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Temperature and Dissolved Oxygen Lead to Behavior and Respiration Changes in Juvenile Largemouth Bass (*Micropterus salmoides*) during Transport

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Abstract: The study aimed to investigate the effects of temperature and dissolved oxygen on juvenile largemouth bass during transportation. The experiment involved four temperature groups: 20, 15, 10, and 5 °C. We analyzed the effects of acute and uniform cooling on fish behavior to determine the optimal approach for cooling. Then, we simulated transport under different temperature conditions while measuring the dissolved oxygen level and metabolic rate until all the fish died. The results showed that acute cooling significantly influenced the tail-beat frequency of fish compared with uniform cooling, while abnormal behaviors such as increased swimming, attempted jumping out of the water, and loss of balance were observed. As the transport temperature reduced, the oxygen consumption rate of fish significantly changed at 10 °C, being 2.6 times lower than at 15 °C, with values of 0.10 ± 0.02 and 0.47 ± 0.07 mg·g^{-1}·h^{-1}, respectively. The critical oxygen threshold (P_{crit}) of fish were 1.90 ± 0.12, 1.61 ± 0.04, 1.15 ± 0.09, and 1.12 ± 0.25 mg·L^{-1} at 5, 10, 15, and 20 °C. In addition, below P_{crit}, hypoxia-led behavior changes and oxygen consumption rate reduction were observed at every transport temperature. The findings suggest that the optimal low temperature can reduce metabolism and improve the hypoxia tolerance of juvenile largemouth bass. We recommend transporting largemouth bass at an optimal low temperature (15 °C), monitoring fish behavior, and maintaining oxygen levels above P_{crit} during transport to prevent stress.

Keywords: live transport; behavior; oxygen consumption rate; hypoxia tolerance; temperature

Key Contribution: 1. The cooling method significantly influences largemouth bass tail-beat frequency and behavior. 2. Low-temperature transport can reduce largemouth bass metabolic rate and P_{crit}, improving hypoxia tolerance. 3. With oxygen reduction, largemouth bass behavior changes relevant to hypoxia when below P_{crit}. 4. We demonstrate that behavioral and respiratory metabolism can reflect the effects of temperature and dissolved oxygen on largemouth bass.

Citation: Gui, F.; Sun, H.; Qu, X.; Niu, S.; Zhang, G.; Feng, D. Temperature and Dissolved Oxygen Lead to Behavior and Respiration Changes in Juvenile Largemouth Bass (*Micropterus salmoides*) during Transport. *Fishes* **2023**, *8*, 565. https://doi.org/10.3390/fishes8120565

Academic Editor: Gioele Capillo

Received: 4 November 2023
Revised: 18 November 2023
Accepted: 19 November 2023
Published: 21 November 2023

1. Introduction

Live transport is an integral component of aquaculture [1], encompassing the transportation of juvenile fish from hatcheries to farms and adult fish from farms to markets [2,3]. During transportation, fish are exposed to various environmental factors that can significantly impact their survival and physiological well-being, presenting challenges to this process [4].

Temperature plays a critical role in the transportation of live fish [5]. Proper temperature control can significantly reduce the harm caused by environmental factors to fish and improve their survival rates [6]. For instance, when transported for 30 h at a temperature ranging from

1.6 to 2.7 °C, the survival rate of Chinese sturgeon is notably higher than that at temperatures from 4 to 6 °C [7]. Prior temporary low-temperature rearing can lower the stress response of fish during transportation, enhancing their survival rates. However, the temporary rearing temperature should be much lower than the living temperature of fish [8,9]. Rapid changes in environmental conditions can affect fish and trigger their immune mechanism, which is detrimental to subsequent transportation [10]. Therefore, it is necessary to select an appropriate cooling method for the temporary low-temperature rearing of fish. Furthermore, temperature jointly affects the metabolic intensity and dissolved oxygen saturation of fish [11]. An increase in temperature reduces the solubility of oxygen in water and increases the metabolic rate of fish [12,13]. A reduction in oxygen levels in water to below 2 mg·L^{-1} is defined as hypoxia, which can have detrimental effects on fish physiology, metabolism, antioxidant capacity, and immune function [11,14]. Hypoxia triggers the release of catecholamines, cortisol, and other hormones in fish [15], leading to oxidative stress and the accumulation of reactive oxygen species (ROS) free radicals in cells [14]. Fish under stress exhibit a decline in their oxygen consumption rate, which persists as oxygen levels drop below a critical threshold (P_{crit}) [16,17]. In addition, a range of behavioral responses to hypoxia was exhibited in fish, enabling them to maintain oxygen uptake and aerobic metabolism even at hypoxia [18–20], which also indicated the stress condition.

The largemouth bass (*Micropterus salmoides*) is an economically significant freshwater fish, originally from the United States, which was introduced to China in 1983 [21]. Its cultivation is widespread globally, with an annual output of 610,000 tons in China alone [22,23], making it one of the fastest-growing varieties. Hence, comprehending the influence of transportation on largemouth bass is of utmost significance. However, systematic scientific research on live transport is still in its early stages [5], and the effect of transport temperature and dissolved oxygen concentration on the oxygen consumption rate and behavior of largemouth bass is still not fully understood. This study aimed to investigate the effects of temperature and dissolved oxygen changes on the behavior and respiratory metabolism of juvenile largemouth bass. The results contribute to a deeper understanding of the respiratory metabolism and related behaviors of juvenile largemouth bass and provide essential data and a theoretical underpinning for its live transport management.

2. Materials and Methods

2.1. Fish Preparation

Juvenile largemouth bass, with a body length ranging from 13 to 15 cm and a weight of 15 to 30 g, were procured from a local aquaculture company in Zhoushan. The specimens were then transported in oxygenated bags to the National Marine Facility Aquaculture Engineering Technology Research Center laboratory of Zhejiang Ocean University. The bass were temporarily housed in a circulating water aquaculture tank for two weeks. Before use, the water body was thoroughly aerated, and the water quality was monitored daily to maintain a dissolved oxygen concentration of no less than 7 mg·L^{-1}. The water temperature was maintained at 23 °C, while the pH was maintained at 7.5. The fish were fed twice daily, and the pool was regularly cleaned to remove excrement. All experimental and sampling procedures were conducted according to the Guidelines of the Animal Care of the Zhejiang Ocean University and were approved by the Ethics Committee of the university (number 2023088).

2.2. Experiment Equipment

The experiment equipment is shown in Figure 1. The simulation of transport involves a high-power chiller (Hitachi Home Appliances (China) Co., Ltd., Guangzhou, China), a submersible pump (DC-1020 200 L·h^{-1} Sensen Group, Zhoushan, China), and a simulated transport tank. The transport box comprises an outer water tank measuring 84.5 × 53 × 51 cm and a sealed tank measuring 43 × 31.8 × 26 cm. The outer transport box is filled with water cooled by a high-power chiller. The fish were placed within the sealed tank, and the submersible pump simulated the water exchange during the transport process, creating a certain impact on the test object.

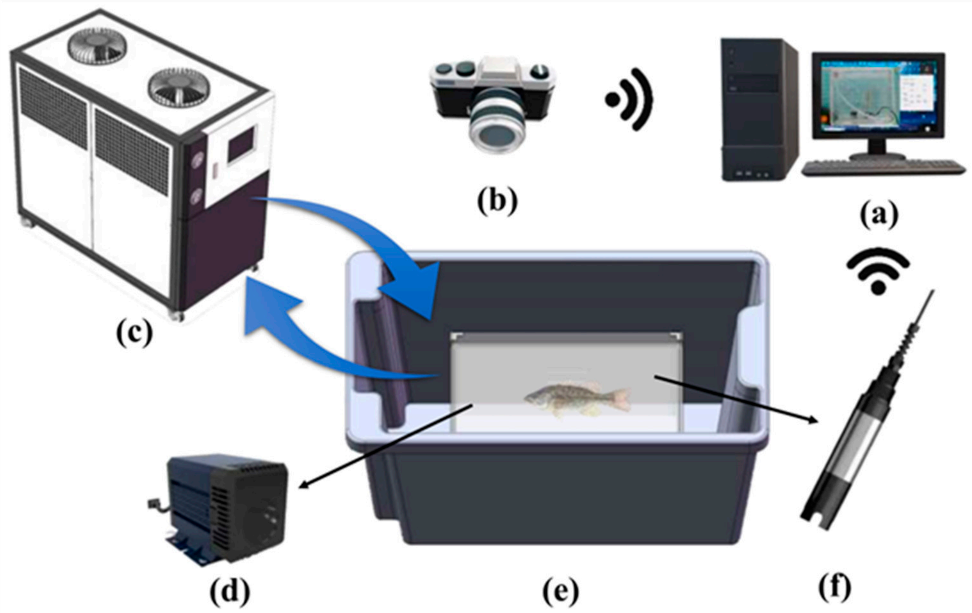

Figure 1. Experiment equipment: (**a**) computer, (**b**) HD camera, (**c**) high-power chiller, (**d**) submersible pump, (**e**) simulated transport tank, and (**f**) dissolved oxygen sensor.

The data collection equipment, consisting of a Lenovo Thinkpad, an HD camera (Nikon, Beijing, China), and a dissolved oxygen sensor (Raymond Instrument, Shanghai, China), was used to record relevant behavioral data of test subjects, as well as changes in dissolved oxygen levels in the transport water during cooling and simulating transportation. The dissolved oxygen sensor was placed inside the sealed tank and connected to external data collection devices using sealed connectors. The concentration of oxygen molecules was calculated based on the fluorescence quenching principle by measuring the phase difference between the excitation red light and the reference light and comparing it with the internal calibration value. This method provides stable results, requires less frequent cleaning, is less prone to interference, and has a faster response time than traditional methods for measuring dissolved oxygen.

2.3. Experiment Design

Juvenile largemouth bass, with an average weight of 25.60 ± 4.60 g, were meticulously selected and placed in an aquaculture water tank. Before the commencement of the experiment, the bass were kept in the tank for 48 h without any feeding while changing the water once every 24 h and inflating the air pump. The water temperature was maintained at 23 °C throughout the experiment. The bass exhibited no signs of physical injury and displayed normal behavior.

For the cooling experiment, four temperature groups were formed: 20, 15, 10, and 5 °C. For each temperature group, three parallel groups were established, and each group consisted of eight fish. Three fish were randomly chosen from each parallel group to calculate their tail-beat frequency. The test object was directly placed in the simulated transport tank at the corresponding temperature. In the uniform cooling group, the temperature was gradually decreased to the desired level at a rate of 1 °C per hour. The tail-beat frequency and other abnormal behaviors of largemouth bass were compared between two cooling methods, and the cooling method that had less impact was selected for the follow-up test.

For the simulated transport, we set four different temperatures (20, 15, 10, and 5 °C) and established three parallel groups for each temperature. Each group contained eight fish, which were placed in a transport box and cooled down to the test temperature (water volume 20 L transport density 10 g·L^{-1}). The transport box was sealed, and the submersible pump was turned on to simulate transportation. During the test, the transport box was shaken every hour to replicate the shaking that occurs during transportation. We recorded any abnormal behavior of the fish during the test and measured the dissolved oxygen concentration using a dissolved oxygen sensor until all the test subjects had died and the test ended.

2.4. Determination of Tail-Beat Frequency

To ensure the well-being of the specimen, it is imperative to transfer it promptly from the circulating water aquaculture tank to the transport container to ensure optimal comfort levels. The tail-beat frequency of the specimens was measured at various intervals based on temperature. These intervals consisted of 0–1 min (early), 2–3 min (middle), and 4–5 min (late). The average number of tail fin movements per minute was calculated as the tail-beat frequency.

2.5. Relate Index

The oxygen consumption rate was measured using closed respirometry [24]. The term "Oxygen consumption rate" refers to the volume of oxygen consumed per hour and is determined using formula (1). "Do_n" represents the concentration of dissolved oxygen in the transportation container throughout a specific period ($mg \cdot L^{-1}$), where "n" denotes a specific hour after the initiation of the experiment, "v" represents the volume of the sealed tank (L), and "w" denotes the body mass of the juvenile largemouth bass (g). The critical oxygen threshold ($mg \cdot L^{-1}$) is the concentration of dissolved oxygen that corresponds to the beginning of a consistent reduction in the oxygen consumption rate of the test subject. During an experiment, the average oxygen consumption rate ($mg \cdot g^{-1} \cdot h^{-1}$) is calculated from the start of the experiment until the critical oxygen threshold. Formula (1) is used to calculate this value. The time taken to reach the critical oxygen threshold is denoted by "t" (h). If the test subject dies, the dissolved oxygen concentration at the time of death is called the death concentration ($mg \cdot L^{-1}$). In order to ensure accurate measurements, we confirmed the absence of microbial respiration by measuring dissolved oxygen in a sealed tank without fish [25].

$$R_o = \frac{(DO_{n+1} - DO_n) \times v}{w \times t}, \tag{1}$$

2.6. Statistical Analysis

The results were analyzed using the SPSS 23.0 statistical software package. Experimental data were expressed as mean ± SEM (standard error of the mean). A one-way analysis of variance was used to analyze the tail-beat frequency and oxygen consumption rate, followed by Duncan's multiple range tests to identify specific differences between pairs of means. We referred to the measurement method in [26] to determine the critical oxygen threshold (P_{crit}) for this experiment. Specifically, a piece-wise regression was conducted on the oxygen consumption rate measurements every hour; when the result was significant between both points, the second was regarded as P_{crit} ($p < 0.05$).

3. Results

3.1. Effects of Cooling on Juvenile Largemouth Bass Behavior and Tail-Beat Frequency

The experiment conducted aimed to investigate the effects of temperature variation on fish behavior. The results indicated that a decrease in water temperature from 23 °C to 20 °C did not significantly affect fish behavior. The fish remained stationary at the bottom of the water tank or swam occasionally, irrespective of whether the cooling was direct or uniform. However, when the temperature was acutely cooled to 15 °C, the fish demonstrated continuous slow behavior that persisted throughout the experiment. Nonetheless, there was no marked change in their behavior when uniformly cooled to 15 °C. Conversely, when the temperature was acutely cooled to 10 °C, there was a significant increase in abnormal behavior among the subjects. These included heightened swimming activity, attempts to leap out of the water surface, and loss of balance in their bodies. Similarly, when uniformly cooled to 10 °C, the subjects briefly lost their balance. Lastly, when the temperature was rapidly cooled to 5 °C, the subjects attempted to leap out of the water surface but ultimately became motionless at the bottom of the tank. They showed minimal gill movement and no response when lightly touched on their bodies. Under uniform cooling conditions at 5 °C, only immobility at the bottom with no response to light touches was observed.

The impact of rapid and uniform cooling on the tail swing frequency of largemouth bass is illustrated in Figure 2. As demonstrated in Figure 2a, when the temperature drops rapidly to 5 and 10 °C, the tail-beat frequency at the outset significantly increased compared with that at 15 and 20 °C ($p < 0.05$) with an average value of 1.11 ± 0.21 and 1.26 ± 0.19 Hz, respectively. Nonetheless, the tail-beat frequency in the mid and late stages tended to approach zero. This was due to the loss of balance in the fish's body and resting on the bottom. The tail-beat frequency in the early stage was significantly ($p < 0.05$) higher than that at 20 °C, and the tail-beat frequency in the middle and late stages decreased with the increase in adaptation time, but it was higher than other temperatures. When the temperature dropped quickly to 20 °C, the tail wagging frequency was lower than the other temperatures, as the temperature was close to the temporary water temperature, and the juvenile largemouth bass were not affected by the temperature change. Figure 2b demonstrates the tail-beat frequency for uniform cooling. The tail-beat frequency of the test subjects was not affected by uniform cooling, indicating that controlling the temperature change rate is beneficial for largemouth bass to adapt to different transport temperatures.

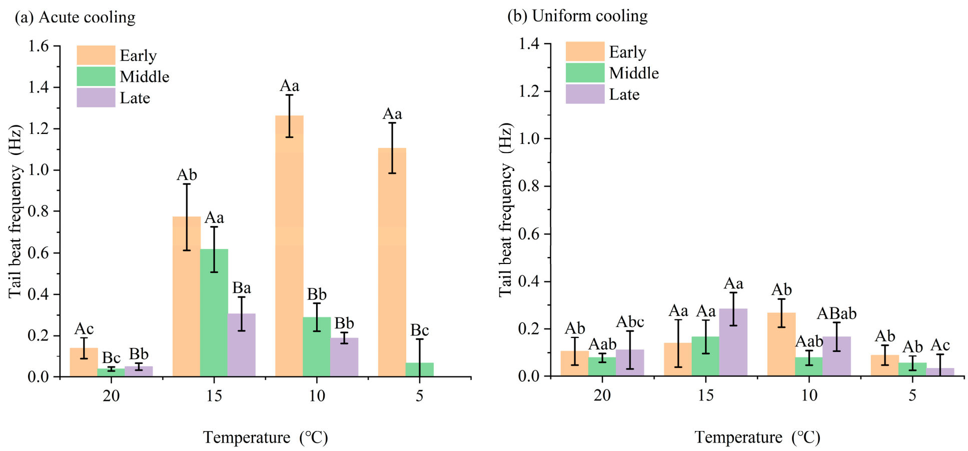

Figure 2. Effect of different cooling methods on the tail-beat frequency of largemouth bass: (**a**) acute cooling and (**b**) uniform cooling. The same letter indicates no significant difference ($p > 0.05$); capital letters indicate different periods of the same temperature; and lowercase letters indicate different temperatures at the same time.

3.2. Effects of Temperature and Dissolved Oxygen on Juvenile Largemouth Bass

3.2.1. Effects of Temperature and Dissolved Oxygen on Oxygen Consumption Rate

The present study investigates the effect of transport temperature on the average oxygen consumption rate of juvenile largemouth bass. Figure 3 displays the results of this study, which reveal that the average oxygen consumption rate increased with rising temperatures. Specifically, the rate increased from 0.19 ± 0.02, 0.18 ± 0.02, 0.47 ± 0.07, to 0.52 ± 0.08 mg·g^{-1}·h^{-1} at 5, 10, 15, and 20 °C, respectively. It was found that the average oxygen consumption rate at 15 and 20 °C was significantly higher than at 5 and 10 °C ($p < 0.05$). The rate increase was especially pronounced between 10 and 15 °C, with the latter being 2.6 times higher than the former. Figure 4 presents the oxygen consumption rate at different dissolved oxygen concentrations. It was observed that the rate continued to decrease as the dissolved oxygen concentration decreased under the conditions of 5, 10, 15, and 20 °C. These findings suggest that transport temperature plays a crucial role in determining the oxygen consumption rate, with higher temperatures leading to an increase in oxygen consumption rate.

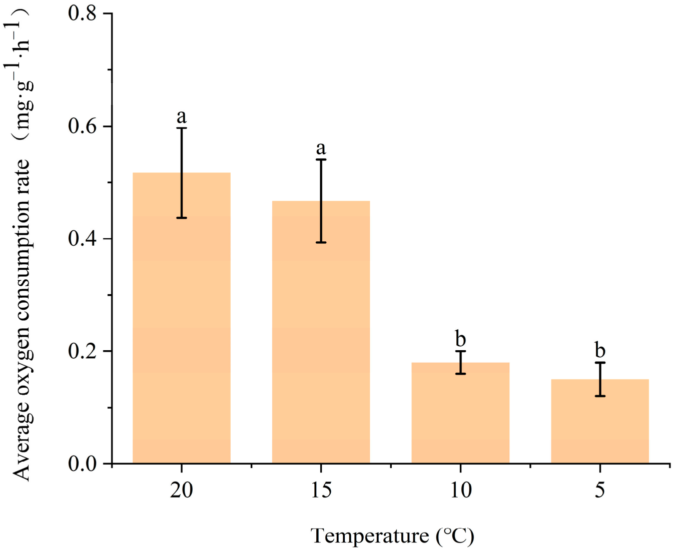

Figure 3. The average oxygen consumption rate of largemouth bass at different transport temperatures. Different lowercase letters indicate significant differences between groups ($p < 0.05$).

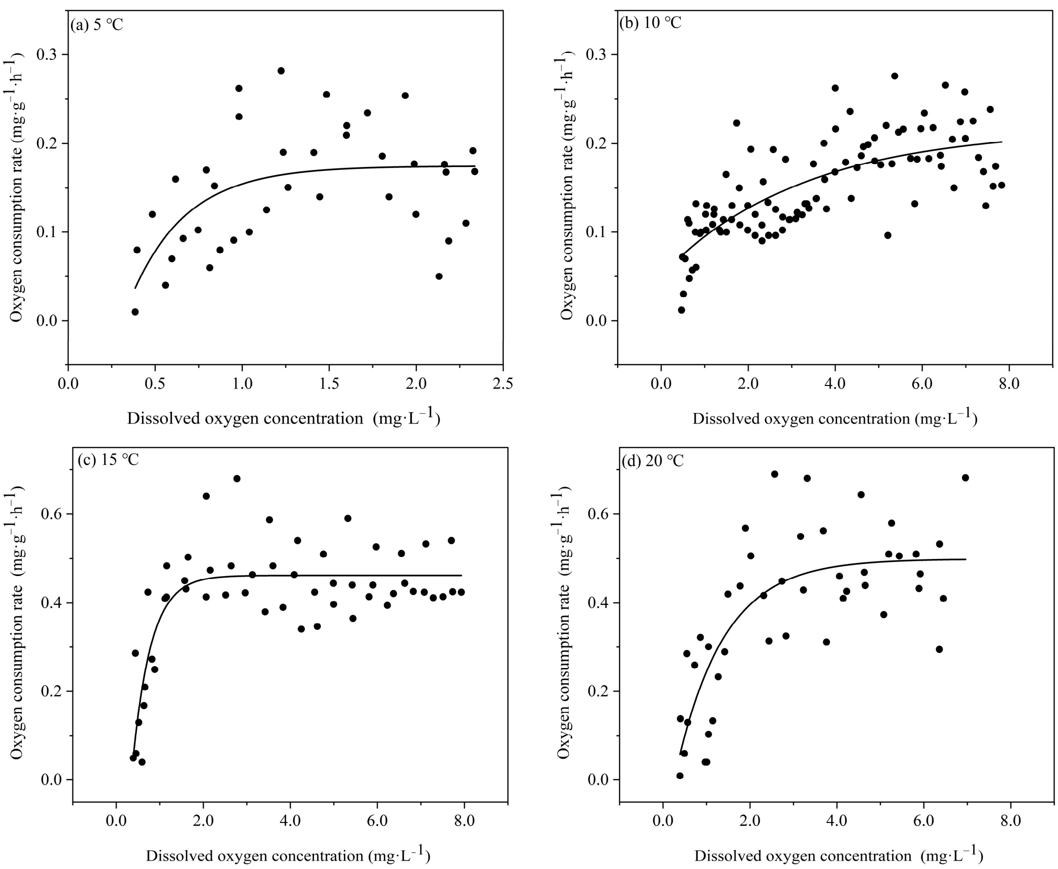

Figure 4. The oxygen consumption rate of largemouth bass at different dissolved oxygen concentrations: (**a**) oxygen consumption rate of 5 °C, (**b**) oxygen consumption rate of 10 °C, (**c**) oxygen consumption rate of 15 °C, and (**d**) oxygen consumption rate of 20 °C. The oxygen consumption rate at each data point in the figure corresponds to different levels of dissolved oxygen concentration, and the curve is derived through linear regression analysis of the relationship between dissolved oxygen concentration and oxygen consumption rate.

Figure 5 depicts the critical threshold of oxygen and the concentration at death for juvenile largemouth bass at different transport temperatures. The critical oxygen thresholds at 5, 10, 15, and 20 °C were 1.12 ± 0.25, 1.15 ± 0.09, 1.61 ± 0.04, and 1.90 ± 0.12 mg·L^{-1}, respectively. The P_{crit} demonstrated a decreasing trend as the temperature decreased, with 5 and 10 °C being significantly lower ($p < 0.05$) than 15 and 20 °C. The corresponding death concentrations at 5, 10, 15, and 20 °C were 0.58 ± 0.22, 0.54 ± 0.09, 0.48 ± 0.10, and 0.62 ± 0.31 mg·L^{-1}, respectively. No significant difference was observed among the different temperatures ($p > 0.05$).

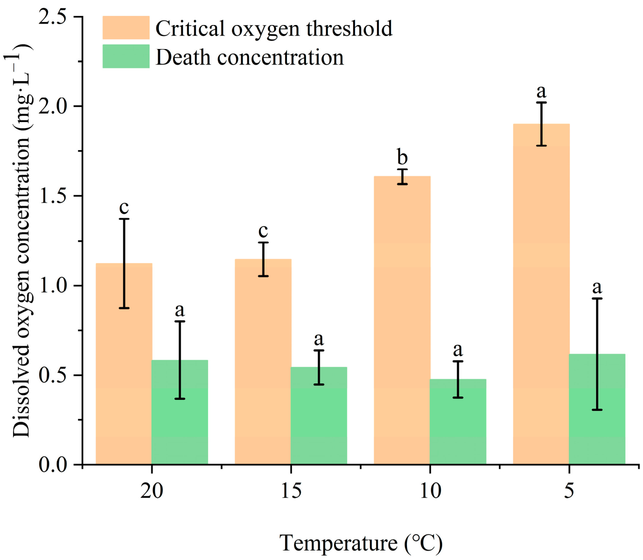

Figure 5. The critical oxygen threshold (P_{crit}) and death point of largemouth bass at different transport temperatures. Different lowercase letters indicate significant differences between groups ($p < 0.05$).

3.2.2. Effect of Temperature and Dissolved Oxygen on Behavior

Table 1 presents the results of an experiment that examined the effects of decreasing concentrations of dissolved oxygen on the behavior of largemouth bass. The experiment observed that at temperatures of 5, 15, and 20 °C, no abnormal behavior was observed at the critical oxygen threshold. However, at 10 °C, some test subjects exhibited abnormal behavior, including floating heads and increased swimming. As the concentration of dissolved oxygen further decreased to 0.72, 0.90, and 1.49 mg·L^{-1} at 5, 15, and 20 °C, respectively, the experimental subjects exhibited abnormal behavior, such as increased swimming, floating heads, and gill movement. Moreover, at 10 °C, when the dissolved oxygen concentration ranged from 0.99 to 0.61 mg·L^{-1}, the test subjects remained stationary in the water. It is important to note that if the dissolved oxygen concentration continued to fall, similar abnormal behavior would be observed as in the other temperatures.

Table 1. Description of abnormal behavior of largemouth bass after P_{crit}.

Temperature °C	Oxygen Level mg·L^{-1}	Behavior Description
20	2.02 to 1.49	Normal
	1.49 to 0.62	Increased swimming, floating head, gill movement amplitude and frequency
15	1.65 to 0.90	Normal
	0.90 to 0.54	Increased swimming, floating head, gill movement amplitude and frequency
10	1.22 to 0.99	Increased swimming, floating head
	0.99 to 0.61	Standing still in the water
	0.61 to 0.48	Increased swimming, floating head, gill movement amplitude and frequency
5	1.41 to 0.72	Normal
	0.72 to 0.62	Floating head and gill movement increased in amplitude and frequency

4. Discussion

Lowering temperature before transport is essential to reduce fish metabolic intensity and prevent the deterioration of water quality [27]. However, as shown in this study, acute cooling can lead to abnormal behaviors such as increased swimming, attempts to jump out of the water, and loss of balance. These changes in behavior are due to the impact of rapid temperature changes on the membrane excitability and synaptic transmission sensitivity of fish, which can negatively affect the normal functioning of the central nervous system [28,29]. Additionally, in a study of Atlantic salmon (*Salmo salar*), acute cooling from 8 to 1 °C significantly changed cardiorespiratory physiology and swimming capacity compared with those acclimated at 1 °C [25]. Acute cooling can damage the plasma membrane of fish, as demonstrated in a study of killifish (*Fundulus heteroclitus*) [30]. However, controlling the cooling rate can improve the adaptability of plasma membrane lipids, helping fish maintain the function of ion transport regulation and adapt to temperature changes to reduce abnormal behaviors [31]. In this study, uniform cooling from 1 °C per hour to 20, 15, 10, and 5 °C had no significant effect on the tail-beat frequency of largemouth bass.

Several studies have demonstrated that observing fish behavior can effectively aid in managing water quality and assessing fish living conditions in aquaculture [9,32,33]. Tail-beat frequency is a crucial metric in monitoring the physiological status of fish and evaluating the impact of environmental changes on them [34]. In Bartolini's study [35], the effect of ethanol on zebrafish (*Danio rerio*) tail-beat frequency was investigated, and the results revealed that a high ethanol concentration significantly reduces fish movement, indicating ethanol's adverse effect. In another study, Xiao calculated the tail-beat frequency of crucian fish exposed to three glyphosate concentrations, and the results indicated that this index can be used to monitor environmental changes and their impact on fish [36]. Additionally, this study revealed that largemouth bass's tail-beat frequency significantly increases when the temperature rapidly drops to 20, 15, 10, and 5 °C. Thus, the tail-beat frequency can be used as an evaluation index to regulate cooling rates and mitigate adverse temperature effects when assessing the impact of cooling on largemouth bass.

The oxygen consumption rate of fish is directly influenced by temperature and reflects all the biochemical processes in fish [37]. Within an appropriate temperature range, fish demonstrate a rapid increase in the rate of oxygen consumption. For instance, the Marcian fish (*Tor tambroides*) exhibits a four-fold increase in oxygen consumption at 30 °C as compared with 28 °C, which indicates that 30 °C is the optimal temperature for normal physiological activities in this species [38]. Our study results demonstrate that temperature significantly impacts the average oxygen consumption rate of juvenile largemouth bass, with the rate at 20 °C being significantly higher than at 5 and 10 °C ($p < 0.05$). Moreover, 15 °C is 2.6 times higher than at 10 °C. It is noteworthy that the normal physiological activity of juvenile largemouth bass can be maintained above 15 °C, as per other research [39]. Therefore, the transportation temperature of 15 °C is suitable for juvenile largemouth bass as it facilitates their normal respiratory metabolism. However, during uniform cooling, it was observed that largemouth bass experienced an imbalance and reduced responsiveness to touch as the temperature dropped to 5 and 10 °C, respectively. These findings align with Wang's research [40], which showed that juvenile largemouth bass exhibit abnormal behaviors such as loss of balance, abnormal breathing, struggling, split gills, and reduced transportation survival rates when the temperature drops to 10 °C and 4 °C. These results suggest that excessively low temperatures can have a detrimental effect on fish.

The respiratory metabolism of fish is impacted by the levels of oxygen present in their environment [17,20]. Fish that maintain a consistent oxygen consumption rate, regardless of changes in dissolved oxygen concentrations, are called "oxygen conformers". In contrast, those that exhibit a decrease in oxygen consumption rate with a decrease in dissolved oxygen concentration are known as "oxygen regulators" [41]. As dissolved oxygen levels decline, fish may display symptoms of hypoxia and transition from being "oxygen regulators" to "oxygen conformers". For instance, when the levels of dissolved oxygen drop to 1.9 and 2.6 mg·L^{-1} at temperatures of 15 and 20 °C, respectively, the Yellowtail Kingfish

(*Seriola lalandi*) shifts from being an "oxygen regulator" to an "oxygen conformer", indicating the onset of hypoxia [26]. Our study on juvenile largemouth bass showed that their oxygen consumption rate remains relatively stable when the dissolved oxygen concentration is sufficient. However, the oxygen consumption rate declines when this concentration falls below a certain point, known as P_{crit}, indicating the onset of hypoxia. This has been observed in other fish species, such as Atlantic salmon, which also exhibit a decline in oxygen consumption rate as the dissolved oxygen concentration falls [17]. Hypoxia triggers fish cells to increase the synthesis of reactive oxygen species (ROS), leading to oxidative stress, which can have a detrimental effect on the growth and survival of fish [42]. Therefore, it is essential to maintain the dissolved oxygen concentration adequately.

The effect of temperature on the tolerance of fish to hypoxia is significant. Lower temperatures can slow down metabolic rates and increase oxygen saturation concentration in water, which can delay hypoxia damage in fish. Studies have shown that increasing temperature in low-oxygen environments can lead to the apoptosis of liver and gill tissues of juvenile largemouth bass, resulting in a decline in hypoxia tolerance [43]. This research examined P_{crit} levels for juvenile largemouth bass at 5, 10, 15, and 20 °C and found that these thresholds were 1.12 ± 0.25, 1.15 ± 0.09, 1.61 ± 0.04, and 1.90 ± 0.12 mg·L^{-1}, indicating increased hypoxia tolerance that is temperature-dependent. Furthermore, this study observed changes in behavior such as surfacing, increased frequency and amplitude of gill movement, and abnormal swimming by juvenile largemouth bass under hypoxia, which attempt to augment oxygen intake and adapt to a hypoxic environment during transport. These hypoxia-induced changes in behavior are similar across various fish species, including tilapia (*Oreochromis niloticus*), zebrafish, and Dover sole (*Solea solea*) [44–46]. Therefore, it is crucial to oxygenate the environment during transportation when the dissolved oxygen concentration drops to the P_{crit} of the corresponding temperature. Additionally, one should observe abnormal behaviors such as surfacing, increasing frequency and amplitude of gill movement, and abnormal swimming to prevent hypoxia's impact on juvenile largemouth bass during transportation.

5. Conclusions

Compared with fish at the farm, live transport, including the effects of cooling and hypoxia, represented the main stressor in our study. The cooling method, temperature, and oxygen levels likely contributed to the effect on the behaviors and metabolism of juvenile largemouth bass. It was found that keeping transport temperatures low can lead to a decrease in the respiratory metabolism and P_{crit} of juvenile largemouth bass while enhancing their hypoxia tolerance. Nevertheless, it is worth noting that abrupt temperature fluctuations may have a significant impact on the tail-beat frequency and trigger unusual behavior. In addition, the unusual behavior and respiration metabolism found after P_{crit}, which caused by hypoxia. We demonstrate that behavioral and respiratory metabolism can reflect the effects of temperature and dissolved oxygen on largemouth bass. Therefore, to reduce the effects of cooling stress and improve hypoxia tolerance, we suggest transporting juvenile largemouth bass at an optimal low temperature (15 °C). At the same time, we suggest using uniform cooling to reduce temperature and supervising behaviors and oxygen levels (above P_{crit}).

Author Contributions: Visualization, data curation, and writing—original draft, H.S. Project administration, funding acquisition, and resources, F.G. Project administration, resources, and supervision, X.Q., G.Z. and D.F. Validation and supervision, S.N. and G.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ("Spearhead", "Leading Yan" research and development program of Zhejiang Province) grant number (2023C02029) and (National key research and development plan) grant number (22020YFE0200100).

Institutional Review Board Statement: All experimental and sampling procedures were conducted according to the Guidelines of the Animal Care of the Zhejiang Ocean University and were approved by the Ethics Committee of the university (Approval code: 2023088).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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