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Effects of Constant Water Flow on Endurance Swimming and Fatigue Metabolism of Large Yellow Croaker

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Abstract: A trend in large yellow croaker (*Larimichthys crocea*) aquaculture is to establish production sites suitable for extreme weather conditions. However, continuous and strong currents can harm fish welfare. To determine a suitable site location, the swimming ability of large yellow croakers must be assessed. This study aims to provide novel insights into the physiological characteristics of large yellow croaker swimming and a reference for fishing and cage site selection. Currently, research on large yellow croakers has focused on behavior analysis. Herein, we investigate the effects of swimming on large yellow croakers' metabolites by examining the preferred speed of the group and the sustained swimming ability of single-tailed fish. We evaluated factors that influence the large yellow croaker's swimming fatigue by quantifying the metabolite contents and constructing a sustained swimming model. The results showed that large yellow croaker populations tend to grow in low-velocity environments, similar to their traditional habitat. The samples were taken at different swimming times at a flow velocity of 0.35 m/s. According to the results of the metabolite content analysis, blood glucose levels are closely associated with the swimming ability in large yellow croakers. The content of liver glycogen, which regulates blood glucose concentration, decreased in a certain linear relationship. The sustained swimming model of the large yellow croaker was constructed according to the changes in liver glycogen content. Based on our findings, we recommend the following: (1) for large yellow croakers with a size of approximately 13.5 cm (approximately 1 year old), the water velocity inside the cage should not exceed 2.6 BL/s; (2) the concentration of liver glycogen limits the sustained swimming ability of the large yellow croaker, providing a reference for studying the swimming ability of other fish.

Keywords: sustained swimming; swimming preference; fatigue metabolism; hepatic glycogen; *Larimichthys crocea*

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1. Introduction

Marine aquaculture has become an important part of the production of high-quality aquaculture products due to the growing demand for high-quality protein [1]. China is vigorously developing its marine aquaculture industry, with its aquaculture sites expanding from nearshore to distant seas. This change has placed new demands on culturing equipment and management models. More importantly, changes in culturing sites generated interest in studying the swimming ability of cultured fish species [2].

The large yellow croaker (*Larimichthys crocea*), *Sciaenidae*, occupies the Pacific Northwest and is the most productive marine cultured fish species in China, with production exceeding 120,000 tons in 2019 and growing steadily [3,4]. While offshore aquaculture has suitable water quality and a habitat closer to nature, fish exposed to the open environment often experience strong currents [5]. Constant currents test the swimming ability of cultured fish. Usually, living in an environment with strong currents will affect fish growth, and possibly due to swimming fatigue and getting stuck on cage walls, cause death

and economic losses [6]. At present, research on the swimming ability of fish is extensive. Different swimming speeds can be divided into preferred swimming speed [7], critical swimming speed [8], and sustained swimming speed [9]. The study of the group's spontaneous preferred swimming speed provides insights into the nature of the migration of wild populations [5,10]. Currently, the swimming ability of fish is evaluated in different ways. Among these, the critical swimming speed (U-crit), referring to the maximum swimming speed achieved by fish in a certain time step, is the most widely used. These results are affected by time steps and speed increments (typically 0.5 body lengths per second). In most cases, however, strong currents last longer, and hence it is necessary to determine the distance at which fish swim at multiple constant flow rates.

The swimming ability of fish varies among species, and is influenced by various factors including body size, red muscle content, muscle mitochondrial density, and mitochondrial respiration rate [11]. In high-flow aquaculture environments, fish must accordingly increase their swimming speed, which often leads to earlier fatigue. Acid–base and endocrine disturbances caused by excessive swimming have been reported to cause mortality in Atlantic salmon (*Salmo salar*) [12]. Zhu et al. investigated the yellow catfish (*Pelteobagrus vachelli*) and identified a link between fish swimming ability and metabolites in the body. The authors also showed that muscle and blood lactate concentrations increased over time, while liver, muscle, and blood glucose levels decreased [13]. However, in American redfish, the concentration of muscle glycogen was not significantly different in the tested swimming periods [14]. These findings suggest differences in the metabolic capacity of different fish.

Fish often have group swimming behaviors closely associated with fish migration and habitat selection [15,16]. Sustained swimming speed is an important factor limiting the distribution of river fish and amphibians [17]. The critical swimming speed of juvenile *Argyrosomus japonicus* in the estuaries is 1.7 body lengths s^{-1} (BL/s) [18]. Coral reefs in low flow areas are populated by fish with relatively weak sustained swimming ability (<1.11 BL/s), including *Dischistodus prosopotaenia*, *Apogon trimaculatus*, and *Apogonid A* [9]. The U_{crit} of masu salmon migrating from the ocean to the upper reaches of the river is 1.89 BL/s [19]. *Salmo salar* cultured in open water with stronger currents have a U_{crit} of 2.74 BL/s [7]. These studies show that the environment in which fish are cultured must be adapted to their swimming ability.

Currently, the Chinese aquaculture industry is constructing deep-water cages in the open sea to meet the growing demand for seafood [20,21]. Atlantic salmon can only initially adapt to the culture environment of open-sea cages at the age of 1 year [6]; hence, this study conducted a flow preference test and a continuous swimming ability test for a large yellow croaker population in the same period. Changes in the levels of five metabolites at six time points at an endurance swimming time of 150 min were also assessed. The continuous swimming model of large yellow croaker was constructed based on changes in the levels of main metabolites that limit swimming ability. This study provides suggestions for the site selection of large yellow croaker aquaculture cages and the cage culture strategy in open sea, and will facilitate future physiological and metabolic research on fish swimming.

2. Materials and Methods

2.1. Materials

Large yellow croakers were purchased from Zhoushan Breeding Station in Zhejiang Province. A total of 400 large yellow croakers, with an initial weight of 28.4 ± 0.9 g, were temporarily reared for 1 week in 5 automatic pump oxygen water tanks (length × width × height: 1 × 1 × 1 m, water depth: 0.5 m). During the temporary rearing period, the water temperature was constant at 20 °C, the dissolved oxygen concentration was 6.5 mg/L, and the photoperiod cycle was 12 h day:night. Fish were fed twice daily (at 3% of their total weight) with a commercially available floating pellet diet for marine fish (12.71% moisture, 10.24% fat, 45.08% crude protein) at 7:00 and 19:00. After two hours of feeding, a siphon was used to remove residual feed and excreta. The daily change of water accounts for one third of the total. The experimental environment is the same as the

domestication environment. Experimental fish were anesthetized in an aqueous solution of 60 mg/L eugenol. The blood was drawn and then dissected. The large yellow croakers died due to blood loss during the blood collection. Every effort was made to minimize the suffering of the experimental fish in this study.

2.2. Laboratory Equipment

The experiment was carried out in a multifunctional two-body water tank (Figure 1, drawn by 3DMAX 2022, Autodesk, California, USA). The total length of the water tank was 17.13 m and it was divided into two tunnels. The two parts were isolated, each with a separate water flow speed. The cross-sectional area of channel C varies with location, serving as a site for the velocity preference test of fish. The cross-sectional area gradually decreases from 1 to 2, while the flow velocity increases, forming a stable flow velocity change. In Figure 1, the area was 80 cm × 80 cm at 1 and 20 cm × 80 cm at 2, and the flow velocity at 1 was the smallest. The cross-sectional area of channel G in the endurance swimming test did not change, the water flow speed was the same, and the cross-sectional area was 50 cm × 80 cm. Studies have shown that the choice of experimental equipment has a significant impact on fish swimming ability [22]. In the experiment, a metal cage was set up in a water tank to simulate the conventional cage culture environment, and an air pump was used to provide sufficient oxygen. Electric motors and mesh rectifiers generated a stable and uniform circulating water flow to test the swimming ability. The flow meter was placed at the center of the water channel with a 15 cm probe from the bottom of the water channels G and C (1 site) to measure the water flow speed in real time. We also set up 3 cameras to record the whole swimming process.

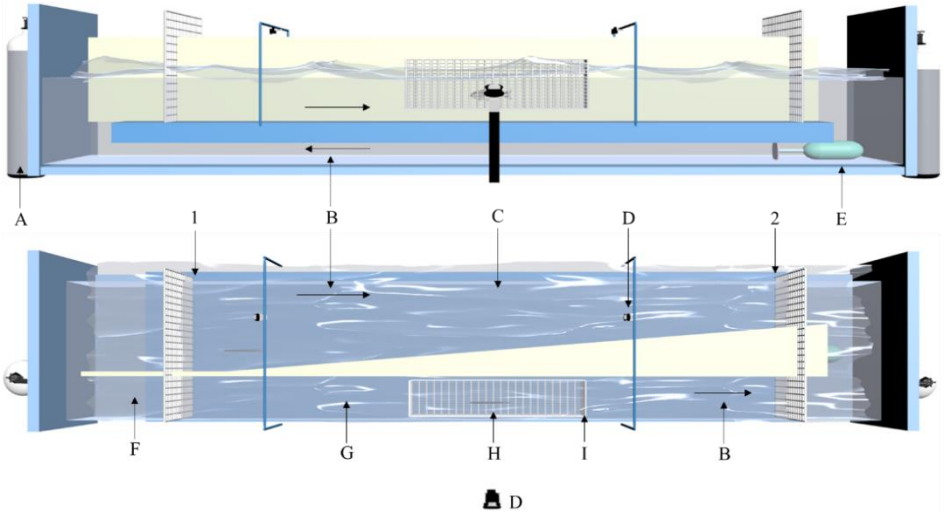

Figure 1. Swimming ability test flume. (**A**) Air pump. (**B**) Flow direction. (**C**) Sink 1. (**D**) Camera. (**E**) Motor. (**F**) Rectifier section. (**G**) Sink 2. (**H**) Fish. (**I**) Net cage.

2.3. Swimming Preference

A total of 64 large yellow croaker juveniles with a body length of 13.47 ± 2.45 cm were placed in tank C as a group and adapted in still water for more than 24 h to reduce the stress associated with environmental changes. Five groups of flow rates were set up (1 site of tank C), and the flow rates at different positions of tank C were represented as

$$V = \frac{8R}{8 - 0.6X}$$

where V is the flow rate at any site in the tank C (m/s), R is the flow rate measured at 1 site (m/s), and X is the distance from any site to 1 (m). Before the formal test, we adjusted the flow velocity from 0 m/s to 0.024 m/s within 1 min (the flow velocity refers to the

flow velocity at 1 point where the cross-sectional area of the water tank is the largest, while the flow velocity in the flow rate preference test refers to the flow velocity at this place) for 200 min. The distribution of fish in the tank at 200 min was observed and recorded using a camera (Nikon, Tokyo, Japan; 25 frames/s). The tested fish were put back into the automatic pump oxygen water tank for acclimation, and the same experiment was not carried out again. The experiment was terminated, the water flow was readjusted to 0 m/s, and another group of fish with no significant difference in body length underwent the same acclimation process for 24 h in still water. We adjusted the flow velocity to 0.038 m/s and observed and recorded the fish distribution at 200 min (before each change in flow velocity, we adjusted the flow velocity to 0 m/s to acclimate for more than 24 h). The flow velocities were 0.024, 0.038, 0.05, 0.078, and 0.095 m/s, and a total of 320 large yellow croakers were tested. The testing area was completely closed to reduce the influence of the external environment and noise. For detailed experiments, please refer to Lou Yudong et al. [23].

2.4. Sustained Swimming Capacity Test

The swimming ability of fish is often measured by swimming speed or time. Under laboratory conditions, fish behaviors, such as sticking to the net and swimming against the wall, interfere with the experiments. These behaviors can be effectively avoided in the experiment using the metal cage set in the tank G. The endurance swimming experiment was intended to control the water flow speed and measure the swimming time at a given flow rate. The test results of the endurance swimming time provide a basis for the subsequent measurement of physiological indicators. The water depth of the tank was set to 0.40 m. The flow rates of the six experiments were set to 0.15, 0.25, 0.35, 0.4, 0.45, and 0.5 m/s. The swimming speed was defined as the current flow rate when swimming against the current. The groups are shown in Table 1. During the experiment, the large yellow croaker with no significant difference in body length ($p > 0.05$) was selected from the culture pond and placed into a tank with a flow rate of 0 m/s for acclimation for more than 1 h, after which the flow velocity was uniformly accelerated from 0 m/s to the design flow velocity within 1 min, and the swimming state was monitored using a camera. The fish touched the net for more than 10 s to mark the end of the experiment. Five large yellow croakers of the same body length were used for each flow rate, and each fish was tested once. Six flow rates were tested with a total of 30 fish. During the experiment, to avoid mutual interference of large yellow croaker, only one fish was taken at a time that was not subjected to the follow-up swimming experiment. After the experiment, the body weight and length were measured. From this, Fulton's body condition factor K was estimated as

$$K = \frac{100{,}000 \times W}{L^3}$$

where W is the weight (g) and L is the body length (mm) [24]. K is determined by measuring the weight and length of individual fish. It is used to study the relationship between the morphological index and the physiological condition of fish [25]. For detailed experiments, please refer to Chao Shuai et al. [14].

According to the results of the endurance swimming experiment, a swimming speed of 0.35 m/s was selected for the subsequent experiments (the average swimming time of large yellow croaker was 214.4 min at this speed). To determine the changes in the main metabolite concentrations of large yellow croaker during the whole swimming process, six sampling time points were set in the experiment: 0, 30, 60, 90, 120, and 150 min. Before the experiment, fish were placed into tank G for 1 h of acclimation, after which the flow velocity was uniformly accelerated from 0 m/s to the design flow velocity (i.e., 0.35 m/s) within 1 min. When the fish reached the set sampling time node, the experiment was immediately terminated, and the fish were taken out and anesthetized. Using a 1 mL sterile syringe, 0.3 mL blood samples were collected from the tail vein, centrifuged at 3000 r/s to obtain serum, and stored at 4 °C. The liver and back muscles were dissected in a sterile

environment and stored in a −80 °C refrigerator. The blood glucose (kit number: F006-1-1) concentration was measured via the glucose oxidase method, while the concentrations of liver glycogen (kit number: A043-1-1), muscle glycogen (kit number: A043-1-1), muscle lactate (kit number: A019-2-1), and blood lactate (kit number: A019-2-1) were measured via colorimetry. All kits were purchased from Nanjing Jiancheng Bioengineering Institute and operated according to the instructions. Each sampling node was repeated 5 times, and a total of 30 fish were sampled.

Table 1. Body length, weight, and *K* of large yellow croaker.

Index	Group						F	*p*
	1	2	3	4	5	6		
Absolute swimming speed (m/s)	0.15	0.25	0.35	0.40	0.45	0.50	\	\
Relative swimming speed (BL/s)	1.10	1.85	2.60	2.96	3.33	3.70	\	\
Swimming time (min)	632 ±22.5 ^{a}	317 ±16.3 ^{b}	190 ±10.5 ^{c}	127 ±7.5 ^{d}	76 ±5.7 ^{e}	27 ±2.1 ^{f}	654.2	<0.001
Body length (cm)	13.62 ± 0.10	13.58 ± 0.06	13.64 ± 0.17	13.08 ± 0.05	13.66 ± 0.04	13.70 ± 0.03	1.57	0.21
Weight (g)	27.92 ± 1.33	27.90 ± 1.87	28.46 ± 0.63	27.74 ± 1.10	28.10 ± 0.64	27.94 ± 1.25	0.042	0.99
K	1.11	1.11	1.13	1.11	1.10	1.09	0.377	0.859

In the same row, values with different superscript letters indicate significant differences ($p < 0.05$). Data are shown as mean ± SE.

2.5. Data Analysis

Data are presented as mean ± standard error. All experimental data were recorded and analyzed using Excel 2021 and IBM SPSS Statistics 26; images were generated using origin 2021. The data were assessed for normality using the Shapiro–Wilk method, while one-way analysis of variance (ANOVA) was used to analyze the differences in body length, body weight, swimming ability, and biochemical characteristics of the experimental groups. When one-way ANOVA indicated a significant difference of $p < 0.05$, the data were further compared using Tukey's test.

3. Results

3.1. Swimming Preference

Figure 2 shows the distribution of the large yellow croaker in the water tank at 200 min. The density of large yellow croakers are represented by different colors; the higher the degree of red, the denser the distribution of large yellow croakers. The five panels all show that the number of large yellow croakers in the first 2 m of the tank accounted for more than 85% of the total at 200 min. The results show the following: (1) viewed along the x-axis (longitudinal), the large yellow croaker population prefers to be distributed in places with low flow velocity (1 site in tank C); (2) viewed along the y-axis (horizontal), the fish prefer to be distributed on both sides of the tank when swimming, that is, swimming against walls or obstacles. Overall, the distribution results in the tank were not significantly different.

3.2. Swimming Time

To assess the relationship between the sustained swimming time and flow velocity, we used absolute and relative swimming speed to describe the endurance swimming ability of large yellow croaker (Figure 3). The swimming time of large yellow croaker was significantly different at different flow rates ($p < 0.05$). Specifically, when the absolute swimming speeds were 0.15, 0.25, 0.35, 0.40, 0.45, and 0.50 m/s (relative swimming speeds were 1.10, 1.85, 2.60, 2.96, 3.33, 3.70 BL/s), the corresponding endurance swimming times of large yellow croaker were 631, 316, 191, 127, 76, and 27 min, respectively. According to the results, we used the power function for fitting analysis, and obtained the absolute swimming speed as $Y = 1529 * e^{\left(\frac{-x}{19}\right)} - 74$ and the relative swimming speed as $Y = 1501 * e^{\left(\frac{-x}{1.4}\right)} - 79$.

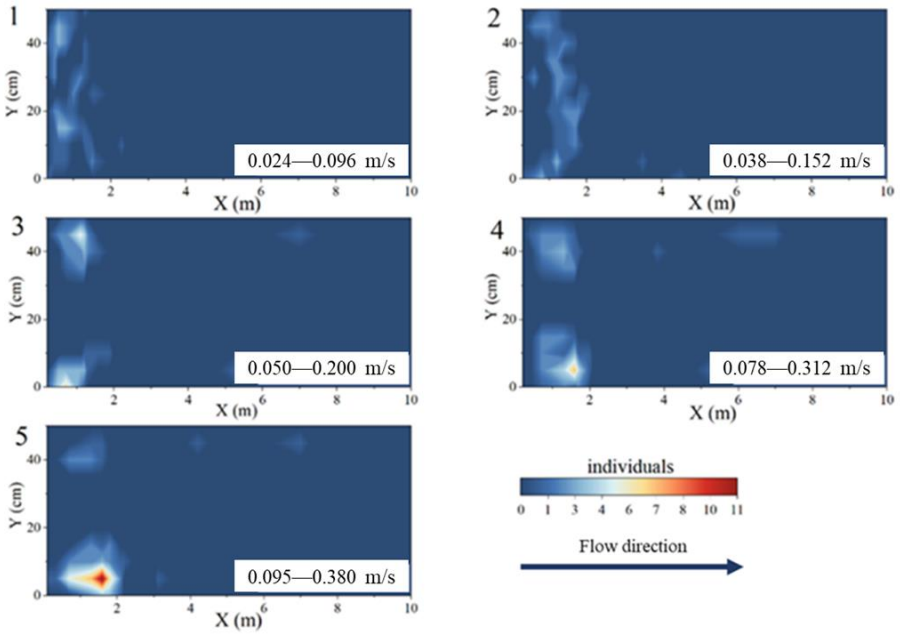

Figure 2. Distribution of large yellow croaker at 200 min. (**1**–**5**) in the figure represent the distribution of large yellow croaker under the five flow rate gradients respectively. The density of large yellow croakers are represented by different colors; the higher the degree of red, the denser the distribution of large yellow croakers.

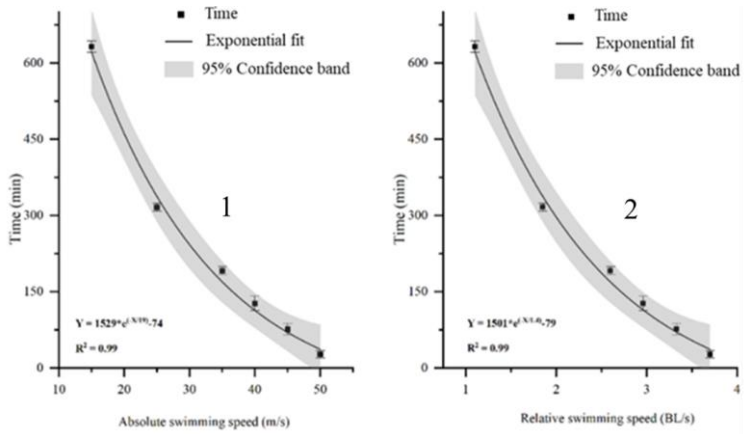

Figure 3. The relationship between absolute swimming speed (**1**) and relative speed (**2**) of large yellow croaker and time under constant flow rate.

3.3. Biochemical Characteristics

Figure 4 lists the concentrations of five metabolites of large yellow croaker at six time points when the flow rate was 0.35 m/s. Compared to 0 min, the levels of glycogen and lactate were significantly different in large yellow croaker after swimming for 150 min ($p < 0.05$). The concentrations of liver glycogen in adjacent experimental groups were significantly different ($p < 0.05$), while no significant difference was observed in muscle glycogen concentration between individual adjacent experimental groups (e.g., 0 and 30 min, 120 and 150 min; $p > 0.05$). However, there were significant differences in muscle glycogen concentration between each experimental group at 60 min intervals (e.g., 0 and 60 min, 30 and 90 min; $p < 0.05$). Glucagon, muscle lactate, and blood lactate levels showed similar trends. The concentrations of the three physical and chemical indexes significantly increased with the increase in swimming time ($p < 0.05$). In the experimental group with 60 min intervals, the concentration difference was significant ($p < 0.05$).

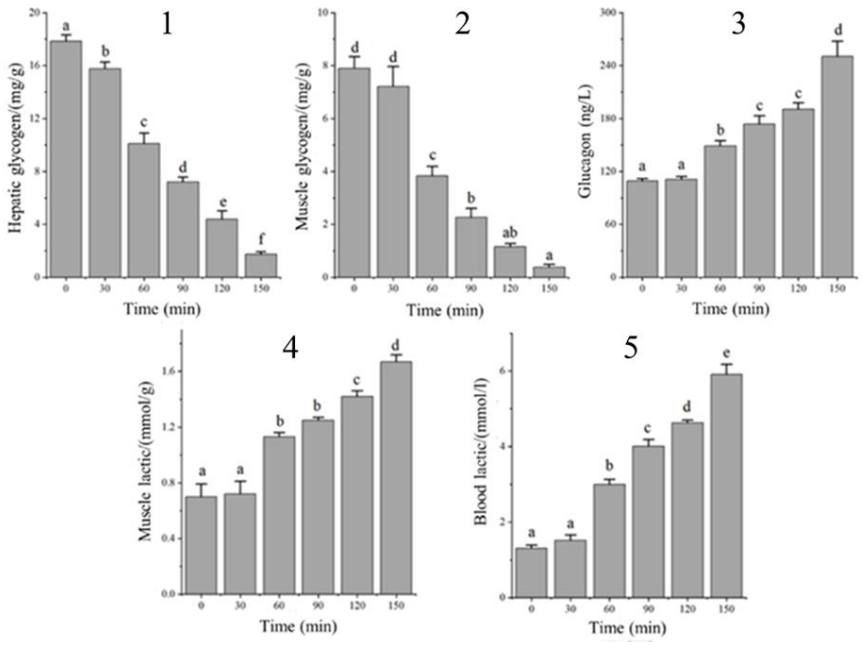

Figure 4. Metabolite concentrations at six time points in 0.35 m/s flow rate. In the same metabolism, values with different superscript letters indicate significant differences ($p < 0.05$). (**1**–**5**) are subfigures of changes in concentrations of five metabolites. (**1**): Hepatic glycogen; (**2**)**:** Muscle glycogen; (**3**): Glucagon; (**4**): Muscle lactic; (**5**)**:** Blood lactic.

Five metabolite concentrations were analyzed using principal component analysis (Figure 5). The results showed that the first principal component (PC 1) significantly differentiated the concentration changes in glucagon compared with glycogen and lactate, while PC 2 significantly differentiated the changes in glycogen and lactate concentrations. During swimming, the concentration changes of the same metabolites were consistent, while the change trends of different metabolites showed obvious differences. Changes in muscle and blood lactate were grouped together, and so were muscle and liver glycogen, while those in glucagon concentration were not.

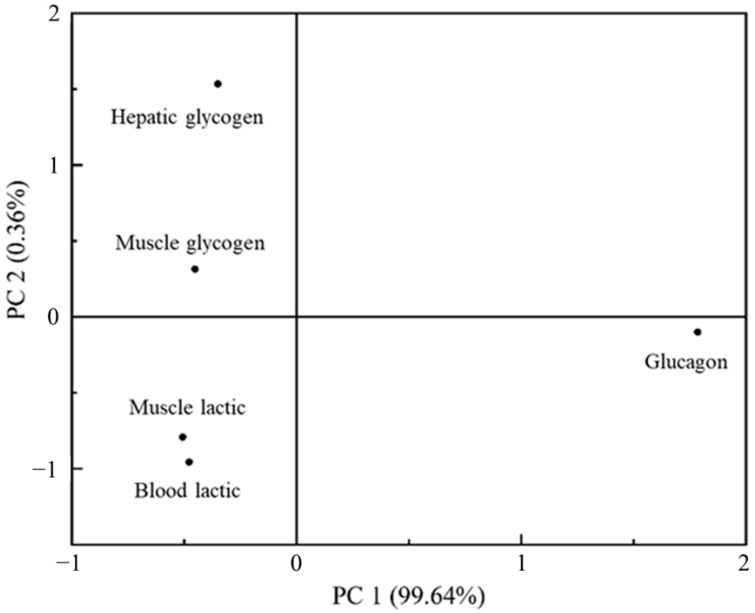

Figure 5. Principal component analysis plot.

3.4. Metabolite Multiple Linear Regression Analysis

To further evaluate the effect of the five metabolites on the swimming fatigue of large yellow croaker, we constructed a swimming fatigue model and used multiple linear regression analysis (ML), one of many linear models used to study the prediction of one or more variables. However, ML regression requires normality, homogeneity of variance, and non-collinearity among variables [23]. The results showed that the sample data supported at least a normal distribution and homogeneity of variance. However, the differences in blood and muscle lactate and muscle glycogen were not significant ($p > 0.05$); that is, they did not significantly affect swimming fatigue of large yellow croaker (Table 2). Hepatic glycogen and glucagon require further analysis.

Table 2. Multiple linear regression analysis of swimming fatigue of large yellow croaker.

	β	Standard Error	t	p	*VIF*	R^2
Constant	96.837	41.086	2.357	0.027		0.974
Hepatic glycogen	−6.320	2.063	−3.063	0.005	25.301	/
Muscle glycogen	1.592	2.755	0.578	0.569	23.571	/
Muscle lactic	−44.090	28.189	−1.564	0.131	37.304	/
Blood lactic	14.803	6.566	2.255	0.100	26.306	/
Glucagon	0.199	0.070	2.861	0.009	4.556	/

The data in Table 3 show that the two independent variables in the standard model have a significant predictive effect on the time of the dependent variable according to the statistical results of ANOVA ($F = 382.128$, $p < 0.05$). The multicollinearity (*VIF*) results of liver glycogen and glucagon were 4.243 ($VIF < 10$); that is, the two variables were not correlated with each other. Standard regression analysis showed that the fitting degree of the prediction model to the dependent variable was $R^2 = 0.966$.

Table 3. Multiple linear regression analysis of swimming fatigue of large yellow croaker.

	β	Standard Error	t	p	*VIF*	R^2	p	F
Constant	98.075	17.509	5.601	0	/			
Hepatic glycogen	−6.625	0.639	−10.375	0	4.243	0.996	<0.05	382.128
Glucagon	0.243	0.072	3.37	0.002	4.243			

The absolute value of β in Table 3 indicates the order of independent variable importance. The variable with the highest β value (in absolute value) is the relatively most important independent variable. By examining the contribution of the independent variables to the model, we found that the concentration of liver glycogen had the greatest contribution ($\beta = 6.625$). Glucagon, which contributed the least to the model, had a β value of 0.243 due to the nature of the regression analysis (any significant predictive effect on the dependent variable must be included in the calculation model).

According to the analysis results, the following regression equation was obtained:

$$T = 8.075 - 6.625H + 0.243G$$

where T, H, and G represent swimming time, hepatic glycogen concentration, and glucagon concentration of large yellow croaker, respectively.

4. Discussion

4.1. Swimming Behavior of Large Yellow Croaker

A previous study found that the critical swimming speed of large yellow croaker was 3.0 BL/s at a temperature range of 19.0–21.0 °C [26]. The tank flow rate range designed in this study was 0.17–2.8 BL/s, with a maximum flow rate of < 3.0 BL/s. The tank was free of obstacles for the test fish to swim back and forth. Notably, this study considered

various flow rate environments for large yellow croakers voluntarily swimming, which is significantly different from the design of the endurance swimming ability experiment. Therefore, the location distribution of the large yellow croaker population in the tunnel at 200 min is attributed to the preference for flow velocity.

The large yellow croaker has a group activity habit, and the preference test of the swimming speed of single-tailed fish has no direct value to traditional fisheries. Studies have shown that group swimming affects the metabolic rate of fish and the behavior of fish while swimming [27]. Our results showed that, in the non-uniform flow field, the number of large yellow croaker was significantly higher than that in other environments in the environment where the flow rate was < 0.74 BL/s. In addition, within 20 min of the trial, the fish had a higher probability of swimming back and forth in the tank; after one hour, the fish gathered in the low-velocity area and maintained a circle-like formation [6]. There is no catchable food in the tank to replenish energy, and the fish gradually seek the most energy-efficient swimming speed. Fish in a certain formation can reduce the cost associated with transport and increase swimming time [28,29]. Studies have also shown that when *Scomber japonicus* and *Dicentrarchus labrax* swim, the frequency of tail beats in fish at the rear of the school significantly decreases, thereby causing a 9–23% reduction in oxygen uptake [29,30]. However, in *Acipenser brevirostrom*, there was no difference in U_{crit} between individual and group test fish [31]. Therefore, the ability to exploit school swimming appears species-specific; hence, research on group swimming behavior of yellow croaker remains warranted.

The preference of the fish school for the low flow environment is consistent with that of *Schizothorax oconnori* [32], and the same result also appeared in the swimming preference experiment of black sea bream [23]. Research suggests that fish preference for flow velocity may correlate with optimal cruising speed, a notion that was confirmed in a study of Atlantic salmon respiration measurements and minimal transport costs. However, American redfish disperse at low flow rates and aggregate at a flow rate of 2.1 BL/s [22]. Although swimming speed preferences vary with fish species, the test of large yellow croaker populations can help to study their living habits and facilitate future studies on large yellow croaker aquaculture cage site selection and fish welfare.

4.2. Swimming Ability of Large Yellow Croaker

According to the five flow velocity experiments, the average swimming time of large yellow croaker was 20 min when the flow velocity was 0.50 m/s. In contrast, Atlantic salmon have a sustained swimming speed range of 0.96–1.02 m/s [33]. *Acipenser brevirostrum* with a body length of 23.8 cm can swim for 170–200 min at a speed of 1.47 BL/s [34]. The critical swimming speed of American redfish reaches 3.9 BL/s. The critical swimming speed of sea bass (*Lateolabrax japonicus*) is 60 cm/s. These data indicate that the swimming ability of large yellow croaker is at a moderate level compared with Atlantic salmon, i.e., not strong.

Furthermore, in the initial stage of swimming, the large yellow croaker was closer to the front of the experimental device and continuously adjusted its position in a small range, possibly searching for the most energy-efficient location, eventually moving to the back end of the cage. The change in the position of the large yellow croaker in the cage is consistent with the findings of Hvas on Atlantic salmon, which showed that when the fish swim at an environmentally determined speed, they develop top-current swimming and maintain a fixed position [35]. This behavior is possibly associated with the fish's preferred swimming speed and group swimming behavior [36].

4.3. Biochemical Changes

Fatigue in constant speed test is thought to be caused by substrate depletion [37]. The swimming process of fish is mainly based on aerobic respiration, with glycogen as the primary source of energy. In this study, we measured the concentration of glucagon in fish and found an increase in glucagon concentration of approximately 140 ng/L, suggesting an increased blood glucose requirement for fish while swimming. Sources of blood sugar

include gluconeogenesis and glycogenolysis; studies have shown that blood sugar levels are significantly higher in American redfish and black snapper when they continue to swim [38]. Although studies on rainbow trout have shown that glucagon has limited ability to regulate blood glucose, the fact that glucagon concentrations are significantly different in different periods of continuous swimming in large yellow croakers suggests that blood glucose concentrations are closely associated with fish exercise fatigue [39].

The correlations of changes in concentrations of the five metabolites were analyzed using principal component analysis. The results showed that the concentration changes of the same metabolites tended to be consistent in the swimming process of large yellow croaker. Both liver and muscle glycogen concentration changes significantly decreased over time, and the two glycogen concentration changes were similar. The effect of exercise fatigue on muscle glycogen concentration remains controversial. For example, a study on rainbow trout (*Salmo gairdneri*) showed that short periods of vigorous swimming dropped muscle glycogen concentrations by >50% [40]. Similarly, the decrease in muscle glycogen concentration of coalfish (*Gadus virens L.*) was proportional to the increase in swimming speed [41]. In contrast, muscle glycogen concentration in redfish was not significantly related to swimming time. These results suggest that the energy sources of fish are diverse during swimming, and the effects of the same metabolic substrate on different species of fish also differ. Although muscle glycogen has not been verified to significantly affect the swimming ability of fish, the results of liver glycogen are more consistent; that is, the concentration of liver glycogen is closely related to swimming fatigue, and significantly decreases with the increase in fatigue degree [41]. This is mainly since the increase in glucagon concentration promotes the breakdown of liver glycogen into blood sugar, which provides energy for swimming. The lactic acid concentration in fish after fatigue is an important indicator to measure the anaerobic exercise capacity of fish [42]. Experiments show a strong correlation between the concentration changes of blood lactic acid and muscle lactic acid. It is speculated that there may be a "lactic acid release" mechanism in large yellow croaker [43], that is, a Cori cycle in large yellow croaker. However, lactate is not readily exchanged between muscle and blood; both lactate and hydrogen ions remain in the white muscle for in situ metabolism, and lactate is oxidized or undergoes gluconeogenesis [44]. These findings are consistent with the results of this study. As shown in Figure 4, the ratio of lactate to blood in muscle is 1:1000, which is similar to the findings of Milligan, who assessed the changes in blood lactate concentration after swimming fatigue in salmon [45]. Compared with benthic species such as *Pleuronectiformes*, the muscles of flounder hardly release lactic acid and have extremely low blood lactate levels [46]. The mechanism of action of the Cori cycle in ectothermic animals such as fish remains unclear [47], and so does the extent of the effect of lactic acid on fish fatigue. The muscle and blood lactic acid concentrations of large yellow croaker in this study were found to be significantly increased. This indicates that the degree of fatigue was positively correlated with the lactic acid concentration.

4.4. Relationship of Physiological Parameters with Swimming Ability

Swimming is an energy-intensive process, and the aerobic metabolism of muscles provides the energy required for swimming. In fish, sugar is stored in the muscles and liver as glycogen and in the blood as glucose [48]. Fish with strong swimming abilities are known to have higher blood glucose levels, whereas fish with slower movement have lower blood glucose levels [49]. This study tested the changes in the concentration of five metabolites in large yellow croaker, aiming to explore the relationship between its physiological factors and swimming ability. The study used multiple linear regression analysis to obtain two main metabolites affecting the endurance swimming of large yellow croaker: liver glycogen and glucagon. Compared with Wang's research, which is a physical model of the physical energy distribution of American redfish [14], this study analyzed the statistical significance of five metabolites' concentrations and endurance swimming ability, and the influence of single liver glycogen concentration was extended to two key

physiological indicators. Herein, we determined whether changes in the concentrations of five metabolites in the standard model were significantly correlated with the prediction of swimming fatigue. Firstly, the assumptions required by the multiple linear regression analysis were assessed, and regression analysis was conducted on the data that meet these assumptions. The predictability of the standard model for the dependent variable was 0.992; from these coefficients, the dependent variable predicts the model well. In terms of the contribution of independent variables to the model, the concentration of liver glycogen showed the greatest effect on swimming fatigue of large yellow croaker, which was 6.625, followed by glucagon at 0.243. Overall, the large yellow croaker can be cultured in waters with a short-term flow rate not exceeding 0.35 m/s or 2.6 BL/s.

5. Conclusions

Marine aquaculture, including purse seine and cage aquaculture, occupies an important position in China's marine fishery production. Oftentimes, it is necessary to resist the influence of long-lasting tidal currents, and hence, understanding group behavior and maximum sustained swimming speed of cultured species is necessary. In this study, the large yellow croaker was taken as the research object to investigate the group flow rate preference and the changes in metabolite concentrations during continuous swimming. Based on principal component and multiple linear regression analyses, a linear relationship model for predicting swimming fatigue of large yellow croaker with metabolite concentration was established. Notably, the conclusion drawn in this study is based on laboratory setting, which did not consider the complex flow of water in real farming facilities; therefore, further studies with large sample sizes and additional variables are warranted.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and all procedures in this study were conducted on fish, and animal care was performed strictly following the Management Rules for Laboratory Animals (Chinese Order No. 676 of the State Council, revised 1 March 2017).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data generated or analyzed during this study are provided in full within the published article.

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