases, and the physiological function of the
276 body can be regulated to a certain extent through this cycle [46]. Conversely, when the body
277 temperature is low, the utilization of oxygen may be affected, the synthesis of hemoglobin is
278 reduced, and the hematocrit is also reduced. The experimental results are consistent with this
279 conclusion.
280   The overall health of fish is directly reflected in their growth performance and survival rate.
281 Our test results are shown in Table 1. The survival rate of crucian carp under hyperoxia
282 conditions was significantly higher than that under mesoxia and hypoxia conditions. The growth
283 indexes SGR and WGR were the highest at 24 ℃, followed by 30 ℃, and the lowest at 18 ℃ for
284 the crucian carp. The optimal growth temperature was 25 °C, which is similar to the results of
285 previous studies [47]. When the ambient temperature was the same, the growth under hyperoxia
286 conditions was higher than that under mesoxia and hypoxia conditions. Therefore, growth may
287 be inhibited by hypoxia [48, 49]. It was also reported that the growth rate of the crucian carp
288 showed a trend of increasing first and then decreasing within a specific range [50]. The results
289 showed that the growth rate of the H24 crucian carp was the highest, and the growth rate of the
290 L30 carp was the lowest. In addition, when the temperature was set to 30 °C, the growth rate
291 under hyperoxia conditions was higher than that of mesoxia and hypoxic conditions, suggesting
292 that hyperoxia may protect the crucian carp from the adverse effects of high temperature.

Conclusions

294   Crucian carp had better growth performance under hyperoxia (9.3 mgL^{-1}) than under
295 medium oxygen (5.4 mgL^{-1}) or hypoxic (2.1 mgL^{-1}) conditions. High temperature and hypoxia
296 stress may reduce the antibacterial properties of crucian carp, and various physiological
297 indicators may become disordered as a result. However, these adverse effects can be mitigated to
298 some extent by increasing the oxygen concentration. Therefore, according to the changing trends
299 of various parameters in the nine treatment groups, the optimal growth environment for crucian
300 carp was the H24 treatment. These results indicate that under high oxygen conditions, the water
301 temperature was best kept at 24 ℃.
302   This study was limited by a small sample size, which may have affected the accuracy of the
303 conclusions. Therefore, future studies with a larger sample size are required for further
304 verification.

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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 8ucius 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.

366 20. Jinghua Li.; Kai Huang.; Lida Huang.; Yanyu Hua.; Kai Yu.; Ting Liu. Effects of dissolved oxygen on the growth
367   performance, haematological parameters, antioxidant responses and apoptosis of juvenile GIFT (Oreochromis niloticus).
368   *Aquaculture Research* **2020**, *51*, 3079–3090. DOI 10.1111/are.14684.
369 21. Brittney G. Borowiec.; Graham R. Scott. Rapid and reversible modulation of blood haemoglobin content during diel cycles
370   of hypoxia in killifish (Fundulus heteroclitus). *Comparative Biochemistry and Physiology, Part A* **2021**, *261*, 111054. DOI
371   10.1016/j.cbpa.2021.111054.
372 22. Daiani Kochhann.; Carolina G. Sarmento.; Jomara C. de Oliveira.; Helder L. Queiroz.; Adalberto L. Val.; Lauren J.
373   Chapman. Take time to look at the fish: Behavioral response to acute thermal challenge in two Amazonian cichlids. *J. Exp.*
374   *Zool* **2021**, *335*, 735-744. DOI 10.1002/jez.2541.
375 23. Neal J.Dawson.; Caroline Millet.; Colin Selman.; Neil B. Metcalfe. Measurement of mitochondrial respiration in
376   permeabilized fish gills. Journal of Experimental Biology **2022**, *4*, 223. DOI 10.1242/jeb.216762.
377 24. Tingting Zhao.; Aijun Ma.; Shuangshuang Yang.; Zhihui Huang. Integrated metabolome and transcriptome analyses
378   revealing the effects of thermal stress on lipid metabolism in juvenile turbot Scophthalmus maximus. *Journal of Thermal*
379   *Biology* **2021**, *99*, 102937. DOI 10.1016/j.jtherbio.2021.102937.
380 25. Conall McNicholl.; François Oberhaensli.; Angus Taylor.; Isak Holmerin.; Peter W. Swarzenski.; Marc Metian.
381   Deoxygenation reduces growth rates and increases assimilation of essential trace metals in gilthead seabream (Sparus
382   aurata). *Environmental Pollution* **2021**, *288*, 117786. DOI 10.1016/j.envpol.2021.117786.
383 26. Miao Dong.; Yimin Liang.; Rajkumar Ramalingam.; Sze Wing Tang.; Wei Shen.; Rui Ye.; Singaram Gopalakrishnan.;
384   Doris Wai Ting Au.; Yun Wah Lam. Proteomic characterization of the interactions between fish serum proteins and
385   waterborne bacteria reveals the suppression of anti-oxidative defense as a serum-mediated antimicrobial mechanism. *Fish*
386   *& Shellfish Immunology* **2017**, *62*, 96-106. DOI 10.1016/j.fsi.2017.01.013.
387 27. Na Li.; Hua-Qiong Huang.; Gen-Sheng Zhang.; Wei Cui. Effect of 5- Azn-2 '-deoxycytidine on proliferation of human
388   lung adenocarcinoma cell line A549 in vitro. *Asian Pacific Journal of Tropical Medicine* **2013**, 982-985. DOI
389   10.1016/S1995-7645(13)60176-5.
390 28. Milad Adel.; Amir Hossein Omidi.; Mahmoud A.O. Dawood.; Behnaz Karimi.; Seyed Pezhman Hosseini Shekarabi.
391   Dietary Gracilaria persica mediated the growth performance, fillet colouration, and immune response of Persian sturgeon
392   (Acipenser persicus). *Aquaculture* **2021**, *530*, 735950. DOI 10.1016/j.aquaculture.2020.735950.
393 29. Cuifeng Jiang.; Cong Zhang.; Juan Song.; Xiaojie Ji.; Wei Wang. Cytidine-gold nanoclusters as peroxidase mimetic for
394   colorimetric detection of glutathione (GSH), glutathione disulfifide (GSSG) and glutathione reductase (GR).
395   *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **2021**, *250*, 119316. DOI
396   10.1016/j.saa.2020.119316.
397 30. Małgorzata Witeska.; Elzbieta Kondera.; Katarzyna Ługowska.; Bartosz Bojarski. Hematological methods in fish – Not
398   only for beginners. *Aquaculture* **2022**, *547*,737498. DOI 10.1016/j.aquaculture.2021.737498.
399 31. Fatma Ahmed.; , Faiza M. Soliman.; Mohamed A. Adly.; Hamdy A. M. Soliman.; Mansour El-Matbouli.; Mona Saleh.
400   Dietary Chitosan Nanoparticles: Potential Role in Modulation of Rainbow Trout (Oncorhynchus mykiss) Antibacterial
401   Defense and Intestinal Immunity against Enteric Redmouth Disease. *Mar. Drugs* **2021**, *19*, 72. DOI 10.3390/md19020072.
402 32. Miguel L. Grilo.; Ana Pereira.; Carla Sousa-Santos.; Joana I. Robalo.; Manuela Oliveira. Climatic Alterations Influence
403   Bacterial Growth, Biofilm Production and Antimicrobial Resistance Profiles in Aeromonas spp. *Antibiotics* **2021**, *10*, 1008.
404   DOI 10.3390/antibiotics10081008.
405 33. Daniel J. Ritchie.; Christopher R. Friesen. Invited review: Thermal effects on oxidative stress in vertebrate ectotherms.
406   *Comparative Biochemistry and Physiology, Part A* **2022**, *263*, 111082. DOI 10.1016/j.cbpa.2021.111082.
407 34. Bing Liu.; Youling L. Xiong.; Jiang Jiang.; Dongyou Yu.; Gang Lin. Cellular antioxidant mechanism of selenium-enriched
408   yeast diets in the protection of meat quality of heat-stressed hens. *Food Bioscience* **2021**, *39*, 100798. DOI
409   10.1016/j.fbio.2020.100798.
410 35. Ponma Kuzhiyil Aswathy.; Chokki Veetil Priyatha.; John Nikhil.; Kumari Chidambaran Chitra. Triclosan at environmental
411   concentration alters the hepatic antioxidant defence system in the fish, Anabas testudineus (Bloch, 1792). *Aquaculture*
412   *Research*. **2021**, *52*, 6641–6655. DOI 10.1111/are.15534.
413 36. Matheus D. Baldissera.; Carine F. Souza.; Carla Cristina Zeppenfeld.; Maiara C. Velho.; Bruna Klein.; Lorenzo B. Abbad.;
414   Aline F. Ourique.; Roger Wagner.; Aleksandro S. Da Silva.; Bernardo Baldisserotto. Dietary supplementation with
415   nerolidol nanospheres improves growth, antioxidant status and fillet fatty acid profiles in Nile tilapia: Benefits of
416   nanotechnology for fish health and meat quality. *Aquaculture* **2020**, *516*, 734635. DOI 10.1016/j.aquaculture.2019.734635.
417 37. Matheus D. Baldissera.; Carine F. Souza.; Sharine N. Descovi.; Tiago G. Petrolli.; Aleksandro S. da Silva.; Bernardo
418   Baldisserotto. A caffeine-supplemented diet modulates oxidative stress markers and prevents oxidative damage in the livers
419   of Nile tilapia (Oreochromis niloticus) exposed to hypoxia. *Fish Physiol Biochem* **2019**, *45*, 1041–1049. DOI
420   10.1007/s10695-019-00616-7.
421 38. Kai Yang.; Qixue Fan.; Lei Zhang.; Bo Li.; Yinai Gao.; Kewei Zeng.; Qingyun Wang.; Sihua Zhu.; Gang Fang. Effect of
422   dissolved oxygen levels on growth performance, energy budget and antioxidant responses of yellow catfish, Pelteobagrus
423   fulvidraco (Richardson). *Aquaculture Research* **2015**, *46*, 2025–2033. DOI 10.1111/are.12359.
424 39. Danilo da Costa Barroso.; Vera Maria Fonseca Almeida-Val.; Adalberto Luis Val. Temperature and food availability alters
425   the physiology and aerobic capacity of tambaqui (Colossoma macropomum). *Comparative Biochemistry and Physiology,*
426   *Part A* **2020**, *245*, 110704. DOI 10.1016/j.cbpa.2020.110704.
427 40. Zhilu Zhang.; Ke Lu.; Xu-Fang Liang.; Jiao Li.; Shulin Tang.; Yanpeng Zhang.; Wenjing Cai.; Qiaoqiao Xiao.; Qiwei
428   Zhang. Effects of early low temperature exposure on the growth, glycolipid metabolism and growth hormone (gh) gene
429   methylation in the late stage of Chinese perch (Siniperca chuatsi). *Comparative Biochemistry and Physiology, Part B* **2022**,
430   *259*, 110705. DOI 10.1016/j.cbpb.2021.110705.

431 41. Muhammad A.B. Siddik.; Md Javed Foysal.; Ravi Fotedar.; David S. Francis.; Sanjay K. Gupta . Probiotic yeast
432   Saccharomyces cerevisiae coupled with Lactobacillus casei modulates physiological performance and promotes gut
433   microbiota in juvenile barramundi, Lates calcarifer. *Aquaculture* **2022**, *546*, 737346. DOI
434   10.1016/j.aquaculture.2021.737346.
435 42. Ying-Ying Wang.; Yu Zhou.; Huang-Cui Fu.; He-Zhong Huang.; Ze Li.; Rui-Ming Jin.; Xiao-Zhe Fu.;Ning-Qiu Li.
436   Transcriptomic and proteomic analyses of the immune mechanism in pathogenetic and resistant mandarin fish (Siniperca
437   chuatsi) infected with ISKNV. *Aquaculture* **2021**, *545*, 737198. DOI 10.1016/j.aquaculture.2021.737198.
438 43. Shannon G. Moorhead.; Austin J. Gallagher.; Liza Merly.; Neil Hammerschlag. Variation of body condition and plasma
439   energy substrates with life stage, sex, and season in wild-sampled nurse sharks Ginglymostoma cirratum. *J Fish Biol* **2021**,
440   98, 680–693. DOI 10.1111/jfb.14612.
441 44. Daniel F. Gomez Isaza.; Rebecca L. Cramp.; Craig E. Franklin. Exposure to Nitrate Increases Susceptibility to Hypoxia in
442   Fish. *Physiological and Biochemical Zoology* **2021**, *94*, 124–142. DOI 10.1086/713252.
443 45. Brittney G. Borowiec.; Graham R. Scott. Rapid and reversible modulation of blood haemoglobin content during diel cycles
444   of hypoxia in killifish (Fundulus heteroclitus). *Comparative Biochemistry and Physiology, Part A* **2021**, *261*, 111054. DOI
445   10.1016/j.cbpa.2021.111054.
446 46. Gail D. Schwieterman.; Jodie L. Rummer.; Ian A. Bouyoucos.; Peter G. Bushnell.; Richard W. Brill. A lack of red blood
447   cell swelling in five elasmobranch fishes following air exposure and exhaustive exercise. *Comparative Biochemistry and*
448   *Physiology, Part A* **2021**, *258*, 110978. DOI 10.1016/j.cbpa.2021.110978.
449 47. Justyna Sikorska.; Elżbieta Kondera.; Rafał Kamiński.; Katarzyna Ługowska.; Małgorzata Witeska.; Jacek Wolnicki.
450   Effect of four rearing water temperatures on some performance parameters of larval and juvenile crucian carp, Carassius
451   10ucius10us, under controlled conditions. *Aquaculture Research* **2018**, *49*, 3874–3880. DOI 10.1111/are.13855.
452 48. Anne Beemelmanns.; Fábio S. Zanuzzo.; Xi Xue.; Rebeccah M. Sandrelli.;Matthew L. Rise.; A. Kurt Gamperl. The
453   transcriptomic responses of Atlantic salmon (Salmo salar) to high temperature stress alone, and in combination with
454   moderate hypoxia. *BMC Genomics* **2021**, *22*, 261. DOI 10.1186/s12864-021-07464-x.
455 49. Truong Quoc Phu.; Bui Thi Bich Hang.; Dang Diem Tuong.; Vila-Gispert Anna.; Toyoji Kaneko.; Nguyen Thanh Phuong.;
456   Do Thi Thanh Huong. Effects of size and nitrite exposure on respiration, oxygen partitioning, and growth of obligate
457   air-breathing fsh Channa striata. *Fisheries Science* **2022**, *88*, 149–159. DOI 10.1007/s12562-021-01562-1.
458 50. Dariusz P. Fey.; Martyna Greszkiewicz. Effects of temperature on somatic growth, otolith growth, and uncoupling in the
459   otolith to fish size relationship of larval northern pike, Esox 10ucius L. *Fisheries Research* **2021**, *236*, 105843. DOI
460   10.1016/j.fishres.2020.105843.

461
462 Legends:
463 Figure 1:
464 Schematic diagram of oxygen regulation system

465
466 Figure 2:
467 The growth rates of *Aeromonas* in crucian carp serum were compared by two-way ANOVA under the interaction conditions of
468 three temperatures (18 ℃, 24 ℃, and 30 ℃) and three oxygen levels (2.1 mgL^{-1}, 5.4 mgL^{-1}, and 9.3 mgL^{-1}). Values are expressed
469 as mean ± SD of triplicate pooled data for each treatment. Means of different letters in the same row were significantly different ($P < 0.05$).

471
472 Figure 3:
473 The two-way ANOVA was used to detect the changes of serum antioxidant enzyme activities of crucian carp at different
474 temperatures and oxygen levels (n = 9). Capital letters represent significant differences under different oxygen conditions at the
475 same temperature ($P < 0.05$). Lowercase letters represent significant differences under the same oxygen and different temperature
476 conditions ($P < 0.05$). Values are expressed as mean ± SD of triplicate pooled data for each treatment. L represents hypoxic
477 conditions; M represents medium oxygen conditions; H represents hyperoxic conditions; (A) CAT; (B) GPx; and (C) SOD.

478
479 Figure 4:
480 The two-way ANOVA was used to detect the changes of serum biochemical parameters of crucian carp at different temperatures
481 and oxygen levels (n = 9). Capital letters represent significant differences under different oxygen conditions at the same temperature
482 ($P < 0.05$). 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
484 medium oxygen conditions; H represents hyperoxic conditions. TP, total protein, TG, triglycerides. (A) glucose; (B) TP; and (C)
485 TG.

486
487 Figure 5:
488 The two-way ANOVA was used to detect the changes of hematological variables in crucian carp at different temperatures and
489 oxygen levels (n = 9). Different capital letters indicate significant differences ($P < 0.05$) under different oxygen conditions at the
490 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
492 medium oxygen conditions; H represents hyperoxic conditions. HCT, hematocrit; MCH, mean hemoglobin content; HGB,
493 hemoglobin. (A) HCT; (B) MCH; and (C) HGB.

494
495 Table 1:

496 Summary of the two-way ANOVA results for the growth performance of crucian carp under different interaction treatments.
497 Values are expressed as mean ± SD of triplicate pooled data for each treatment. Means in the same column with different letters
498 are significantly different (P < 0.05).

Figure 1

Schematic diagram of oxygen regulation system

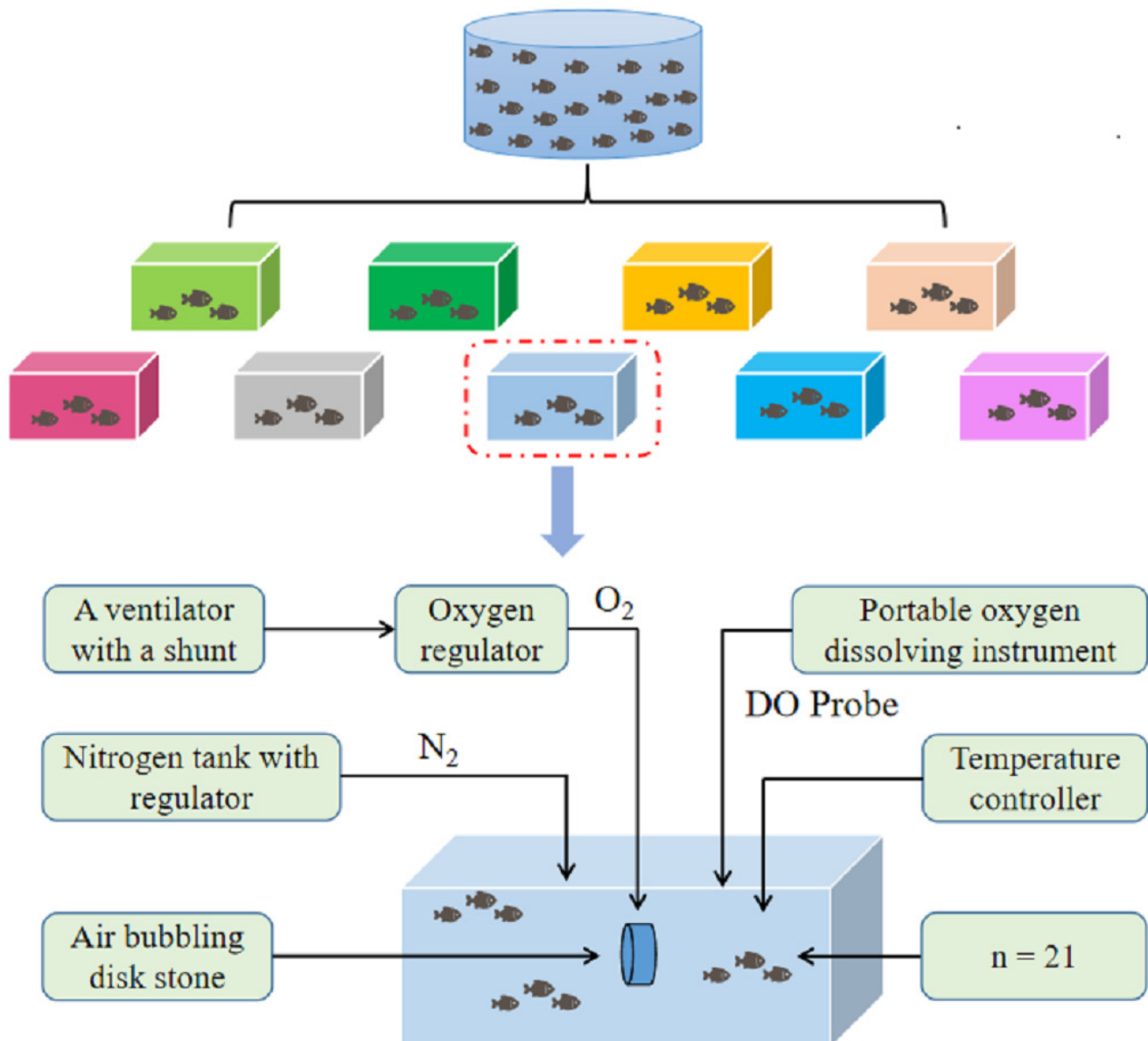



Figure 2

The growth rates of *Aeromonas* in crucian carp serum were compared by two-way ANOVA under the interaction conditions of three temperatures (18 ℃, 24 ℃, and 30 ℃) and three oxygen levels (2.1 mgL $^{-1}$, 5.4 mgL $^{-1}$, and 9.3 mgL [sup]-

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).

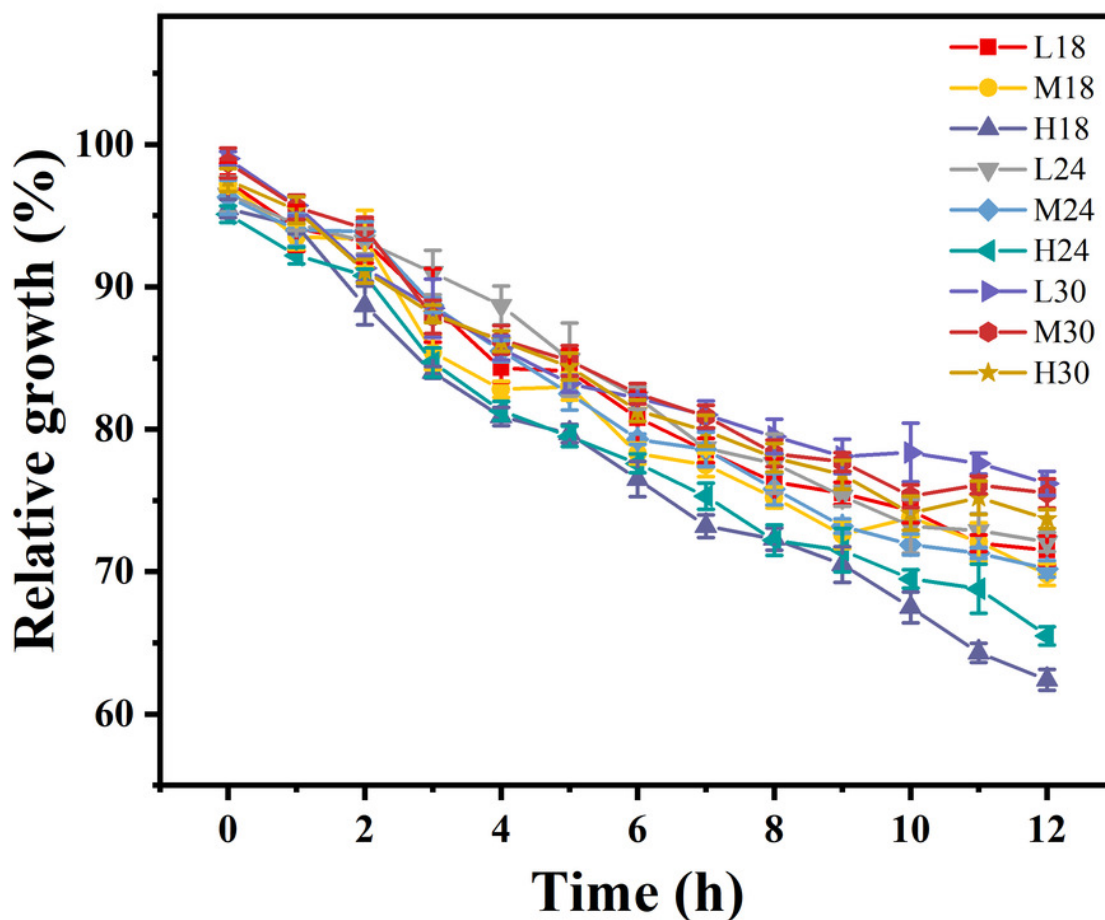



Figure 3

The two-way ANOVA 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; (A) CAT; (B) GPx; and (C) SOD.

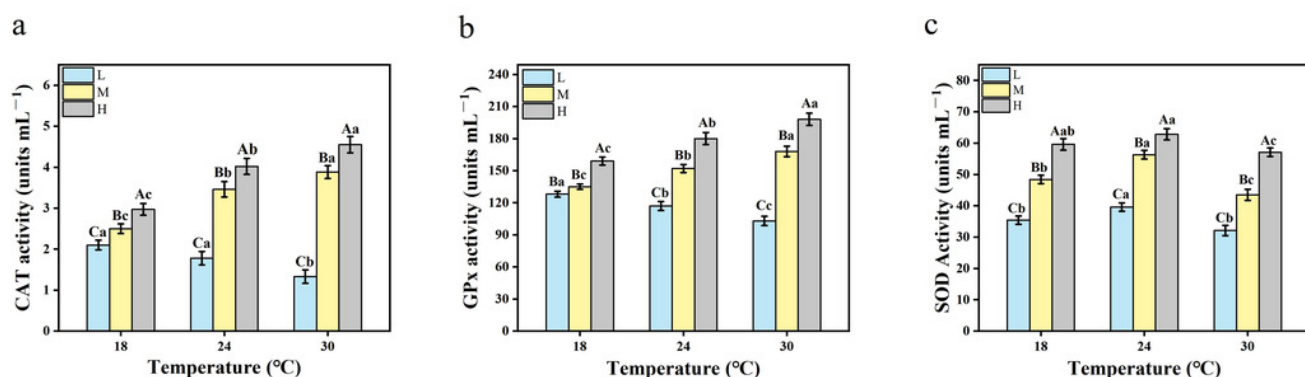



Figure 4

The two-way ANOVA 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. (A) glucose; (B) TP; and (C) TG.

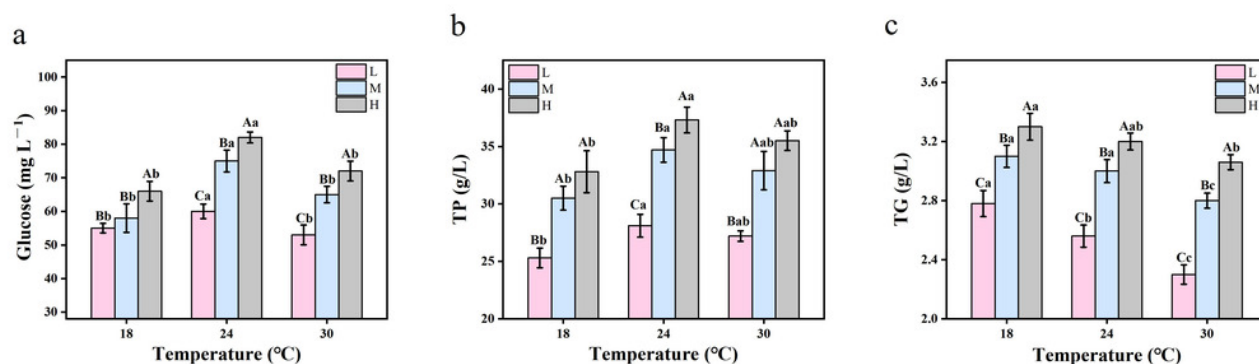
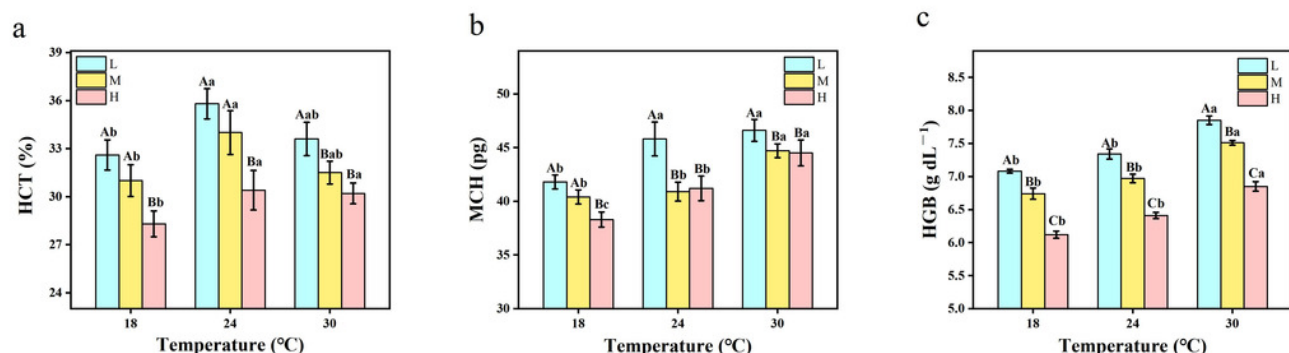



Figure 5

The two-way ANOVA 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. (A) HCT; (B) MCH; and (C) HGB.

Summary of the two-way ANOVA results for the 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