Study on Respiratory Metabolism of Wild *Acanthopagrus schlegelii* in South China Sea under Different Temperatures and Weights

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Abstract: Respiratory metabolism is an important part of bioenergy research. The experimental device of indoor closed flowing respiratory metabolism designed and authorized under an invention patent was used to acquire the changes of physiological activity of wild *Acanthopagrus schlegelii* in the open sea. Specifically, with this device, we conducted respiratory metabolism experiments of wild *Acanthopagrus schlegelii* in the South China Sea with various sizes (body length 96-122 mm, body weight 9.99-17.36 g) at different temperatures (16-32℃) and further analyzed the changes of oxygen consumption rate and ammonia excretion rate of *Acanthopagrus schlegelii* and its metabolic substrates. It could be seen from the results that the changes of water temperature have significant effects on the oxygen consumption rate and ammonia excretion rate of *Acanthopagrus schlegelii* in the South China Sea. The oxygen consumption rate increases with the rise of water temperature, while the ammonia excretion rate shows a fluctuating increase. Under the same water temperature, the oxygen consumption rate and ammonia excretion rate of *Acanthopagrus schlegelii* were significantly negatively correlated with weights ($P<0.01$). Namely, the oxygen consumption rate and ammonia excretion rate of large *Acanthopagrus schlegelii* are lower than those of small *Acanthopagrus schlegelii*, and the variation of oxygen consumption rate and ammonia excretion rate of *Acanthopagrus schlegelii* with different weights conform to the power exponential growth model. The average oxygen consumption rate and ammonia excretion rate per unit weight of *Acanthopagrus schlegelii* are 0.11 mg/(g·h) and 4.48 μg/(g·h) respectively. The change of Q10 coefficient of *Acanthopagrus schlegelii* shows that the Q10 value of oxygen consumption rate per unit weight of *Acanthopagrus schlegelii* is the minimum of 1.31 within the temperature of 24-28℃, and the maximum of 1.69 within 16-20℃. The Q10 value of ammonia excretion rate is the minimum of 1.05 within 28-32℃, and the maximum of 1.78 within 24-28℃. The O: N ratio of *Acanthopagrus schlegelii* in different combinations varies from 15.37 to 44.37. This paper studied the relationship between important environmental factors affecting the respiratory metabolism of *Acanthopagrus schlegelii* and the metabolism of organisms, which is conducive to a better understanding of the metabolic pattern of *Acanthopagrus schlegelii*, thus providing basic data for the proliferation and conservation of *Acanthopagrus schlegelii* resources.

1 INTRODUCTION

Acanthopagrus schlegelii, which belongs to sparidae of perciformes, is distributed chiefly in the western part of the North Pacific Ocean, and also distributed in Bohai Sea, Yellow Sea, East China Sea and South China Sea. *Acanthopagrus schlegelii* is a eurythermic and euryhalinous fish species with excellent adaptability to the environment. Its survival temperature is within 4.3℃-34.0℃, and the suitable

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temperature for its growth is within 17.0℃-25.0℃. Because of its delicious meat, *Acanthopagrus schlegelii* has become an important marine commercial fish and an excellent species for aquaculture which has been widely cultivated in recent years (Lin 2001). *Acanthopagrus schlegelii* is an important breeding species with high economic value and development prospects. In recent years, studies on its breeding have been increasing and made some progresses (Liu 2002, Bai 1999, Xu 2008). As the focus of bioenergy research, respiratory metabolism is also vital to the study of commercial species breeding of marine and freshwater fishery resources and offshore marine pasture. However, there is few research on respiratory metabolism of *Acanthopagrus schlegelii*, especially the wild individuals. In this paper, the respiratory metabolism of wild *Acanthopagrus schlegelii* in the South China Sea was investigated through indoor experiments in simulated marine environment. The results can further enrich the basic data of the study and provide theoretical guidance and basis for the conservation and utilization of wild *Acanthopagrus schlegelii* in the South China Sea and various coastal areas.

2 MATERIALS AND METHODS

2.1 Experimental Materials

Wild *Acanthopagrus schlegelii* used for experiments was caught from the natural waters of Daya Bay in Shenzhen. Healthy and fresh individuals with body length of 96 to 122 mm and weight of 9.99 to 17.36 g were selected. Temporary cultivation was carried out in a disinfected square pond and then the selected individuals were moved to the indoor tank for 48 hours before the experiment. The temperature of seawater in the temporary pond was 24±0.5℃ with salinity of 30.7±.55, and the natural light cycle was maintained during the temporary cultivation period by continuous aeration using oxygenation pumps. The experimental seawater was taken from the natural seawater of Dapengao sea area in Daya Bay and was used after precipitation with salinity of 31.25 and pH of 8.07.

2.2 Experimental Method

The experiment was conducted indoor under natural illumination, and the experiment started at the same time every day to ensure consistent experimental conditions. The experimental device of closed flowing respiratory metabolism was used for measuring the relatively static respiratory metabolism of an organism when it performed respiratory metabolism and its respiratory chamber was completely isolated from the outside air. The experimental device is a self-designed closed flowing experimental device, which is used to measure the oxygen consumption rate and ammonia excretion rate of *Acanthopagrus schlegelii* (Feng 2018). The water temperature was controlled by the cold and warm water exchanger to ensure that the fluctuation of water temperature was ±0.5℃ during the experiment. The seawater entered the experimental respiratory chamber through the panel-type flowmeter, and the sample water was collected and preserved by the outlet sampling bottle. During the experiment, the water velocity was kept at (12±0.4) L/h according to the physiological characteristics of *Acanthopagrus schlegelii* and the pre-experimental results. The experimental subjects were divided into 10 groups based on their weights, with 3 parallel samples in each group. At the same time, 5 water temperature gradients were set, namely 16℃, 20℃, 24℃, 28℃ and 32℃ respectively. When the *Acanthopagrus schlegelii* adapted to the environment and the water flow became stable after a period of time, the water samples were collected from the outlet and fixed on the spot to measure the dissolved oxygen value, and the ammonia nitrogen value was taken under the temperature of -20℃ and then the *Acanthopagrus schlegelii* was kept in the laboratory for measurement. After sampling, the *Acanthopagrus schlegelii* was taken out from the experimental device, drained and dried in an electric thermostatic oven at 60℃ to the constant weight to measure the body length and weight. Dissolved oxygen (DO) was measured by iodometric method, and ammonia nitrogen was measured by FIAstar™ 5000 flow injection analysis system of FOSS. The sodium hypobromite oxidation method in the marine survey specification (GB 1274-2007) was also used for testing and calibration. Each experimental sample was measured twice, and the average value of three groups of parallel samples was taken.

2.3 Analysis Method

2.3.1 Calculation Formula for Oxygen Consumption Rate and Ammonia Excretion Rate

$$Q_0 = \frac{V \times (A_1 - A_2)}{W} \quad (1)$$

$$Q_T = \frac{V \times (N_T - N_0)}{W} \quad (2)$$

Formula (1) is for the calculation of oxygen consumption rate, and Formula (2) is for the calculation of ammonia excretion rate. In Formulae (1) and (2), Q_0 is oxygen consumption rate (mg/g•h), Q_T is ammonia excretion rate (μg/g•h), W is the body weight (g), V is the water velocity (L/h), A_1 is the initial dissolved oxygen (mg/L), A_2 is the dissolved oxygen (mg/L) after a period of time, N_0 is the initial ammonia and nitrogen concentration (ug/L), and N_T is the ammonia and nitrogen concentration (μg/L) after a period of time.

2.3.2 Calculation Formula for Metabolic Impact Intensity

$$Q_{10} = \left(\frac{M_2}{M_1}\right)^{\frac{10}{(T_2-T_1)}} \quad (3)$$

In Formula (3), Q_{10} is the intensity of the effect of water temperature on metabolism, indicating the change rate of respiratory metabolism of acanthopagrus schlegelii for every 10°C increase in water temperature. M_1 and M_2 are the metabolic rates of acanthopagrus schlegelii at temperatures T_1 and T_2 respectively.

2.3.3 Calculation Formula for the Analysis of Respiratory Metabolic Substrates

$$O{:}N = 1000 \times \frac{Q_0}{Q_T} \quad (4)$$

In Formula (4), Q_0 and Q_T are oxygen consumption rate and ammonia excretion rate at the same temperature, respectively.

3 RESULTS AND ANALYSIS

3.1 Relationship between Respiratory Metabolism and Water Temperature of *Acanthopagrus Schlegelii*

The results of oxygen consumption rate of *Acanthopagrus schlegelii* at different temperatures are shown in Fig. 1. Within the temperature ranging from 16 to 32°C, the oxygen consumption rate of *Acanthopagrus schlegelii* increases with the rise of water temperature. According to the figure of the relationship between oxygen consumption rate of *Acanthopagrus schlegelii* and the water temperature, the oxygen consumption rate of *Acanthopagrus schlegelii* with certain body weight has no obvious change, when the experimental water temperature ranges from 24 to 32°C. This might be related to the temperature suitable for the survival of *Acanthopagrus schlegelii*. The oxygen consumption rate of black seabream is the maximum at the experimental water temperature of 32°C. The one-way ANOVA test shows that the effect of temperature on the oxygen consumption rate of *Acanthopagrus schlegelii* reached a highly significant level ($F=18.231$, $P<0.01$) within the range of experimental water temperature.

Within the experimental water temperature ranging from 16 to 32℃, the variation of ammonia excretion rate and oxygen consumption rate of *Acanthopagrus schlegelii* are similar (as shown in Fig. 2). However, with the rise of water temperature, the ammonia excretion rate of *Acanthopagrus schlegelii* demonstrates a fluctuating increase in the experimental water temperature ranging from 24 to 32℃. It is possible that the high temperature affects the excretion of *Acanthopagrus schlegelii* thus causing disorder. The one-way ANOVA test revealed that the effect of temperature on ammonia excretion rate of *Acanthopagrus schlegelii* reached a significant level ($F=3.496$, $P<0.05$) within the range of experimental water temperature.

The results of average oxygen consumption rate and ammonia excretion rate per unit of weight of *Acanthopagrus schlegelii* at different temperatures also indicates that the oxygen consumption rate and ammonia excretion rate shows a fluctuating increase as the water temperature keeps rising (as shown in Tab. 1). The average oxygen consumption rate per unit of weight of *Acanthopagrus schlegelii* is 0.11 mg/g·h and its ammonia excretion rate per unit of weight is 4.48 μg/g·h when the experimental water temperature is 16℃, 20℃, 24℃, 28℃ and 32℃, respectively.

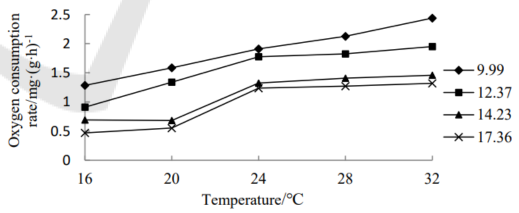

Figure 1: Variation Relationship between oxygen consumption rate and water temperature of *Acanthopagrus schlegelii.*

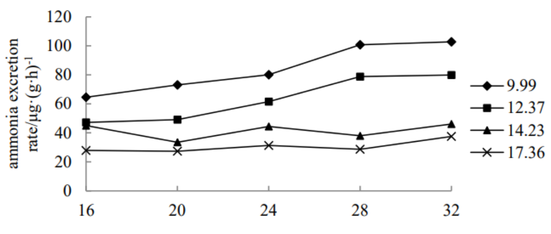

Figure 2: Variation Relationship between ammonia excretion rate and water temperature of *Acanthopagrus schlegelii.*

Table 1: Oxygen consumption rate and ammonia excretion rate per unit of weight under different water temperature of *Acanthopagrus schlegelii.*

Experimental water temperature (℃)	16	20	24	28	32	Average value
Oxygen consumption rate per unit of weight (mg/g•h)	0.07	0.09	0.12	0.13	0.14	0.11
Ammonia excretion rate per unit of weight (μg/g•h)	3.81	3.66	4.33	5.27	5.34	4.48

3.2 Relationship between Respiratory Metabolism and Weight of *Acanthopagrus Schlegelii*

Under the same water temperature, the results of oxygen consumption rate of *Acanthopagrus schlegelii* with different weights are shown in Fig. 3. With the increase of experimental individuals, the oxygen consumption rate of *Acanthopagrus schlegelii* keeps decreasing, i.e., they show a negative correlation. The correlation analysis shows that there is a significant negative correlation between the weight and oxygen consumption rate of *Acanthopagrus schlegelii* at the level of 0.01 ($P<0.01$) within the range of experimental water temperature. Under different water temperatures, the change of oxygen consumption rate of *Acanthopagrus schlegelii* with different weights is consistent to the power exponential growth model (as shown in Tab. 2). The range of value a is 3.6459 to 10.129 and the range of value b is -0.172 to -0.079 in the change of oxygen consumption rate of *Acanthopagrus schlegelii* at the water temperature of 16 to 32℃.

The results of ammonia excretion rate are similar to oxygen consumption rate. The ammonia excretion rate of small *Acanthopagrus schlegelii* is higher than that of large *Acanthopagrus schlegelii*, and the larger the *Acanthopagrus schlegelii*, the smaller the ammonia excretion rate per unit weight (Fig. 4). The correlation analysis shows that there is a significant negative correlation between body weight and ammonia excretion rate of *Acanthopagrus schlegelii* at 0.01 level ($P < 0.01$). Under different water temperatures, the change of ammonia excretion rate of *Acanthopagrus schlegelii* with different weights is consistent with the power exponential growth model as shown in Tab. 3. The range of value a is 197.4 to 843.32 and the range of value b is -0.197 to -0.107 in the change of ammonia excretion rate of *Acanthopagrus schlegelii*.

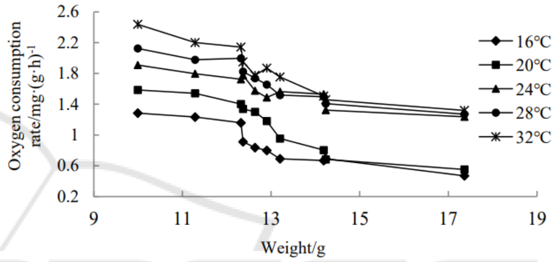

Figure 3: Variation Relationship between oxygen consumption rate and body weight of *Acanthopagrus schlegelii.*

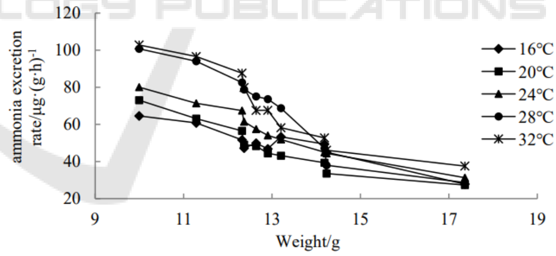

Figure 4: Variation Relationship between ammonia excretion rate and body weight of *Acanthopagrus schlegelii.*

Table 2: The relationship between oxygen consumption rate and body weight of *Acanthopagrus schlegelii.*

Temperature (℃)	Equation	R^2	a	b
16	$y = 6.0098e^{-0.151x}$	0.8807	6.0098	-0.151
20	$y = 10.129e^{-0.172x}$	0.8677	10.129	-0.172
24	$y = 3.6459e^{-0.064x}$	0.8476	3.6459	-0.064
28	$y = 4.7016e^{-0.079x}$	0.8621	4.7016	-0.079
32	$y = 6.1059e^{-0.093x}$	0.8834	6.1059	-0.093

Table 3: The relationship between ammonia excretion rate and body weight of *Acanthopagrus schlegelii.*

Temperature (℃)	Equation	R^2	a	b
16	$y = 197.40e^{-0.107x}$	0.8720	197.40	-0.107
20	$y = 296.11e^{-0.143x}$	0.9321	296.11	-0.143
24	$y = 324.32e^{-0.136x}$	0.9681	324.32	-0.136
28	$y = 843.32e^{-0.197x}$	0.8935	843.32	-0.197
32	$y = 518.85e^{-0.157x}$	0.8929	518.85	-0.157

3.3 Changes in Q_{10} Coefficients Caused by the Metabolism of *Acanthopagrus Schlegelii*

The effect of water temperature on the respiratory metabolism of *Acanthopagrus schlegelii* could be expressed by Q_{10} value (Tab. 4). The results of different experimental water temperatures indicates that the Q_{10} value of oxygen consumption rate per unit weight of *Acanthopagrus schlegelii* is the minimum of 1.31 within the temperature of 24 to 28℃; and the maximum of 1.69 within the temperature of 16 to 20℃. The Q_{10} value of ammonia excretion rate per unit weight of *Acanthopagrus schlegelii* is the minimum of 1.05 within the temperature of 28 to 32℃, and the maximum of 1.31 within the temperature of 24 to 28℃.

3.4 Analysis of Respiratory Excretion Substrate

The relationship between weight, water temperature and O: N of *Acanthopagrus schlegelii* was investigated by the analysis of respiratory excretion substrate. The O: N ratio of *Acanthopagrus schlegelii* under different water temperatures varies (Fig. 5), and the variation of O: N ratio of *Acanthopagrus schlegelii* ranges from 15.37 to 44.37, and the O: N ratio of metabolic substrate is the minimum at 16℃ and the maximum at 28℃. In small *Acanthopagrus schlegelii* and the large *Acanthopagrus schlegelii* with the experimental water temperature below 24℃, the energy-supplying substances of respiratory excretion are a mixture of protein and fat. When the experimental water temperature reached 24℃ or even higher, the energy-supplying substances in the excretion substrate of large *Acanthopagrus schlegelii* are mainly a mixture of fat and carbohydrate.

Table 4: The Q_{10} coefficients of *Acanthopagrus schlegelii* ammonia excretion rate.

Temperature (℃)	Oxygen consumption rate Q_{10}	Ammonia excretion rate Q_{10}
16~20	1.69	1.36
16~24	1.64	1.31
16~28	1.52	1.45
16~32	1.49	1.34
20~24	1.59	1.26
20~28	1.44	1.49
20~32	1.43	1.33
24~28	1.31	1.78
24~32	1.36	1.37
28~32	1.41	1.05

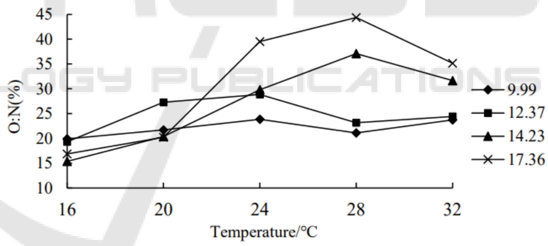

Figure 5: The relationship between body weight, water temperature and O: N on *Acanthopagrus schlegelii.*

4 DISCUSSIONS

Water temperature is an important environmental factor affecting the respiratory metabolism of fish, and its fluctuation is the key focus of biological respiratory metabolism (Spanopoulos-Hernández 2005). The oxygen consumption rate varies among different species. Generally speaking, the oxygen consumption rate and the water temperature are positively correlated within a certain range of water temperature (Song 1997, Wang 2002). The results of oxygen consumption rate of *Acanthopagrus schlegelii* reveal that its average oxygen consumption rate is lower than that of certain fish in the same area, which is related to the maturity of fish and interspecific genetic factors. The oxygen consumption rate increases with the rise of water temperature. As the water temperature rises, the activity performance and biochemical reaction speed of animal tissues and organs also increase, which leads to the acceleration of respiration and excretion. This is the common feature of ectotherm (Wang 2010). The external temperature at which the maximum respiratory metabolism occurs is the optimum living temperature of the organism. The fitted curve of the effect of water temperature change on the respiratory metabolism of *Acanthopagrus schlegelii* studied in this paper basically leveled off at 28℃. At this temperature, the physiological activity of *Acanthopagrus schlegelii* reached its peak and the respiratory metabolic rate was fast, indicating that the temperature at around 28℃ might be the optimum temperature for *Acanthopagrus schlegelii*. In the experiment of *Acanthopagrus schlegelii*, the results are similar to those of Zheng Jianmin et al. on juvenile acanthopagrus schlegelii at 17.5 to 21.0°C, and the experimental subjects are farmed *Acanthopagrus schlegelii* (Zheng 1991). We can speculate that there is little difference in oxygen consumption rate between farmed and wild *Acanthopagrus schlegelii*.

The change of ammonia excretion rate of *Acanthopagrus schlegelii* with water temperature is similar to that of oxygen consumption rate, which indicates that the change of metabolism in *Acanthopagrus schlegelii* caused by temperature is also influenced by enzyme activity and activity of internal body organ. As the water temperature rises, the basal metabolism of *Acanthopagrus schlegelii* also increases, showing the enhancement of body excretion. In the excretion study of hybrid *Acanthopagrus schlegelii* and juvenile *Acanthopagrus schlegelii*, Yan Fuyun et al. found that the ammonia excretion rate increases with the rise of water temperature in juvenile hybrid *Acanthopagrus schlegelii* and juvenile *Acanthopagrus schlegelii* at water temperature from 13 to 28°C (Yan 2010).

Organisms control energy metabolism through the regulation of biological functions by weight. The results show that the oxygen consumption rate of *Acanthopagrus schlegelii* decreases with the increase of weight, which may be related to the change of the proportion of tissues that sustain the life of fish in the body. Tissues are used to sustain the life of fish, such as: brain, kidney, and gonads, have high oxygen consumption. While tissues do not directly sustain life, such as bones, muscles, and fats, have low oxygen consumption (Li 2009). Small fish under growth and development stage usually has large proportion of tissue in the front part and small proportion of tissue in the back part. In contrast, large fish often has small proportion of the front part and large proportion of the back part. For this reason, the metabolic activity of small fish is more vigorous than that of large fish (Wang 2011). The oxygen consumption rate of *Acanthopagrus schlegelii* decreases successively with the increase of weight, which is similar to that of the fry of *Fugu obscurus* (Wang 2002) and *Perca fkuviatilis* (Zakęś 2003). There is a negative correlation between ammonia excretion rate and the weight of fish. Since mature fish are well-developed with strong anti-interference, external changes have less impact on them than that of juvenile fish. This was also found in other aquatic organisms such as *Strongylocentrotus intermedius* (Bi 2000), *Penaeus japonicas* (Zhu 2001), *Oratosquilla oratoria* (Jiang 2000) and *Apostichopus japonicas* (Sun 2012), suggesting a more pronounced effect of weight on respiratory metabolism.

The value of Q_{10} reflects the extent to which the metabolic intensity is affected by temperature (Bayne 1983), which indicates the change in oxygen consumption rate caused by every 10°C increase in water temperature. The Q_{10} value of oxygen consumption rate of *Acanthopagrus schlegelii* varies from 1.31 to 1.69. The larger the Q_{10} value, the more sensitive it is to the change of water temperature within such temperature range, and the oxygen consumption rate changes significantly. The Q_{10} value of ammonia excretion rate varies from 1.05 to 1.78, and high Q_{10} values indicates that there is an upper limit exceeding the temperature tolerance threshold of experimental fish in this temperature range, which affects its physiological activities.

The energy substances metabolized by animals are proteins, fats and carbohydrates, which are eventually metabolized into CO_2, water and nitrogen while releasing energy. Excretion is one of the basic physiological activities of energy metabolism in organisms. The ratio of metabolic substrate O: N can be used to deduce the source of energy substances. Changes in O: N ratio are closely related to the environmental factors to which the organisms are subjected and can be used to determine the growth of organisms under specific conditions (Widdows 1978). When the energy supply of the body is provided by proteins, the O: N ratio is about 7 to 10. When proteins and fats are oxidized for energy supply, the O: N ratio is about 24. An infinitely increasing O: N ratio is presumed to be a combined energy supply of fats and carbohydrate (Mayzaud 1978, Ikeda 1974, Conover 1968). In the experimental results, the O: N ratio of metabolic substrates of *Acanthopagrus schlegelii* suggests that the metabolic energy substances are supplied by a mixture of protein and fat, together with a small amount of carbohydrates. This is consistent with the research on basic metabolic law of juvenile *Paralichthys olivaceus* by Wang Bo et al. Their research results show that the average O: N ratio of metabolic substrate of juvenile *Paralichthys olivaceus* at different temperatures is 38.8, and the main energy supply is a nutrient mixture such as proteins and fats, followed by some carbohydrates (Wang 2004). When the O: N ratio of metabolic substrate reaches the maximum, the mixed metabolic difference of proteins, fats and sugars in aquatic organisms is the largest, and the growth rate is the highest. Many scholars regard the O: N ratio of substrate as an indicator of biological adaptation to the environment and the determination of suitable conditions (Xu 2008). In this study, the O: N ratio of *Acanthopagrus schlegelii* fluctuates significantly with water temperature, and the results reveal that the growth rate of *Acanthopagrus schlegelii* is the highest at 28℃, indicating that this temperature is the optimum for the growth of *Acanthopagrus schlegelii* under experimental conditions.

5 CONCLUSIONS

The growing market demand for aquatic products has stimulated the development of aquaculture in the whole world, China included. Due to overfishing and marine environmental pollution, China's aquaculture production continues to decline, bringing great environmental pressure to the aquaculture industry. Since *Acanthopagrus schlegelii* has high economic value, it is of great importance to reasonably protect, utilize and vigorously farm it. The study on the respiratory metabolism of wild *Acanthopagrus schlegelii* in this article fills the gap in the research on the respiratory metabolism of wild *Acanthopagrus schlegelii* in the South China Sea and enriches the data on its growth. It provides reference for the assessment of the resources of *Acanthopagrus schlegelii* and snapper, the growth of offshore *Acanthopagrus schlegelii* aquaculture and the assessment on the proliferation capacity of offshore marine pastures.

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