



## Effects of Acute Temperature Change and Temperature Acclimation on the Respiratory Metabolism of the Snakehead

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### Abstract

The effects of acute temperature change and the following two weeks of acclimation from the initial 25°C to 15, 20, 30, and 35°C on the respiratory metabolism of the snakehead (*Channa argus*), a species tolerant to wide temperature range were examined in this study. The resting metabolic rate (*RMR*), ventilation frequency (*VF*), and several hematological parameters were determined. *RMR* of the fish post either acute temperature change or chronic acclimation was positively correlated with temperature, and exhibited V-shaped  $Q_{10}$  values change vs temperature, which suggests that the snakehead has a varying thermal metabolic sensitivity dependent on temperature range. The decreased  $Q_{10}$  value for *RMR* post chronic acclimation suggests that the metabolic thermal sensitivity of the snakehead can be reduced by acclimation. The *VF* increased with increasing temperature slower than *RMR*, which suggests that regulation of *VF* alone may not be sufficient to satisfy the oxygen demand of the snakehead as temperature increases. Cold compensation was observed in *VF*, but not *RMR*, which could be due to a decreasing oxygen exchange capacity of the gill during cold acclimation. Thus, the compensation in *VF* of cold acclimated fish may be necessary to maintain oxygen supply.

**Keywords:** Fish, gill, oxygen consumption, thermal compensation.

### Introduction

The snakehead (*Channa argus*), an eurythermal fish, has a wide geographic distribution in China (Liu, Cui, & Liu, 1998), with a wide habitat temperature range (0-30°C) (Courtenay & Williams, 2004). As an air-breather, it can survive up to four days on land as long as their skin remains moist and survive colder temperatures by burrowing into the mud (Hilton, 2002; Courtenay & Williams, 2004). Snakehead can also jump out of water and burrow in the land near ponds in extremely hot seasons (Xie, Li, Luo, Lin, Zheng, & Lin, 2002). Therefore, the snakehead may experience significant daily acute temperature changes and seasonal temperature changes. Besides, the effects of hot temperature on fish may be intensified by the global warming due to climate changes in future. An annual temperature increase of approximately 3.5 C will be predicted in the Yangtze River Basin, the important habitats of the snakehead, in the end of the twenty-first century (Gu, Yu, Wang, Ju, Yang, & Fan, 2015). Moreover, even being an important aquaculture species in China, the snakehead becomes an invasive species with high risks to aquatic ecosystems in Europe and North America (Courtenay

& Williams, 2004; Lapointe, Odenkirk, & Angermeier, 2013) and may face to relative cool temperature in those invasion regions.

Temperature is one of the most important environmental factors influencing the resting metabolic rate (*RMR*) of fish and an increasing temperature generally induced an increasing *RMR* (Jobling, 1994). Fish adjust respiration to match the change in oxygen demands with changing temperature in several ways, e.g., by increasing ventilation frequency (*VF*) (Millidine, Metcalfe, & Armstrong, 2008; Frisk, Skov, & Steffensen, 2012), improving the oxygen exchange capacity of the gill (Sollid, Weber, & Nilsson, 2005), and regulating the oxygen binding and transportation ability of blood (De Pedro, Guijarro, López-Patiño, Martínez-Álvarez, & Delgado, 2005; Morgan, Thompson, Auchinachie, & Migaud, 2008; Qiang, Yang, Wang, Kpundeh, & Xu, 2013). Co-regulation among these processes may be necessary in the case of severe temperature changes.

Fish may experience acute temperature changes (e.g., thermocline, flooding, and diurnal change) and long term temperature changes (e.g., seasonal and climate changes) (Johnson & Bennett, 1995; Zeng,

Zhang, Fu, & Cao, 2011). Acute temperature change can cause a corresponding change in *RMR* of fish (Dowd, Brill, Bushnell, & Musick, 2006; Perez-Casanova, Afonso, Johnson, & Gamperl, 2008). After long-term acclimation, many fish species show compensation for the direct effects of temperature change on metabolism, as reflected by recovery in *RMR* (Johnston & Dunn, 1987; Johnston, Guderley, Franklin, Crockford, & Kamunde, 1994; van Dijk, Tesch, Hardewig, & Pörtner, 1999; Aguiar, Kalinin, & Rantin, 2002; Ibarz, Fernández-Borràs, Blasco, Gallardo, & Sánchez, 2003; Perez-Casanova et al., 2008; Zhao, Dong, Wang, Tian, & Gao, 2011; Sandblom, Gräns, Axelsson, & Seth, 2014). The capacity of thermal compensation has been considered to be related with fitness and biogeographic range of ectothermal species under climate change (Chevin, Lande, & Mace, 2010; Somero, 2010; Schulte, Healy, & Fangue, 2011; Sandblom et al. 2014). Even though, metabolic compensation may not be a general mechanism across species (Clarke & Johnston, 1999; Zakhartsev, De Wachter, Sartoris, Pörtner, & Blust, 2003; Clarke, 2006).

Studying the metabolic response of the snakehead to temperature change may help develop an understanding of the physiological thermal tolerance mechanisms that allow it to survive across wide regions. However, the thermal physiology of this species is poorly understood, with very limited data on metabolism by now (Liu, Cui, & Liu, 2000; Wang, Wang, Huang, Zhang, & Luo, 2012; He, Li, Xie, Liu, & Luo, 2015). We assumed the metabolic rate of the snakehead may be less dependent on temperature and can effectively compensate for temperature changes after acclimation. The objective of this study was to examine the responses of respiratory metabolism in snakehead to both an acute temperature change and a subsequent long term of temperature acclimation.

## Materials and Methods

### Animals

The snakehead juveniles were obtained from a hatchery in Guangdong province and were maintained in a rearing system with continuous aeration for 2 months prior to the experiment. The temperature was set at  $25.0 \pm 0.5$  °C same as in the hatchery. The photoperiod was set at 12L: 12D, oxygen content was kept above  $7 \text{ mg L}^{-1}$ , and the ammonia-N concentration was maintained lower than  $0.015 \text{ mg L}^{-1}$ . During the acclimation period, the fish were fed once daily to satiation with cutlets of fresh silver carp (*Hypophthalmichthys molitrix*) without viscera, head and tail, and the uneaten food was removed after 30 min. At the end of the acclimation period, the body sizes of experimental fish were  $4.5 \pm 0.5$  g.

### Experimental Procedures

The control temperature was set at 25°C, same as the water temperature in the period prior to the experiment, which was also within the optimum temperature range for growth of the snakehead (25 to 30°C) (Liu et al., 1998). Four other temperatures (15, 20, 30, and 35°C) were set to test the effects of both acute temperature change and chronic temperature acclimation on the metabolism of the snakehead. The sample size of each treatment was seven individuals. After 48 h of fasting, fish of each treatment group were weighed and placed into the respiratory chambers overnight. For the control group, *RMR* and *VF* were measured at 25°C. For the acute temperature change groups, water temperature was changed by  $0.5^\circ\text{C h}^{-1}$  from 25°C to 20°C or 30°C, and by  $1^\circ\text{C h}^{-1}$  from 25°C to 15°C or 35°C within 10 h. Then *RMR* and *VF* were measured. The same process was used for the chronic temperature acclimation groups, and the fish were kept in the target temperatures for 2 additional weeks and were hand fed once daily (18:00) to satiation. Then, after 48 h of fasting, the *RMR* and *VF* of the chronic temperature acclimation groups were measured. All variables of each group were measured at 1-h intervals for 12 h, and the three lowest values of each variable were averaged.

The *RMR* was determined using a 15-chamber continuous-flow respirometer, as described by Luo and Xie (2009). One chamber without fish was used as the control. Oxygen consumption rate was used as metabolic rate. The dissolved oxygen concentration was measured at the outlet of the chamber using an oxygen meter (HQ30, Hach Company, Loveland CO, USA). To enhance the accuracy of the oxygen readings, a difference of at least  $0.3 \text{ mg L}^{-1}$  was maintained between the outflow water of the experiment chamber and the control chamber. The flow rate of the water through the respirometer chamber was measured by collecting the water outflow from each tube into a 100 mL beaker and recording the duration in minutes (Cutts, Metcalfe, & Taylor, 2002). *VF* ( $\text{times min}^{-1}$ ) was counted using a stopwatch.

*RMR* ( $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) was calculated as:  $RMR = \Delta\text{O}_2 \nu m^{-1}$ , where  $\Delta\text{O}_2$  represents the differences in the concentration of oxygen ( $\text{mg O}_2$ ) between fish and the control chambers,  $\nu$  ( $\text{L h}^{-1}$ ) represents the velocity of water flow through the chamber, and  $m$  (kg) is the wet mass of the fish. The oxygen consumption per ventilation (*OCPV*,  $\mu\text{g O}_2 \text{ time}^{-1}$ ) was calculated using the following formula:

$$OCPV = 1000 \times m \times RMR / VF / 60.$$

After taking the metabolism measurements, individual blood samples were obtained to determine the size and number of red blood cell (*RBC*) and hemoglobin (*Hb*) contents. The fish were anesthetized using a mixed liquor of  $0.15 \text{ g L}^{-1}$  tricaine methanesulfonate (MS-222) and  $0.3 \text{ g L}^{-1}$  sodium bicarbonate solution ( $\text{NaHCO}_3$ ). After body mass was

measured, blood was collected using a 1 mL pipette containing 1 mg L<sup>-1</sup> heparin sodium. RBC number was determined using a Neubauer hemocytometer following prior dilution of the blood with 0.65% normal saline (Handy & Depledge, 1999). Erythrocyte smears were produced using a Wright-Giemsa staining solution kit (Jinan Baboo Biotech Co., Ltd, Nanjing, China) (Huang, Zhang, Liu, Wang, & Luo, 2013). The Neubauer hemocytometer and the stained smears were observed and photographed under a digital light microscope equipped with a video camera linked to a computer (Aigo Digital Technology Co., Ltd, Beijing, China). The length, width, and area of 50 erythrocytes were measured randomly by software (Image-pro Plus) for each fish. The *Hb* content was determined through the cyanmethemoglobin method (Dacie & Lewis, 1984).

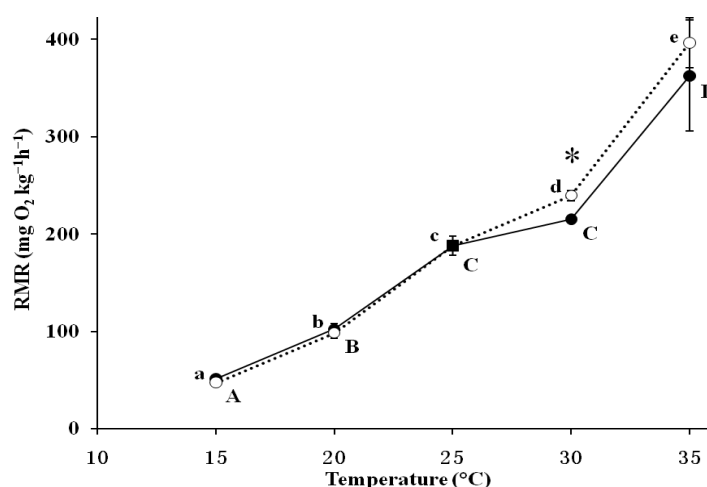
### Statistical Analysis

All statistical analyses were carried out using SPSS, version 11.0 (SPSS Inc., Chicago, IL, USA). The effects of the acute temperature change and chronic temperature acclimation were tested using one-way ANOVA followed by least significant difference (LSD) test. The *t*-test was used to compare the effects of acute temperature change and those of

chronic temperature acclimation at same temperature. The correlations between those variables and temperatures were analyzed by Pearson's correlation. Variables of the acute temperature group and the chronic temperature acclimation group were represented by the subscripts of 'Acute' and 'Accl', respectively. Differences were considered significant when the *P*-value was less than 0.05. All data were presented as the mean ± SE.

### Results

RMR was 188.1 mg O<sub>2</sub> kg<sup>-1</sup>h<sup>-1</sup> at 25°C, and increased to 396.2 and 362.7 mg O<sub>2</sub> kg<sup>-1</sup>h<sup>-1</sup> at 35°C, while decreased to 46.9 and 51.2 mg O<sub>2</sub> kg<sup>-1</sup>h<sup>-1</sup> at 15°C, post acute temperature change and chronic acclimation, respectively (Figure 1). Both RMR<sub>Acute</sub> and RMR<sub>Accl</sub> were positively correlated with temperature ( $r = 0.97$  for RMR<sub>Acute</sub> and  $r = 0.95$  for RMR<sub>Accl</sub>, both  $P < 0.01$ ), and exhibited V-shaped Q<sub>10</sub> values change vs temperature (Table 1). The Q<sub>10</sub> values ranged 1.62 to 4.37 for RMR<sub>Acute</sub> and 1.31 to 4.00 for RMR<sub>Accl</sub> and their lowest values were observed within 25 to 30°C. Significant differences between RMR<sub>Acute</sub> and RMR<sub>Accl</sub> were found only in the fish treated at 30°C, with a lower RMR<sub>Accl</sub> compared to RMR<sub>Acute</sub> ( $P < 0.05$ ). Four individuals died during



**Figure 1.** Effects of acute temperature change (open circles, dashed line) and temperature acclimation (filled circles, solid line) on resting metabolic rate (RMR) of the snakehead. The 25°C is the control (filled square). Data are presented as the mean ± SE. Asterisks denote significant differences between acute change and chronic acclimation at the same temperature ( $P < 0.05$ ). Different superscripts denote significant differences among acute temperature change treatments (lowercase) or temperature acclimation treatments (uppercase) ( $P < 0.05$ ).

**Table 1.** Q<sub>10</sub> values for resting metabolic rate (RMR) and ventilation frequency (VF) of the snakehead post acute temperature change and chronic acclimation

Temperature range	15 to 20°C	20 to 25°C	25 to 30°C	30 to 35°C
Q <sub>10</sub> of RMR <sub>Acute</sub>	4.37	3.68	1.62	2.38
Q <sub>10</sub> of RMR <sub>Accl</sub>	4.00	3.38	1.31	2.84
Q <sub>10</sub> of VF <sub>Acute</sub>	2.51	1.99	1.29	1.99
Q <sub>10</sub> of VF <sub>Accl</sub>	2.01	1.52	1.37	1.08

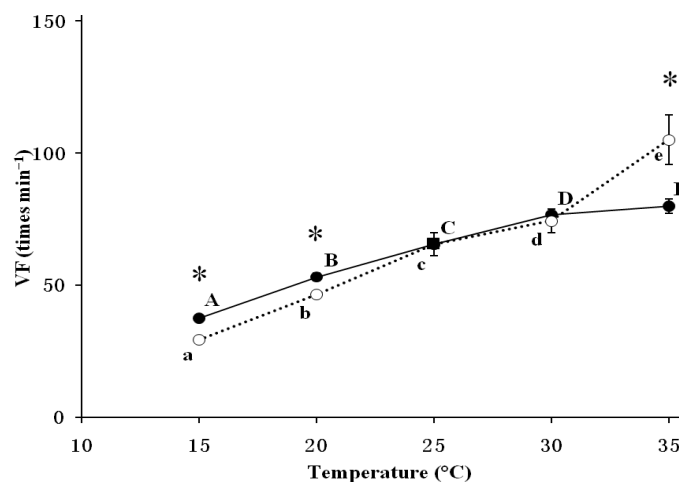
acclimation at 35°C, thus the data were obtained only in three individuals at this temperature.

$VF$  also showed positive correlations with temperature in the fish after acute temperature change (Figure 2). However,  $VF$  tended to increase and level off at higher temperature in the fish after chronic acclimation.  $Q_{10}$  values for  $VF$  changed with temperature in similar tendencies to those for  $RMR$ , with largest values within 15 to 20°C and lowest values between 25 and 30°C, but had smaller values compared to  $RMR$  (Table 1).  $VF_{Accl}$  were larger than  $VF_{Acute}$  at 15°C and 20°C, but  $VF_{Accl}$  were lower than  $VF_{Acute}$  at 30°C. Similarly,  $OCPV$  tended to be higher at higher temperature, except for a lower value of the

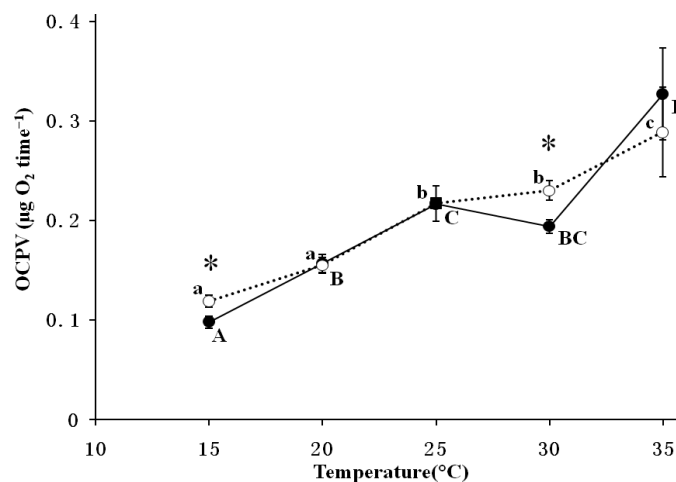
acclimated fish at 30°C compared to those at 20°C (Figure 3).  $OCPV_{Acute}$  were larger than  $OCPV_{Accl}$  at 15°C and 30°C.

The  $RBC$  number of the fish treated by acute change in temperature was significantly lower at 30°C compared to the acute temperature change in other groups ( $P < 0.05$ ) (Table 2). No significant difference of  $RBC$  number was found among those fish after temperature acclimation. No significant difference in  $RBC$  width, length, and area and  $Hb$  content was found among different temperatures (Table 2).

## Discussion



**Figure 2.** Effects of acute temperature change (open circles, dashed line) and temperature acclimation (filled circles, solid line) on ventilation frequency ( $VF$ ) of the snakehead. The 25°C is the control (filled square). Data are presented as the mean $\pm$ SE. Asterisks denote significant differences between acute change and chronic acclimation at the same temperature ( $P < 0.05$ ). Different superscripts denote significant differences among acute temperature change treatments (lowercase) or temperature acclimation treatments (uppercase) ( $P < 0.05$ ).



**Figure 3.** Effects of acute temperature change (open circles, dashed line) and temperature acclimation (filled circles, solid line) on oxygen consumption per ventilation ( $OCPV$ ) of the snakehead. The 25°C is the control (filled square). Data are presented as the mean $\pm$ SE. Asterisks denote significant differences between acute change and chronic acclimation at the same temperature ( $P < 0.05$ ). Different superscripts denote significant differences among acute temperature change treatments (lowercase) or temperature acclimation treatments (uppercase) ( $P < 0.05$ ).

**Table 2.** Hematological parameters of the snakehead after acute temperature change and chronic temperature acclimation

	Control			Acute temperature change			Chronic temperature acclimation		
	25°C	15°C	20°C	30°C	35°C	15°C	20°C	30°C	35°C
Number	7	7	7	7	3	7	7	7	3
Body mass (g)	4.43±0.15	4.42±0.07	4.43±0.12	4.26±0.12	4.47±0.11	4.30±0.13	4.84±0.06	4.14±0.11	4.34±0.04
Hb (mg mL <sup>-1</sup> )	66.54±7.46	56.90±3.10	66.54±5.64	75.09±8.06	73.54±6.27	50.95±3.47	62.56±3.50	54.10±4.35	61.97±0.95
RBC number (10 <sup>9</sup> mL <sup>-1</sup> )	2.33±0.19 <sup>a</sup>	2.44±0.15 <sup>a</sup>	2.19±0.20 <sup>a</sup>	2.95±0.11 <sup>b</sup>	2.24±0.27 <sup>a</sup>	2.4±0.10	2.74±0.10	2.41±0.18	2.74±0.10
RBC length (µm)	9.33±0.19	9.36±0.09	9.50±0.13	9.43±0.60	9.09±0.35	9.01±0.14	9.29±0.12	8.75±0.22	9.29±0.12
RBC width (µm)	6.17±0.15	6.10±0.13	6.31±0.16	6.22±0.15	6.35±0.11	6.08±0.06	6.18±0.16	6.09±0.07	6.18±0.16
RBC area (µm <sup>2</sup> )	45.21±1.83	44.80±1.23	47.57±1.72	45.98±1.13	45.23±2.48	42.96±0.82	45.04±1.45	41.72±0.80	45.04±1.45

Data are presented as the mean±SE. <sup>a,b</sup>: Values with different superscript were significantly different (P<0.05).

*RMR* of the snakehead tended to be higher at higher temperatures (Figure 1), similar to the general metabolic response patterns observed in many other fish species (Jobling, 1994; Clarke & Johnston, 1999). The  $Q_{10}$  values for *RMR* of fishes generally ranged 1.65 to 2.70 as reviewed by Jobling (1994) and was suggested a mean value of 2.4 (Clarke & Johnston, 1999), compared to which the  $Q_{10}$  values of the snakehead varied within wider ranges (range 1.62 to 4.37 for  $RMR_{Acute}$  and 1.31 to 4.00 for  $RMR_{Accl}$ ) with changing temperature (Table 1). The results suggest that the snakehead has a varying metabolic thermal sensitivity dependent on temperature range, which is not consistent with our prediction. The lowest  $Q_{10}$  values of the snakehead were observed between 25 to 30°C, in consistent with its optimum temperature range for growth (25 to 30°C) (Liu *et al.*, 1998). Our results also showed that the metabolic thermal sensitivity of the snakehead can be reduced by acclimation, represented by lower  $Q_{10}$  value for  $RMR_{Accl}$  than that for  $RMR_{Acute}$ , which is consistent with previous results for the sandbar shark (*Carcharhinus plumbeus*), Atlantic cod (*Gadus morhua*), and southern catfish (*Silurus meridionalis*) (Dowd *et al.*, 2006; Perez-Casanova *et al.*, 2008; Zeng *et al.*, 2011). It suggests a down-regulation in metabolism of this fish during acclimation for coping with the challenge due to the higher oxygen demand at higher temperature. In our study, fish were fed to satiation during temperature acclimation. Thus, the effect of temperature on *RMR* may be also partly attributed to different feeding levels and then different nutritional status among temperatures as fish generally eats more at higher temperature.

Fish may increase *VF* to compensate for the problem of increasing oxygen demand and decreasing water oxygen availability as temperature increases (Sollid *et al.*, 2005; Frisk *et al.*, 2012). *VF* was reported to be the main regulatory mechanism of oxygen uptake in pikeperch (*Sander lucioperca*) (Frisk *et al.*, 2012). Increased *VF* has been reported in many studies of fish after either acute temperature increase (Cai & Adelman, 1990; Jayasundara, Healy, & Somero, 2013) or thermal acclimation (Aguilar *et al.*, 2002; Robinson, Egginton, & Davison, 2011; Zhao *et al.*, 2011; Frisk *et al.*, 2012). In the present study, the snakehead presented smaller variations in *VF* (approximately 3.6-fold for acute temperature change and 2.1-fold for thermal acclimation) than in

*RMR* (approximately 8.5-fold for acute temperature change and 7.1-fold for thermal acclimation) as temperature either dropped to 15 °C or increased to 35°C (Figure 1, 2). The  $Q_{10}$  values for *VF* were also smaller than those for *RMR* (Table 1). These results suggest that regulation of *VF* alone may not be sufficient to satisfy the oxygen demand of the snakehead as water temperature increases. Similarly, smaller increases in *VF* than in *RMR* were also reported in spangled perch (*Leiopotherapon unicolor*) (Gehrke & Fielder, 1988), tilapia (*Oreochromis niloticus*) (Maricondi-Massari, Kalinin, Glass, & Rantin, 1998), and grass carp (*Ctenopharyngodon idellus*) (Zhao *et al.*, 2011). Therefore, the increased *OCPV* with increasing temperature (Figure 3) indicates additional regulations, such as ventilatory volume, oxygen extraction, gill perfusion, and gill remodeling, may be required for oxygen exchange at high temperature (Brauner & Randall, 1996; Sollid *et al.*, 2005; Sollid & Nilsson, 2006). Consistent with the results of silver catfish (*Rhamdia quelen*) and rainbow trout (*Oncorhynchus mykiss*) (Lermen, Lappe, Crestania, Vieira, Gioda, Schetinger, Baldisserotto, Moraes, & Morsch, 2004; Guan, Wen, Liu, Wang, & Wang, 2014), *RBC* size and *Hb* content of the snakehead did not change in both acute and chronic temperature acclimation (Table 2). However, whether regulations happen in blood oxygen-carry capacity needs more hematological properties, e.g. hematocrit. It also indicates other regulation factors, such as gas diffusion distance, total respiratory surface area, heart rate, cardiac output and cardiac stroke volume may be more important for oxygen transport at high temperature (Saroglia, Cecchini, Terova, Caputo, & De Stradis, 2000; Saroglia, Terova, Stradis, & Caputo, 2002; Sollid *et al.*, 2005; Sollid & Nilsson, 2006; Dowd *et al.*, 2006; Farrell, Axelsson, Altimiras, Sandblom, & Claireaux, 2007; Farrell, Eliason, Sandblom, & Clark, 2009).

Thermal compensation of metabolic rate have been found in many fish species, such as tilapia (*Alcolapia grahami*), Antarctic eelpout (*Pachycara brachycephalum*), pacu (*Piaractus mesopotamicus*), gilthead seabream (*Sparus aurata*), Atlantic cod, grass carp, and shorthorn sculpin (*Myoxocephalus scorpius*) (Johnston *et al.*, 1994; van Dijk *et al.*, 1999; Aguilar *et al.*, 2002; Ibarz *et al.*, 2003; Perez-Casanova *et al.*, 2008; Zhao *et al.*, 2011; Sandblom *et al.*, 2014). In our study, compensation for temperature

occurred at 30°C rapidly post only two weeks of acclimation, as  $RMR_{Accel}$  was lower than  $RMR_{Acute}$  (Figure 1), which is consistent with our prediction. However, further increasing temperature to 35°C did not result in significant thermal acclimation effects, as no significant difference was produced between  $RMR_{Accel}$  and  $RMR_{Acute}$ . This is an expected outcome, as 35°C exceeds the upper limits of the optimum temperature range of the snakehead (Liu *et al.*, 1998; Xie *et al.*, 2002). Indeed, some individuals died at 35°C, partly due to the rapid temperature change (10 °C in 10h) in our study. Our results did not find a significant difference between  $RMR_{Accel}$  and  $RMR_{Acute}$  in the fish at 20°C or 15°C, suggesting no metabolic compensation at a lower temperature. Similarly, metabolic compensation appearing only at higher temperatures was also reported in gilthead seabream (Ibarz *et al.*, 2003).

An interesting result of our study was that  $VF$  increased in the cold acclimation fish compared to the fish treated by acute cooling (Figure 2), while  $RMR$  remained unchanged, indicating stronger thermal compensations in  $VF$  rather than in  $RMR$  at 15°C and 20°C. Similar compensation in  $VF$  was also found in common carp (*Cyprinus carpio*), Antarctic notothenioid fish (*Pagothenia borchgrevinkii*), and (*Trematomus bernacchii*) by two weeks of temperature acclimation (Cai & Adelman, 1990; Robinson *et al.*, 2011; Jayasundara *et al.*, 2013). A higher  $VF$  under cold temperature might not be energetically advantageous, as opercular movement of gills is energetically expensive for a fish (Perry & Wood, 1989). Thus, the increasing  $VF$  during cold acclimations without changes in  $RMR$  suggests that some other mechanism may happen. A possible explanation could be that oxygen exchange capacity gradually decreases during cold acclimation, as gill remodeling by cold acclimation may reduce respiratory area (Sollid *et al.*, 2005; Sollid & Nilsson, 2006) and increase gas diffusion distance (Saroglia *et al.*, 2000). Thus, a compensation of  $VF$  in cold acclimated fish may be necessary to maintain oxygen supply levels.

In conclusion, our results suggest that the snakehead has a varying thermal metabolic sensitivity dependent of temperature range and that some regulations other than  $VF$ , RBC, and Hb content may be needed to satisfy its oxygen demand as temperature increases. The higher  $VF$  of cold acclimated fish may compensate for the decreasing oxygen exchange capacity of the gill during cold acclimation.

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