Full Length Article

Response of Respiration Physiology and Nitrogen Metabolism under Low Dissolved Oxygen Stress in *Whitmania pigra*

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Abstract

Whitmania pigra is a traditional Chinese medicine used to treat congestion, hypertension, coronary heart disease and tumors; however, its response of respiration physiology and nitrogen metabolism under low-oxygen stress need to be further studied. In this paper, a pattern of decreasing oxygen was adopted to investigate the physiological changes in respiration and nitrogen metabolism. Behavioral responses were observed by measuring oxygen consumption rate (R_{OC}) and ammonia extraction rate (R_{AE}) of leeches (body mass 4.73±2.34 g) under low oxygen conditions in 20 and 30°C water. The results showed that R_{OC} was 0.065 and 0.093 mg·g^{-1}·h^{-1} in 20 and 30°C water, respectively. But under sufficient oxygen, R_{OC} decreased with the decrease of dissolved oxygen. When the dissolved oxygen was lower than 1.8 mg·L^{-1}, the leeches would lengthen the body wandering around, and waiting for an escape, gradually dying below 1.0 mg·L^{-1}. When the oxygen stress increased, the ammonia and nitrite gradually increased, R_{AE} showed the time rhythm characteristics; and the O:N decreased. To sum up, these results indicate that *W. pigra* tends to change the metabolic pattern to adapt to the low-oxygen environment by reducing its respiratory metabolism. © 2021 Friends Science Publishers

Keywords: *Whitmania pigra*; Low oxygen stress; Metabolism; Nitrogen

Introduction

Whitmania pigra Whitman, commonly known as leech, belongs to a species of Annelida, Hirudinea, Gnathobdellida, and Hirudinidae (Kuo and Lai 2019), which exhibit a strong predatory capacity in controlling snails. Hirudin is a naturally occurring peptide *of W. pigra* that has anticoagulant effects, and has widest therapeutic usage worldwide (Sig *et al.* 2017). Wang *et al.* (2017) have found that the anticoagulantion-related gene has a higher restriction fragment length polymorphism (RFLP) in the transcriptome, and the anticoagulat demand is the main evolutionary pressure throughout its evolution by using a transcriptome EST-SSR analysis. In addition, *Hirudo medicinalis* species of leeches, used for medical purpose (Baskova *et al.* 2008), has been applied for treating congestion, hypertension, coronary heart disease and tumors (Wang *et al.* 2018); and its extracts are widely used in cosmetics and health care industry. Thus, artificial culture of leech is widely developed all over the world (Whitaker *et al.* 2004).

Dissolved oxygen is the main source of oxygen for aquatic animals (Butterfield 2018). The amount of dissolved oxygen in ponds directly affects the healthy growth of fish, and is one of the main environmental factors determining the aquacultural yield (Chen *et al.* 2018). *W. pigra* spends most of their time in water, and their respiration mainly depends on the absorption of oxygen via the skin. Shi *et al.* (2005) have measured the oxygen consumption rate of a *W. pigra* by using a flowing water breathing chamber method, and reported an internal oxygen consumption rate where an average mass of 10 g was 0.049 ~ 0.094 mg·g^{-1}·h^{-1} at a temperature of 15 ~ 35°C; while the suffocation point was 0.90 ~ 1.51 mg·L^{-1}. The oxygen consumption rate increased with the increase of water temperature, and the suffocation point was negatively correlated with water temperature and body weight. The oxygen consumption rate of the *W. pigra* is lower than that of crucian, grass carp and other fish, but is higher than that of the *Hyriopsis cumingii* and other shellfishes (Wang *et al.* 2016). The live snails are mainly used as bait during the artificial breeding (Khan *et al.* 2019). It is used for a means of biological control of the exotic invasive snail *Pomacea canaliculata* (Guo *et al.* 2017). In addition, many parts of the axe foot muscles are left after snails are sucked, which can quickly deteriorate the water quality. Therefore, under the conditions of artificial breeding, *W. pigra* are prone to low-oxygen stress, which can lead to slow growth and diseases (Li *et al.* 2018). The mass mortality often occurs during the high temperature

seasons from August to September. A high mortality rate is the most serious bottlenecks in its artificial breeding (Wang *et al.* 2018).

So far, the response of respiration physiology and nitrogen metabolism under low-oxygen stress in *W. pigra*has not been reported. In this paper, a pattern of decreasing dissolved oxygen was adopted to investigate the physiological changes in respiration and nitrogen metabolism of *W. pigra*, aiming to provide theoretical basis for some key technologies such as artificial breeding, disease control and prevention, and live transportation of *W. pigra*.

Materials and Methods

Experiment materials

Whitmania pigra used in this study was an offspring from the first generation collected in the wild river in Taizhou area. *W. pigra* had a body mass of 4.73±2.34 g and were raised in a non-toxic plastic water tank (60×30×30 cm) with a water depth of 20 cm. Feeding water and experimental water were tap water aerated by air pump for more than 72 h with a pH of 7.8±0.3 and a hardness of 126 ± 4.8 mg·L^{-1} measured by $CaCO_3$.

Experimental equipment

The method of hydrostatic respiration was adopted in this experiment. All equipment for the metabolism determination of *W. pigra* was designed according to previously described approach (Wang *et al.* 2016; Fig. 1, Patent grant No.: ZL201920550423.9). Briefly, the breathing room was filled with fully aerated tap water. Then, the rubber tube was connected with water stop clip at the bottom for collecting water samples. The *W.pigra* was then weighted and transferred into the metabolism measurement. Consequently, the device was connected with the water level buffer bin, and was fixed with clamps. The piston was immediately sealed after filling with water to separate the test water from the outside world. The total volume of water in the breathing chamber was 33 L, including 3 L in the water level buffer bin and 30 L in the breathing chamber. Approximately 300 mL of water samples were taken out each time for determination, and the piston moved downward to prevent air from entering the experimental water. A mesh was provided at the joint of the water level buffer to prevent leeches from escaping into the water level buffer bin and damaging the sealing of the piston.

Experimental methods

The experiment was divided into 3 groups: normal temperature group (20 ± 0.5°C, group 1 and 2), high temperature group (30 ± 0.8°C, group 3 and 4) and blank control group (group 5 and 6). The initial dissolved oxygen

was 6.02 ± 0.18 mg/L. Each group included 36 leeches. In addition, leeches from groups 1, 2, 3 and 4 had the body mass of 172.16, 167.31, 168.53 and 172.95 g, respectively; while there was no leeches in group 5 and 6.

The experiment began at 20: 00 pm. A 300 mL water samples were collected every 4 h to determine dissolved oxygen, ammonium nitrogen (ammonium-N) and nitrite nitrogen (nitrate-N) content. A span of 0–8 h indicated a nighttime (20: 00 to 4: 00), while 12 to 20 h (8: 00 to 16: 00) indicated a daytime. The dissolved oxygen in the water was determined by the iodometric method; the ammonium-N was determined by the Nessler's reagent method (Wu and Cao 2013); the nitrate-N was determined by the sulfonamide-naphthalene ethylenediamine hydrochloride method (Wang *et al.* 2019). Each water sample had 3 replications.

All experiments were performed in accordance with the guidelines for animal research established by the Local Ethics Committee of Animal Experiments.

Data statistics and analysis

The oxygen consumption rate was calculated according to the formula (Wang *et al.* 2016):

$$R_{OC} = (B_1 - B_2 - B_0) \times V / (W \times t)$$

Where B_1 represented the dissolved oxygen (mg/L) in water before the experiment; B_2 the dissolved oxygen (mg/L) after the experiment; B_0 the difference of dissolved oxygen in the control group (mg/L); V the actual volume of experimental water (L) that was expressed as the volume of water at the end of the last sample minus the volume of the sample; W the weight (g) of the experimental leech and t represented the time (h).

The ammonia extraction rate was calculated using the following formula (Wang *et al.* 2016):

$$R_{AE} = (A_1 - A_2 - A_0) \times V / (W \times t)$$

Where A_1 represented ammonium-N before the experiment; A_2 the ammonium-N in the water after the experiment, A_0 the difference of ammonium-N in the control group; and V the actual volume of the experimental water (L), which was expressed as the volume of water at the end of the last sample minus the volume of the sample; W the weight (g) of the experimental leech and t was the time (h).

The ratio of oxygen to nitrogen (O:N) was calculated as the ratio of oxygen consumption and ammonia excretion (Mayzaud 1976):

$$O:N = (R_{OC}/16):(R_{AE}/14)$$

All data were sorted in Excel 2010, and the average value was obtained. The analysis was performed by SPSS 19.0 statistical software. ANOVA and Duncan multiple comparisons were used to compare the significance of the mean between the groups. $P<0.05$ represented the significant difference.

Results

Changes of dissolved oxygen concentrations at different water temperature

The dissolved oxygen values in aquatic water of *W. pigra* at different water temperature are shown in Fig. 2. After cutting off the dissolved oxygen source, the dissolved oxygen in the aquatic water at 20 and 30°C showed a gradual declining trend. At 30°C, the dissolved oxygen value decreased faster than that at 20°C; however, no significant differences were observed between the two groups ($P>0.05$). After 4 h, the dissolved oxygen in 20 and 30°C water decreased to 4.53 mg/L and 3.91 mg/L, respectively, both of which were significantly lower than the initial concentration ($P<0.05$).

At 20°C, the dissolved oxygen at 12 h was significantly lower than that of the 4 h ($P < 0.05$), which was only 52.16% of the initial dissolved oxygen value. After 16 h, the dissolved oxygen was 1.80 mg/L, and the difference was not significant from 16 to 24 h ($P>0.05$). Moreover, the dissolved oxygen at 24 h was 1.46 mg/L, which was only 24% of the initial dissolved oxygen value. From the 16 h, the leeches lengthened the body wandering around, and waiting for an escape. At 24 h, the vitality of *W. pigra* significantly decreased, however still no death was observed.

At 30°C, the dissolved oxygen at 8 h was significantly lower than that of the 4 h ($P<0.05$), which was only 42.88% of the initial value. After 12 h, the dissolved oxygen was as low as 1.70 mg/L, and the difference was not significant from 12 to 24 h ($P>0.05$). The dissolved oxygen at 24 h was 0.48 mg/L, which was only 7.80% of the initial dissolved oxygen. Immediately after 12 h, *W. pigra* began to move and swim around looking for escape points. Most of leeches died after 24 h, and the mortality rate was 22.22%. After examining the leeches were unresponsive and very weak. They were gradually dying when put them into the normal water; while the mortality rate reached 57.14% within 24 h.

Oxygen consumption rate at different water temperature

The changes in oxygen consumption rate of *W. pigra* at different water temperature are shown in Fig. 3. Under gradually dissolved oxygen stress, the changes in rates of oxygen consumption were similar to when the ambient temperature was 20 or 30°C. The oxygen consumption rate decreased with a decrease of dissolved oxygen, and no significant differences were observed between 20 and 30°C ($P>0.05$).

At 20°C, the oxygen consumption rate was 0.065 mg•g^{-1}•h^{-1} when the dissolved oxygen was > 4.53 mg/L within 4 h. After 20 h, there was no significant difference in oxygen consumption rate when the dissolved oxygen was > 1.60 mg/L ($P>0.05$), and with an average oxygen consumption rate of 0.037 mg•g^{-1}•h^{-1}. At 24 h, the dissolved oxygen was < 1.50 mg/L, and the oxygen consumption rate
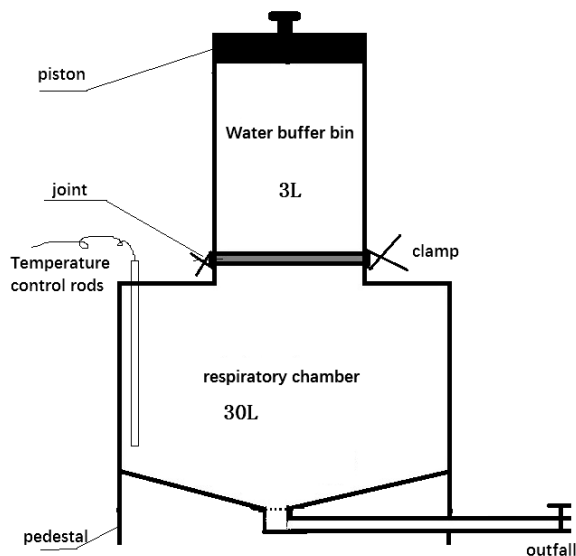

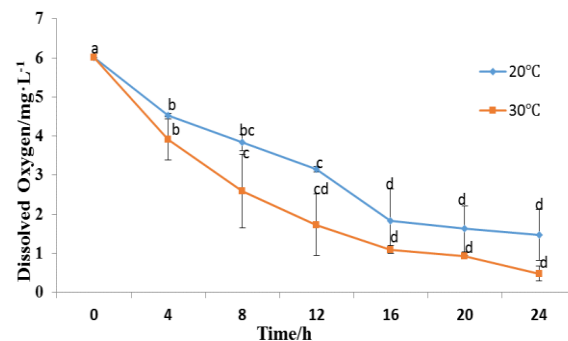**Fig. 1:** Equipment of metabolic measurement

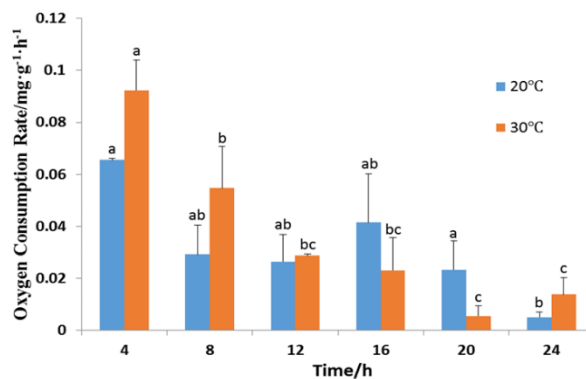**Fig. 2:** Dissolved oxygen concentrations in aquatic water of *W. pigra*, at different water temperature

Fig. 3: Oxygen consumption rate of *W. pigra* under low dissolved oxygen stress at different water temperature

decreased to 0.005 mg/L, which was significantly lower than the oxygen consumption rate at the 4 h ($P<0.05$); but, the difference was not significant form 8 to 20 h ($P>0.05$), with an average oxygen consumption rate of 0.025 mg•g^{-1}•h^{-1}.
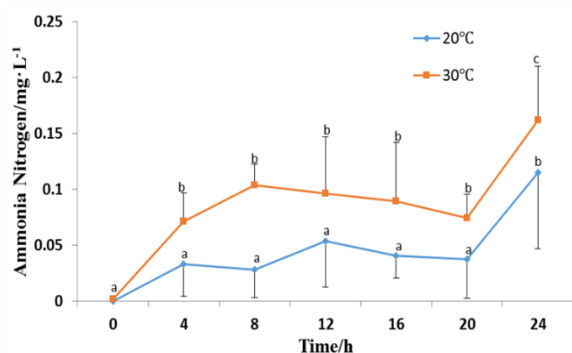
Fig. 4: Ammoniacal nitrogen concentrations in aquatic water of *W. pigra* under low dissolved oxygen stress in different temperature
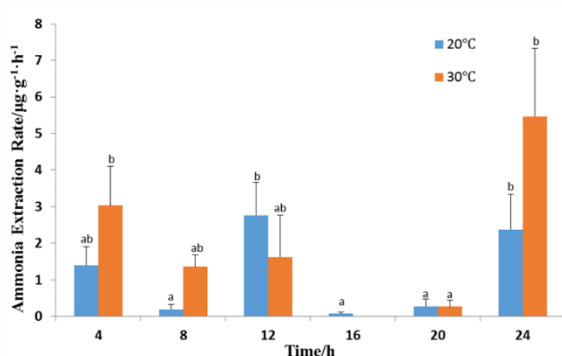

Fig. 5: Ammonia extraction rate of *W. pigra* under low dissolved oxygen stress in different water temperature
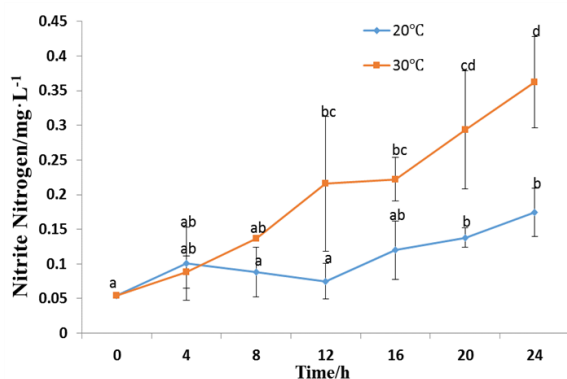

Fig. 6: Nitrite-N concentrations in aquatic water of *W. pigra* under low dissolved oxygen stress in different water temperature

At 30°C environment, the oxygen consumption rate was 0.092 mg•g^{-1}•h^{-1} when the dissolved oxygen was > 3.91 mg/L within 4 h. The oxygen consumption rate at 8 h was 0.055 mg•g^{-1}•h^{-1}, which was significantly lower than that at 4 h ($P<0.05$). The dissolved oxygen value between 8 and 16 h ranged from 1.09 to 2.58 mg/L, and the difference in oxygen consumption rate during this period was non-significant ($P>0.05$). The dissolved oxygen concentration was < 0.93 mg/L at 20~24 h, and the oxygen consumption

rate was significantly lower than that at 8 h ($P<0.05$). When the dissolved oxygen concentration was below 1.72 mg/L at 12~24 h, the difference in oxygen consumption rate during this period was non-significant ($P>0.05$), with an average oxygen consumption rate of 0.014 mg•g^{-1}•h^{-1}. The lowest oxygen consumption rate occurred at 20 h, and the lowest rate was only 5.94% of that at 4 h.

Changes of ammoniacal nitrogen at different water temperature

The changes of ammoniacal nitrogen in the aquatic water of *W. pigra* under different water temperature conditions are shown in Fig. 4. The concentration of ammonium-N in the aquatic water of *W. pigra* gradually increased at temperature of 20 and 30°C; and no-significant ($P>0.05$) differences were observed between 20 and 30°C.

At 20°C, the ammonium-N concentration gradually increased within 20 h; nevertheless, the difference was not significant ($P>0.05$). The concentration reached 0.11 mg/L at 24 h, which was significantly higher than that of every group before 20 h. At ambient temperature of 30°C, the concentration reached 0.07~0.10 mg/L at 4~20 h, and reached 0.10 mg/L at 8 h, which was significantly higher than the initial value ($P<0.05$). The concentration at 24 h reached 0.16 mg/L, which was significantly higher than other times ($P<0.05$).

Variation of ammonia emission rate at different water temperature

The changes of ammonia emission rate of the *W. pigra* under different water temperature conditions are presented in Fig. 5. The variation trend of the ammonia emission rate in the environment temperature of 20 and 30°C was similar, showing a certain time of rhythmic variation. At 14 and 20 h (12:00 ~ 16:00), the ammonia emission rates were lower.

At 20°C, ammonia emission rates at 8, 16 and 20 h were significantly lower than those at 12 and 24 h ($P < 0.05$). At 30°C, ammonia emission rates at 16 and 20 h were significantly lower than those at 4 and 24 h ($P < 0.05$). At 20 and 30°C, the average ammonia emission rate within 24 h was 1.18 and 2.35 μg•g^{-1}•h^{-1}, respectively. In low-oxygen environment at 24 h, the ammonia excretion rates in groups at 20 and 30°C were both higher, but there was no significant difference with that at 4 h with high dissolved oxygen ($P > 0.05$).

Changes of nitrite-nitrogen at different water temperature

The changes of nitrite-N in aquatic water under different water temperature conditions are shown in Fig. 6. At 20 and 30°C, the nitrite-N concentration gradually increased. At 20°C, the concentration of nitrite-N slowly increased and reached 0.22 mg/L within 16 h; however, no significant

($P > 0.05$) differences were observed between different time points. The nitrite-N concentrations at 20 and 24 h were 0.14 and 0.17 mg/L, respectively; both of which were significantly higher than those before 12 h ($P < 0.05$), but were not significantly different from that at 16 h ($P > 0.05$). At 30°C, the nitrite-N concentration reached 0.21 mg/L at 12 h, which was significantly higher than the initial concentration ($P < 0.05$); reached 0.29 mg/L at 20 h, which was significantly higher than the group at 8 h ($P < 0.05$), and reached 0.36 mg/L at 24 h, which was significantly higher than the group at 16 h ($P < 0.05$). The accumulation of nitrite-N at 30°C conformed to the linear function relationship of y=0.05x-0.006, R^2=0.98.

Discussion

When the water temperature was 20 and 30°C, the oxygen consumption rate of *W. pigra* with body mass of 4.73 ± 2.34 g in the environment with relatively sufficient dissolved oxygen was 0.065 and 0.093 mg•g^{-1}•h^{-1}, respectively (Fig. 3). The temperature increase was correlated with the oxygen consumption rate, which is consistent with previous studies (Shi *et al.* 2005). The oxygen consumption rate of *W. pigra* is higher as compared to rotifer (Galkovskaya 1995); and the same to squid *Dosidicus gigas* (Ommastrephidae) (0.094 mg•g^{-1}•h^{-1}) (Trueblood and Seibel 2013); but lower as compared to some other species, such as *Oreochromis niloticus* (0.1122±0.0099 mg•g^{-1}•h^{-1}) (Obirikorang *et al.* 2017). When dissolved oxygen decreased, the oxygen consumption rate of *W. pigra* gradually decreased. This trend was more obvious at higher temperatures. At 20°C, the oxygen consumption rate was only 7.63% of the 6.02 mg/L when the dissolved oxygen was 1.46 mg/L; at 30°C, the oxygen consumption rate was only 14.98% of the 6.02 mg/L when the dissolved oxygen was 0.48 mg/L. These results showed that the oxygen consumption rate and respiration metabolism of the *W. pigra* stressed by low dissolved oxygen were significantly lower than that of the *W. pigra* when the dissolved oxygen was sufficient. Therefore, *W. pigra* might respond to the dissolved oxygen stress by reducing its respiratory metabolism. In this experiment, it was found that the *W. pigra* stretched and wigged its body in the low-dissolved oxygen environment, which may be a performance of its increasing oxygen intake by lengthening its body and increasing its surface area. Zhou *et al.* (2018) have reported similar behavior in *Apostichopus japonicus* when exposed to low-dissolved oxygen (1 mg/L).

Shi *et al.* (2005) have found that when dissolved oxygen drops below 1.80 mg/L, the leech shows discomfort; while at 0.48 mg/L, the mortality rate increases and the dissolved oxygen at the time of death is slightly lower than the suffocating point (1.07 ± 0.22 mg/L) of leeches with body mass of 11.3 g. The smaller the leech, the lower is its suffocating point. Throughout the experimental procedure, the experimental device was improved many times. The soft plastic film was applied on the surface of water to cut off air

as previously described (Wang *et al.* 2016). In this study, when the dissolved oxygen dropped below 1.8 mg/L, *W. pigra* began to move and looked for an opportunity to escape the plastic film. This trend was more evident when dissolved oxygen was less than 1.5 mg/L Moreover, when the dissolved oxygen was lower than 1.0 mg/L, it was almost impossible to prevent the leech from drilling out of the plastic membrane. In several experimental groups, the plastic membrane was drilled through, leading to failure of the experiment. The measuring device was specially designed (Patent grant No.: ZL201920550423.9), using the glass material in order to prevent the drilling, thus completing the experiment. In aquaculture production, *W. pigra* often raised their heads and other upper body parts above the water surface, which may be their way of avoiding the hypoxic environment that is often interpreted as a normal reaction after feeding and has not been paid attention to (Wang *et al.* 2016). It has been shown that when the dissolved oxygen in the breeding water is less than 1.0 mg/L, the *W. pigra* can still feed and grow, that is why the farmers mistakenly believe that the *W. pigra* is resistant to low oxygen, and thus they do not install oxygen increasing equipment.

The nitrogen excreta of aquatic animals include ammonium-N, uric acid and urea, where the formation of ammonium-N through deamination and transamination is the most important way of excretion (Dosdat *et al.* 1996). Generally, aquatic animals can expel ammonia from their body through gills and kidneys, and simple diffusion and ion exchange in the form of ions are the most important ways (Dosdat *et al.* 1996). Ammonia comes mainly from protein, so ammonia excretion rate can reflect protein metabolism level. *W. pigra* does not have gills, and its respiration is mainly performed by the skin (Liu *et al.* 2006); So, the skin may be one of the main ways of excreting nitrogen. In this study we found that at water temperature of 20°C, the ammonia excretion rate of *W. pigra* was 0.07 ~ 2.37 μg. g^{-1}·h^{-1}; at 30°C, the ammonia excretion rate was 0.26 ~ 5.46 μg.g^{-1}·h^{-1} (Fig. 5). The nitrogen metabolism of *W. pigra* was similar with benthic aquatic animals, such as *Perinereis aibuhitensis* Grub (Liu *et al.* 2016), *Apostichopus japonicus* (Zhou *et al.* 2018), *Sipunculus nudu* (Liu *et al.* 2017), *Rhodeus sinensis* (Wang *et al.* 2016); and lower than in some upper aquatic animals, including *Haliotis discus hannai* Ino (Zhang *et al.* 2017), *Epinephelus* SP. (Xing *et al.* 2019), etc. In the process of the gradual decrease of dissolved oxygen in the environment, the total ammonium-N value and nitrite-N in the aquatic water of *W. pigra* were gradually increased, and the ammonia excretion rate showed a temporal rhythm. In this study, the ammonia excretion rate was lower during 12:00 ~ 16:00, but relatively higher during evening and nighttime, which is consistent with the feeding rhythm reported by Shi *et al.* (2006). Low dissolved oxygen stress did not change the rhythmic regulation of nitrogen metabolism of *W. pigra*, which was similar to some type of fishes (Wang *et al.*

Table 1: O:N comparison of *W. pigra* under low dissolved oxygen stress in different temperature

Temperature	Time (h)					
	4	8	12	16	20	24
20°C	40.96±1.33	135.00±74.29	8.38±4.17	516.12±128.67	74.08±50.78	1.85±1.88
30°C	26.67±9.31	35.33±42.43	15.48±0.41	754.90±153.12	18.27±8.26	2.21±1.98

2016). Environmental stress has certain influence on the nitrogen excretion in many aquatic animals (Liu *et al.* 2016; Liu *et al.* 2017; Xing *et al.* 2019). When dissolved oxygen decreases, animals such as *R. sinensis* (Wang *et al.* 2016) and *A. japonicas* (Zhou *et al.* 2018) showed a decrease in their ammonia excretion rate, which might be because that they adapt to the hypoxic environment by lowering the metabolic rate. However, the ammonia excretion rate of *W. pigra* in low dissolved oxygen environment was higher than in the high dissolved oxygen environment, which is different from other animals.

Mayzaud (1976) believed that if O:N is less than seven, the energy is completely provided by protein. If it ranges from 8 to 24, the energy is delivered by protein and fat oxidation; if O:N is larger or even infinite, then the body is mainly powered by fat or sugar (Chen *et al.* 2018). In this study, O:N was calculated by oxygen consumption rate and ammonia excretion rate (Table 1). The average O:N of *W. pigra* was 129 at 20°C, and 142 at 30°C. The *W. pigra* takes the fat or sugar as the main energy source. Shi *et al.* (2015) have examined the digestive enzyme activity of the *W. pigra*, and found the activity of lipase>amylase>protease, which suggests that *W. pigra* can absorb and use algae and other nutrients containing starch, fat, and protein. In addition, Lu *et al.* (2011) have determined the nutritional composition of the *W. pigra* in Weishan Lake and found 78.11% of total protein, 6.6% of total fat, 9.94% of total sugar, which was similar to nutritional composition found in fish. Analysis of fatty acid composition indicated that the fatty acid in *W. pigra* is mainly unsaturated fatty acid, and its content is much higher than in freshwater fish and marine fish, thus it is easily decomposable and utilized.

After exposure to 24 h to low dissolved oxygen stress, the O:N in *W. pigra* was lower than 7 (Table 1), which indicated that the energy was mainly supplied by proteins. Metabolic energy supply systems include sugar oxidation energy supply and fat, protein oxidation energy supply. The sugar oxidation energy supply system consumes a large amount of oxygen and is the main energy supply mode for animals. Fat contains more hydrogen than sugar, and it consumes more oxygen than saccharides during oxidative decomposition; it also releases more energy (Humphries 2006). Protein oxidation demands the least amount of oxygen and is often the main energy supply system for endurance programs such as marathon runners (Feo *et al.* 2003). When exposed to long-term anoxic environment, the *W. pigra* is in a low-oxygen state, therefore, it is possible that it uses an adjusted metabolic mode to adapt to the low-oxygen environment with protein oxidation for energy supply, which is why the ammonia excretion rate sharply

rises. Li (2018) found that Nile tilapia (*Oreochromis niloticus*) can regulate the signaling pathways when exposed to chronic hypoxia environment, promoting glycogen production and lipolysis. It supplies energy with adipose metabolization mainly and anaerobic glycolysis auxiliary for airframe, so as to reduce amino acid metabolism. The Nile tilapia responds to hypoxic stress by altering its nutrient and energy metabolism patterns. The results of this study indicated that *W. pigra* could change the metabolic model to cope with hypoxia stress; nevertheless, the processes underlying metabolic changes still remain unclear, and need to be further investigated. The oxygen consumption rate was 0.065 and 0.093 mg·g^{-1}·h^{-1} in 20 and 30°C water, respectively, under the condition of relatively sufficient dissolved oxygen. In this study, at 20°C, when the dissolved oxygen was lower than 1.5 mg/L, the oxygen consumption rate decreased with the decrease of dissolved oxygen (*P*<0.05), significantly, the average oxygen consumption rate was 0.025 mg·g^{-1}·h^{-1}.At 30°C, when the dissolved oxygen was lower than 1.72 mg/L, the oxygen consumption rate decreased significantly (*P*<0.05), the average oxygen consumption rate was 0.014 mg·g^{-1}·h^{-1}, when the dissolved oxygen was lower than 1.8 mg/L, the leeches would lengthen the body wandering around, and waiting for an escape, gradually dying below 1 mg/L.

When the dissolved oxygen stress increased, the ammonium-N and nitrite-N in the water gradually increased significantly (*P*<0.05). At 20°C, the ammonium-N was 0.11 mg/L in 24 h, and at 30°C, the ammonium-N was 0.10 mg/L in 8 h, and it was up to 0.16 mg/L in 24 h (Fig. 4). The ammonia excretion rate showed the time rhythm characteristics; however, the ammonia excretion rate increased and the O:N ratio decreased in the low dissolved oxygen condition. In different spectral environment and low temperature hibernation environment, *W. pigra* adjusted digestive enzyme and antioxidant enzyme activity to adapt to the variation, so as to protect it from the injurious effects of free radicals.

Conclusion

Low dissolved oxygen stress affected the water quality and metabolism of *W. pigra*. Under these conditions, *W. pigra* tended to change the metabolic pattern to adapt to the low-oxygen environment by reducing its respiratory metabolism.

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Author Contributions

Jianguo Wang and Liangwei Xiong carried out the study, participated in collecting data, and drafted the manuscript. Quan Wang and Zhiqin Zhou performed the statistical analysis and participated in its design. Tianle Tang guided the experiment design and revised the paper. All authors read and approved the final manuscript.

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