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## Effects of temperature acclimation on the critical thermal limits and swimming performance of *Brachymystax lenok tsinlingensis*: a threatened fish in Qinling Mountain region of China

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**Abstract** *Brachymystax lenok tsinlingensis* is an endangered teleost fish species that occurs in the Qinling Mountain region of China. It also happens to represent the southernmost distribution of an endemic Salmonid fish worldwide. Recently, the habitat of this species shifted towards a higher altitude presumably because of climate change, indicating that this species might be suffering from thermal stress. However, information on the thermal physiology of this species is extremely limited. Accordingly, we investigated the effects of acclimation temperature (6, 12, and 18 °C) on ecologically relevant end points such as critical thermal limits, swimming performance and metabolic rate. Our results showed that elevated acclimation temperatures resulted in increased thermal tolerance and decreased swimming efficiency. High temperature (i.e., 18 °C) did not have a marked effect on the critical swimming speed and the maximum metabolic rate but caused an increase in the energetic cost of transport compared with the results at 12 °C. Interestingly, we found that both the acclimation response ratio and the critical thermal maxima of *B. lenok tsinlingensis* were higher than that of many other Salmonidae fishes, suggesting that this species responds plastically to temperature changes and has a high thermal tolerance. These characteristics are hypothesized to be related to the southernmost distribution of this species.

**Keywords** Critical thermal limits · Swimming performance · Thermal sensitivity · *Brachymystax lenok tsinlingensis* · Temperature

### Introduction

Temperature is one of the most important abiotic factors for ectothermic animals (Huey and Stevenson 1979) and has been called the ‘ecological master factor’ for fish (Brett 1971). Changes in the average temperature and the thermal heterogeneity of terrestrial and aquatic environments expose many fish populations to suboptimal conditions (Kelly et al. 2014). Climate models project a global increase in global surface temperature of 0.3–4.8 °C by the end of the 21st century (IPCC 2014), and such changes would expose cold-adapted species to suboptimal temperatures and cause a loss of optimal thermal habitats (Ficke et al. 2007; Farrell 2009). According to Casselman (2002), a 3 °C increase in water temperature is predicted to result in a 20% reduction in the range and abundance of cold-water salmonids. Therefore, the persistence of cold-adapted species and populations may be determined by their capacity to adapt to elevated temperatures (Willi et al. 2006; Chown et al. 2010; Kelly et al. 2014).

Qinling lenok, *Brachymystax lenok tsinlingensis*, is an endangered teleost fish species that belongs to the family Salmonidae and has now been listed as “Threatened” on the International Union for Conservation of Nature (IUCN) Red List as well as a nationally protected wild animal according to the China Red Data Book of Endangered Animals. This species is generally regarded as a post-glaciation invader. It is only found in the cold-water stream segments of the Heihe, Shitou, Xushui and Taibai rivers in the Qinling Mountain region of China (Liu et al. 2013). This area represents the southernmost distribution of Salmonid fish worldwide. Remarkably, the lowest elevation for the distribution of *B. lenok*

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*tsinlingensis* has changed from 900 to 1200 m (Ren and Liang 2004; Zhao and Zhang 2009) likely in response to climate change (1 °C increases over the past 10 years) (Zheng et al. 2010), indicating that this species may be suffering from thermal stress to a certain extent. Nevertheless, information is not available on the thermal physiology of this species. Furthermore, *B. lenok tsinlingensis* strongly prefers swift currents and clear water over a large gravel substrate and it presents migratory behavior that can be triggered by changes in seasonal temperatures (Zhao and Zhang 2009). Accordingly, the maintenance of its swimming performance is critically important for its survival, reproduction and routine activities. However, little is known about the effect of temperature on the swimming performance of *B. lenok tsinlingensis*. Therefore, we investigated the critical thermal limits and swimming performance of *B. lenok tsinlingensis* in response to temperature acclimation.

The critical thermal maxima ( $CT_{max}$ ) is assumed to be the most important factor for determining the distribution of a species (McCullough et al. 2009; Sears et al. 2011). As temperatures increase, the thermal limit may be approached and exceeded for a length of time that compromises the survival of individual fish, especially at lower elevations. Even if the temperature does not approach the lethal limit, individuals may experience sublethal thermal stress that results in decreased growth and reproduction, thus leading to eventual population failures (Bear et al. 2007; Underwood et al. 2012; Whitney et al. 2016). Therefore, knowledge of thermal acclimation and critical thermal limits is important in understanding how a species may respond biologically to different thermal regimes (Dülger et al. 2012; Whitney et al. 2016). Laboratory studies have shown that the critical thermal limits of a fish is dependent on the acclimation temperature, and they indicate that a high physiological plasticity in critical thermal limits may greatly facilitate the ability of a fish species to adapt to a new environment (Underwood et al. 2012; Peng et al. 2014; Chen et al. 2015). To explore the temperature-induced plasticity of the phenotypic characteristics of *B. lenok tsinlingensis*, an investigation of the critical thermal limits of the fish and a comparison of its thermal acclimation capacity with other Salmonid fishes is required.

The thermal-tolerance of swimming performance of a fish is considered a principal characteristic that determines the survival of many fish species because of its impact on food capture, predator avoidance, reproduction and habitat shifts (Plaut 2001; Killen et al. 2010). Swimming performance is highly important ecologically and presumed to be subject to selection pressures that enhance evolutionary fitness (Ohlberger et al. 2008; Tu et al. 2011; Roche et al. 2013; Cooke et al. 2014). Thus, when fish are exposed to long-term temperature changes, they can achieve optimal swimming performance by altering their physiological characteristics by acclimation or adaptation (Guderley and Blier 1988). The most common method of assessing the aerobic swimming capacity of fish is the critical swimming speed ( $U_{crit}$ )

(Brett 1964), which is widely considered to be a predictor for assessing the ecological effects of environmental factors on animal performance (Fu et al. 2012, 2013). We hypothesized that the thermal plasticity of swimming performance is likely important to fish species such as *B. lenok tsinlingensis*. Generally, the swimming performance of a fish will be impaired when the ambient temperature exceeds the optimum temperature (Eliason et al. 2011; Pang et al. 2013). Ecologically, decreased swimming performance, including the swimming speed and the swimming efficiency, might render an animal more vulnerable to predation and affect its foraging efficiency and other daily activities (Blake 2004; Pang et al. 2013; Xia et al. 2015).

The purpose of this study was to (1) determine the critical thermal limits of *B. lenok tsinlingensis* and evaluate its thermal acclimation capacity, (2) investigate the effects of temperature acclimation on swimming performance and metabolic rate of *B. lenok tsinlingensis*, and (3) compare the critical thermal limits and thermal sensitivity of swimming performance in *B. lenok tsinlingensis* with that of other Salmonid fishes.

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## Materials and methods

### Ethical statement

The species used in this study is a nationally protected wild animal in China and has been included in the IUCN Red List of threatened species. All of the experiments were conducted with approval from the National Natural Science Foundation of China (No. 31300340) and the Animal Ethics Committee of Chongqing Normal University. The experimental procedures were performed according to the Guidelines on the Humane Treatment of Laboratory Animals established by the Ministry of Science and Technology of the People's Republic of China.

### Experimental fish

Experimental juvenile *B. lenok tsinlingensis* (14.15–26.55 g, 9.9–12.88 cm,  $n = 32$ ) were collected from the Xushui River National Nature Reserve, Qinling Mountain region, Shaanxi Province, China. The sampling site was located at the coordinates 33°744'N and 107°460'E at an altitude of 1200–1500 m above the sea level. After the experimental fish were collected, they were placed in two fully aerated and thermoregulated tanks (60 L) at the same temperature (13.4–14.2 °C) observed at each collection site and quickly transported to our laboratory. The fish were then maintained in a 360 L re-circulating water tank system at Chongqing Normal University for 3 weeks prior to experimentation. During this time, the temperature of the de-chlorinated freshwater was maintained at 13.5 °C ( $\pm 0.5$  °C), the dissolved oxygen level was maintained

above  $9 \text{ mg L}^{-1}$ , the pH ranged from 6.8 to 7.3, and the ammonia-N levels ranged from 0.0025 to  $0.01 \text{ mg L}^{-1}$ . The fish were fed to satiation daily at 8:00 a.m. with fresh live *Tenebrio molitor* larvae. Uneaten food and feces were removed 20 min after feeding using a siphon, and one-fifth of the water in each tank was replaced daily with fresh water.

### Experimental protocol

Three ecologically-relevant acclimation temperatures (6, 12, and  $18 \text{ }^\circ\text{C}$ ) were used in this experiment. The highest temperature (i.e.,  $18 \text{ }^\circ\text{C}$ ) represents the mean temperature occupied by fish in the wild in the hottest summer months. The reproductive temperature of the species ranges from about 6 to  $12 \text{ }^\circ\text{C}$ ; the fish starts migration to overwintering sites and spawning migration, respectively, when the temperature is less than  $6 \text{ }^\circ\text{C}$  in winter and higher than  $6 \text{ }^\circ\text{C}$  in spring (Zheng et al. 2010). After the 3 week housing period, 24 healthy fish of similar size ( $19.1 \pm 0.5 \text{ g}$ ,  $11.1 \pm 0.1 \text{ cm}$ ) were selected and randomly divided into three groups of 8 individuals ( $n = 8$ ). The fish were transferred from the general holding tanks to one of three identical 120 L re-circulating water tank systems. The water temperature was  $13.5 \text{ }^\circ\text{C}$  when the fish were transferred, and it was then increased or decreased at the rate of  $1 \text{ }^\circ\text{C day}^{-1}$  until the desired temperature was reached. The fish were acclimated to the experimental temperature for 3 weeks. The temperature manipulation was automatically achieved by chillers (model C-1000A, Risheng Group, Guangdong, China).

After the temperature acclimation period was completed, the fish were fasted for 48 h. Individual fish were then placed in a swim tunnel respirometer (Li et al. 2010), and their swimming performance and oxygen consumption rate ( $\dot{M}_{O_2}$ ) during swimming were determined using the  $U_{crit}$  method (Brett 1964). The energetic cost of transport (COT) as subsequently calculated.

The fish were then allowed to recover for two weeks after measuring for the  $U_{crit}$ . Thereafter, 5 healthy and lively fish from each acclimation temperature ( $n = 5$ ) were selected and fasted for 48 h, and the upper critical thermal limits of the individual fish was evaluated using the critical thermal methodology of Becker and Genoway (1979). The results of our preliminary experiment showed that two weeks of recovery time were sufficient to counteract the effects of the  $U_{crit}$  measurement. Because *B. lenok tsinlingensis* is a nationally protected animal, a relatively small sample size was used to determine the critical thermal limits.

### Critical thermal limits

The critical thermal limit of the experimental fish was evaluated using the critical thermal methodology of Becker and Genoway (1979). Individual fish were introduced into a 60 L tank for 1 h of habituation. The water temperature

in the tank was the same as the acclimation temperature, and the dissolved oxygen content during the experiment was maintained above 90% saturation. The oxygen content was measured by an oxygen probe (HQ30, Hach Company, Loveland, CO, USA). Either a water chiller or heater and a voltage regulator were used to achieve the experimental rate of temperature change. After the habituation period, the water temperature was increased at a rate of  $0.3 \text{ }^\circ\text{C min}^{-1}$  (Beitinger et al. 2000). The individual fish were closely observed, and their thermal resistance was evaluated. When subjected to adverse temperature conditions, the experimental fish usually exhibited a “jumping” behavior which was characterized by the whole body of a fish breaking the water surface, presumably in an attempt to flee an undesirable temperature. The temperature at the moment that the fish showed the “jumping” behavior for the first time was considered the upper incipient avoidance temperature ( $AT_{max}$ ). The values of the  $CT_{max}$  and the lethal thermal maxima ( $LT_{max}$ ) were considered the temperatures at which the fish showed a loss of body equilibrium and cessation of gill movement, respectively (Peng et al. 2014).

The acclimation response ratio (ARR) is a convenient index of the magnitude of the thermal acclimation of the organisms. Arithmetically, the ARR was determined by dividing the change in tolerance by the total change in the acclimation temperature (Claussen 1977; Li and Wang 2005; Kelley 2014). The ARR value was used to estimate the effect of the acclimation temperature on the  $AT_{max}$ ,  $CT_{max}$  and  $LT_{max}$ .

### Metabolism and swimming performance

#### $U_{crit}$

The  $U_{crit}$  of individual fish was measured using a Brett-type swimming tunnel respirometer with a swim chamber that had a  $20\text{-cm}^2$  cross-sectional area and a total volume of 3.5 L (for details, see Li et al. 2010). The fish were gently transferred into the swimming tunnel and allowed to habituate to the continuous flow of aerated water for 1 h prior to the  $U_{crit}$  measurement (Fu et al. 2011, 2013). The water temperature in the swimming tunnel was consistent with the acclimation temperature and controlled within  $\pm 0.5 \text{ }^\circ\text{C}$  using a water bath connected to a stainless steel heat exchanger. The water velocity was  $6 \text{ cm s}^{-1}$  (i.e.,  $0.5$  body length  $\text{s}^{-1}$ ) during the habituation period and subsequently increased every 20 min in  $6 \text{ cm s}^{-1}$  increments until the fish became exhausted. Fatigue status was defined as the time at which the fish failed to move off the rear screen of the swimming chamber for 20 s. The  $U_{crit}$  was calculated for each fish using Brett’s equation (Brett 1964):

$$U_{crit} = U + (t/T)\Delta U \quad (1)$$

where  $U$  ( $\text{cm s}^{-1}$ ) is the highest speed at which the fish swam during the full time period (20 min);  $\Delta U$  is the

velocity increment ( $6 \text{ cm s}^{-1}$ );  $T$  is the prescribed period of swimming per speed (20 min); and  $t$  is the time duration for which the fish swam at the final speed (min). Because the cross-sectional area of each fish was  $<20\%$  but occasionally  $>10\%$  of the area of the swimming chamber, the calibrated water speed was corrected for the solid blocking effect according to the following equation (Zeng et al. 2009):

$$\text{Corrected } U_{\text{crit}} = U_{\text{crit}} \left\{ 1 + [0.4BL/0.5(w + d)] \times (0.25\pi dw/A_t)^{1.5} \right\} \quad (2)$$

where  $BL$  is the body length (cm);  $w$  is the maximum fish width (cm);  $d$  is the maximum fish depth (cm); and  $A_t$  is the tunnel cross-sectional area ( $\text{cm}^2$ ). The individual relative critical swimming speed ( $rU_{\text{crit}}$ ,  $\text{BL s}^{-1}$ ) was calculated by dividing the  $U_{\text{crit}}$  value by the body length.

### Metabolism

The oxygen concentration was recorded once every 2 min using an oxygen probe. In order to avoid undue stress on the fish, the water oxygen content in the respirometer was never allowed to fall below 80% oxygen saturation by switching between the open and closed mode of the respirometer (Fu et al. 2013). The  $\dot{M}_{\text{O}_2}$  ( $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) of an individual fish was calculated from the rate of oxygen depletion according to the following equation:

$$\dot{M}_{\text{O}_2} = 60(S - S_0)V/m^{0.75} \quad (3)$$

where  $S$  ( $\text{mg L}^{-1} \text{ min}^{-1}$ ) and  $S_0$  ( $\text{mg L}^{-1} \text{ min}^{-1}$ ) are the values of the slope of the decreased dissolved oxygen content per minute in the water with and without the fish, respectively;  $V$  is the total volume of the respirometer (3.5 L) minus the volume of the fish; and  $m$  (kg) is the body mass of the fish. The slope was obtained from a linear regression of a plot of time (min) versus the dissolved oxygen content ( $\text{mg L}^{-1}$ ). Only slope values with an  $r^2 > 0.95$  were considered in the analysis. The  $\dot{M}_{\text{O}_2}$  was adjusted to a standard body mass of 1 kg using a mass coefficient of 0.75 (Fu et al. 2012). The maximal observed  $\dot{M}_{\text{O}_2}$  during the  $U_{\text{crit}}$  test was defined as the maximum metabolic rate (MMR) (Yan et al. 2013).

### Swimming efficiency

COT ( $\text{J m}^{-1}$ ) was defined as the metabolic energy required to move a unit mass over a given distance (Tucker 1970), and this variable provides an index of the overall swimming efficiency, with lower COT values indicating more efficient locomotion. At each swimming speed, the  $\dot{M}_{\text{O}_2}$  values were converted to  $\text{J kg}^{-1} \text{ m}^{-1}$  using an oxycaloric equivalent of  $13.56 \text{ J mg O}_2$  (Elliott and Davison 1975). The values were then divided by the corresponding swimming speed to obtain the COT.

The COT for an individual swimming fish was calculated according to the following equation:

$$\text{COT} = 13.56 \dot{M}_{\text{O}_2} / (U \times 36) \quad (4)$$

where  $\dot{M}_{\text{O}_2}$  ( $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) is the rate of oxygen depletion of the swimming fish and  $U$  ( $\text{cm s}^{-1}$ ) is the swimming speed.

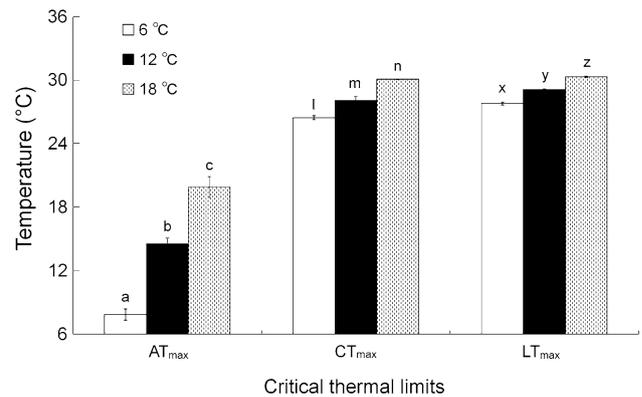
### Data handling and analysis

Statistical analyses were performed using the software program SPSS (version 16.0). The effects of temperature on  $\text{AT}_{\text{max}}$ ,  $\text{CT}_{\text{max}}$ ,  $\text{LT}_{\text{max}}$ ,  $U_{\text{crit}}$ ,  $rU_{\text{crit}}$  and MMR were tested by a one-way analysis of variance (ANOVA) after the data were examined for normality and homogeneity of variance. The ANOVA was followed by Tukey's honest significant difference (HSD) test as a post hoc test (if necessary) to determine differences in the values of different treatment groups. If the data did not show a homogeneity of variance, a Kruskal–Wallis test was conducted followed by a Dunnett T3 test. The effects of temperature and swimming speed on the  $\dot{M}_{\text{O}_2}$  and COT were analyzed by a two-way ANOVA. All of the values are presented as the mean  $\pm$  standard error of the mean (SEM), and a  $p$  value  $< 0.05$  was considered statistically significant.

## Results

### Critical thermal limits

Acclimation temperature had a significant effect on the  $\text{AT}_{\text{max}}$  ( $F = 71.2$ ,  $p < 0.001$ ),  $\text{CT}_{\text{max}}$  ( $\chi^2 = 12.6$ ,  $p = 0.002$ ) and  $\text{LT}_{\text{max}}$  ( $F = 187.7$ ,  $p < 0.001$ ) of the experimental fish. The  $\text{AT}_{\text{max}}$ ,  $\text{CT}_{\text{max}}$  and  $\text{LT}_{\text{max}}$  increased significantly with increases in the acclimation temperature (Fig. 1). Furthermore, the ARR values



**Fig. 1** Effects of acclimation temperature on the upper incipient avoidance temperature ( $\text{AT}_{\text{max}}$ ), the critical thermal maxima ( $\text{CT}_{\text{max}}$ ) and the lethal thermal maxima ( $\text{LT}_{\text{max}}$ ) of *Brachymystax lenok tsinlingensis*. The data are presented as the mean  $\pm$  SEM ( $n = 5$ ). Data with different letters denote statistically significant differences ( $p < 0.05$ ).

**Table 1** Acclimation response ratio (ARR) of *Brachymystax lenok tsinlingensis* within different acclimation temperature ranges

Acclimation temperature (°C)	ARR of AT <sub>max</sub>	ARR of CT <sub>max</sub>	ARR of LT <sub>max</sub>
6–12	1.11	0.27	0.22
12–18	0.89	0.34	0.20

AT<sub>max</sub> upper incipient avoidance temperature; CT<sub>max</sub> critical thermal maxima; LT<sub>max</sub> lethal thermal maxima

indicated that acclimation temperature had a much greater effect on the AT<sub>max</sub> than it did on the CT<sub>max</sub> and the LT<sub>max</sub> (Table 1).

### Metabolism and swimming performance

Both  $U_{crit}$  ( $F = 21.0$ ,  $p < 0.001$ ),  $rU_{crit}$  ( $F = 11.4$ ,  $p < 0.001$ ) and MMR ( $F = 21.3$ ,  $p < 0.001$ ) was significantly affected by temperature acclimation. The values of  $U_{crit}$  and  $rU_{crit}$  and MMR decreased at low temperature (6 °C) compared with the values at 12 °C; however, no significant differences in the  $U_{crit}$ ,  $rU_{crit}$  or MMR were observed between 18 °C (high temperature) and 12 °C ( $p > 0.05$ ) (Fig. 2).

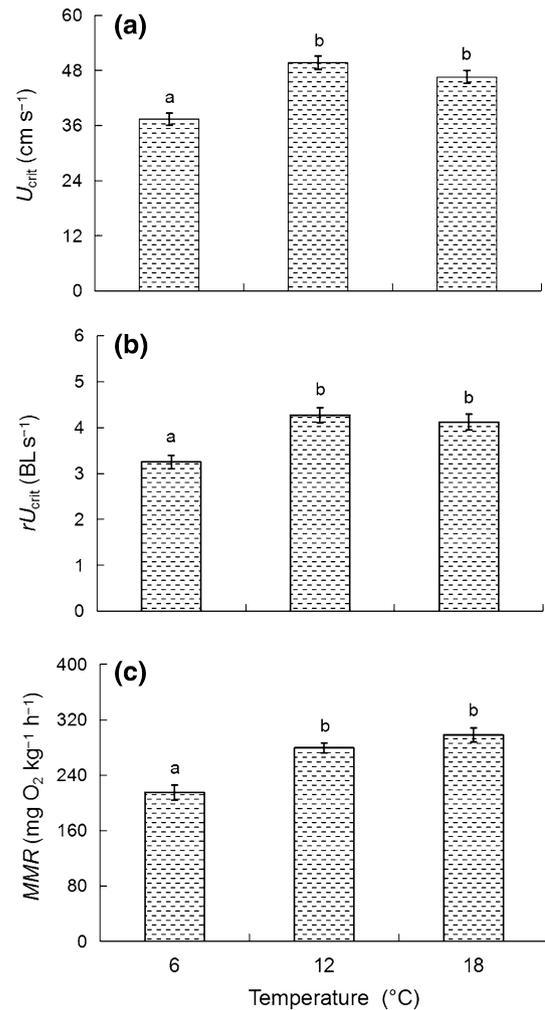
The swimming  $\dot{M}O_2$  was significantly affected by temperature acclimation ( $F = 90.5$ ,  $p < 0.001$ ) and swimming speed ( $F = 38.1$ ,  $p < 0.001$ ). Both a higher acclimation temperature and swimming speed resulted in an increased  $\dot{M}O_2$  (Fig. 3). In addition, temperature acclimation ( $F = 99.3$ ,  $p < 0.001$ ) and swimming speed ( $F = 150.0$ ,  $p < 0.001$ ) significantly affected the COT, which increased with an increase in the acclimation temperature (Fig. 3).

### Discussion

This is the first comprehensive study on the role of acclimation temperature on the critical thermal limits and swimming energetics of endangered *B. lenok tsinlingensis*. Our findings indicate that this species has a high thermal tolerance but its swimming performance was adversely affected by elevated water temperature.

#### Critical thermal limits

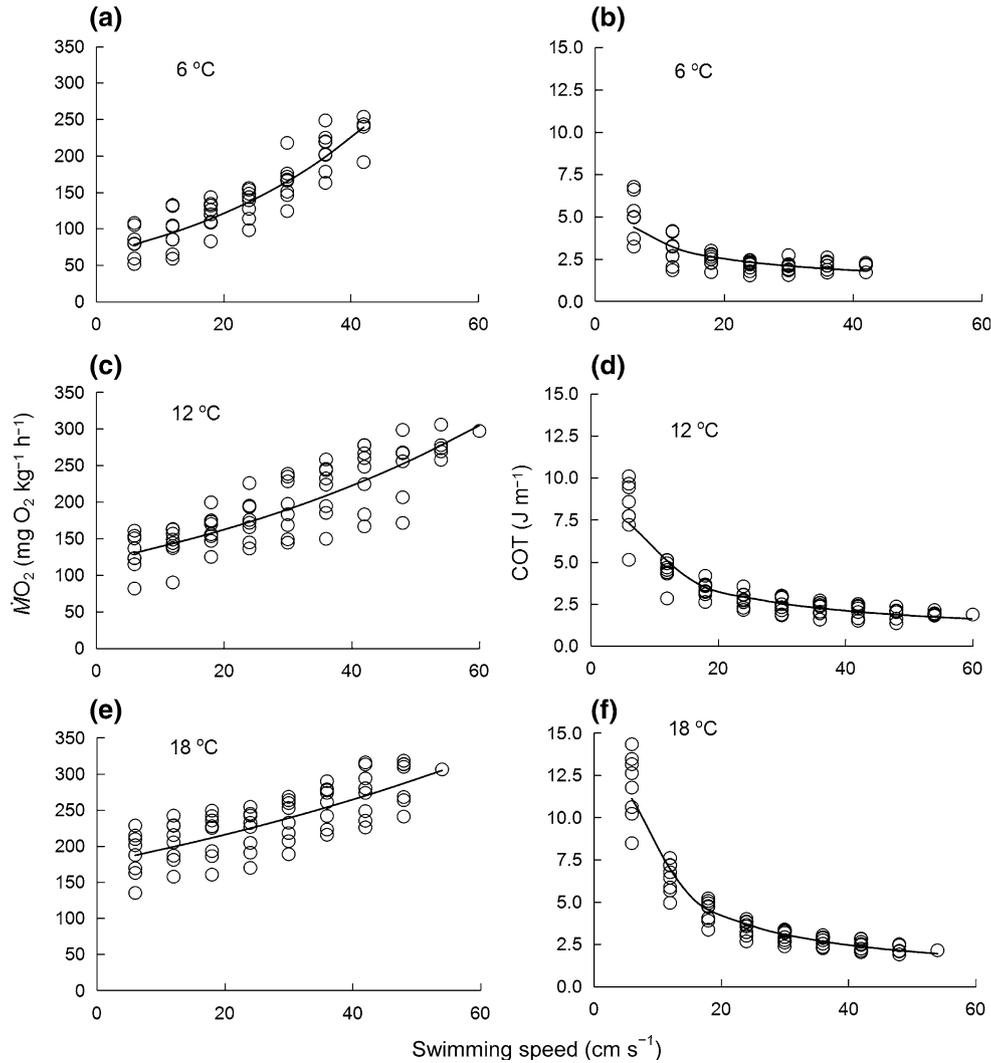
CT<sub>max</sub> is a widely accepted measurement of acute thermal tolerance that can be used to infer an animal's ability to tolerate rapid regional or diurnal temperature increases (Chen et al. 2015). The CT<sub>max</sub> values for *B. lenok tsinlingensis* fell within the range of 26.4–30.1 °C, which are higher than those of many other Salmonidae fishes (Table 2). In particular, the CT<sub>max</sub> values for *B. lenok tsinlingensis* acclimated at 18 °C were higher than 30 °C, which approach the highest value for Salmonidae



**Fig. 2** Effects of acclimation temperature on **a** critical swimming speed ( $U_{crit}$ ), **b**  $rU_{crit}$  and **c** maximum metabolic rate (MMR) of *Brachymystax lenok tsinlingensis*. The  $rU_{crit}$  was calculated by dividing the  $U_{crit}$  value by the body length. The data are presented as the mean  $\pm$  SEM ( $n = 8$ ). Data with different letters denote statistically significant differences ( $p < 0.05$ )

fish, suggesting that this species has a high thermal tolerance.

The ARR is a ratio that estimates the ability of an organism to modify its thermal tolerance level at different acclimation temperatures (Claussen 1977). An animal with a larger ARR value will present a greater increase in its thermal tolerance threshold after acclimation to a higher temperature (Kelley 2014). It has been generally observed that species inhabiting cold and temperate zones have lower ARR values compared with subtropical and tropical species (Dülger et al. 2012; Brahmane et al. 2014), and this difference may have resulted from adaptations in the cold-climate species to greater seasonal fluctuations in temperature over longer time periods (Re et al. 2005). The ARR values and the AT<sub>max</sub>, CT<sub>max</sub> and LT<sub>max</sub> values of *B. lenok tsinlingensis* were relatively high (Kelley 2014) (Tables 1, 2), sug-



**Fig. 3** Effects of acclimation temperature and water velocity on the oxygen consumption rate ( $\dot{M}_{O_2}$ ) and the energetic cost of transport (COT) of *Brachymystax lenok tsinlingensis*. **a, c, e**  $\dot{M}_{O_2}$  and **b, d, f** COT measured in fish at different temperatures

gesting that this species responds plastically to temperature changes and indicating that it has a high thermal tolerance that likely reflects its distribution.

In addition, the values of  $CT_{max}$  and  $LT_{max}$  after thermal acclimation were higher than the  $AT_{max}$  value by more than 10 °C, indicating that this species has the capacity to tolerate a wide thermal range but the range of its optimum temperature is narrow. We therefore hypothesize that this species regularly avoids the high summer/low winter temperatures by migrating to colder/warmer water.

#### Metabolism and swimming performance

Water temperature affects ectothermic animals via changes in the rate of biochemical reactions (Angilletta et al. 2002). Thus, water temperature might influence certain physiological characteristics, such as metabolic

rate and swimming performance. This study clearly demonstrated that both the metabolic characteristics (swimming  $\dot{M}_{O_2}$  and MMR) and the swimming performance of *B. lenok tsinlingensis* were significantly affected by the acclimation temperature. The increased swimming  $\dot{M}_{O_2}$  at high temperatures indicate that greater amounts of energy were required during forced swimming. The COT increased with increasing acclimation temperature, indicating that high temperature resulted in decreased swimming efficiency.

The  $U_{crit}$ , MMR and aerobic scope are considered major parameters that determine the swimming capacity of fish (Fu et al. 2012; Yan et al. 2013). The  $U_{crit}$ ,  $rU_{crit}$  and MMR were all significantly affected by temperature acclimation in this study. The values of  $U_{crit}$ ,  $rU_{crit}$  and MMR decreased by 24.7, 23.8 and 23.1%, respectively, at low temperature (6 °C) compared with the values at 12 °C. In some species, the relationships between  $U_{crit}$  and temperature, and MMR and temperature are rep-

**Table 2** Comparison of the upper thermal limits of several Salmonidae fishes

Species	Acclimation temperature (°C)	$\Delta T$ (°C min <sup>-1</sup> )	CT <sub>max</sub> (°C)	Sources
<i>Brachymystax lenok</i>	6	0.3	26.4	Present study
<i>tsinlingensis</i>	12	0.3	28.1	
	18	0.3	30.1	
<i>Oncorhynchus mykiss</i>	11	0.3	27.5	Myrick and Cech Jr (2005)
	15	0.3	28.4	
	19	0.3	29.6	
<i>Oncorhynchus clarkii pleuriticus</i>	10	0.3	24.6	Underwood et al. (2012)
	15	0.3	26.9	
	20	0.3	29.4	
<i>Oncorhynchus clarkii</i>	10	0.4	27.6	Heath (1963)
	15	0.4	29.1	
	20	0.4	29.9	
<i>Oncorhynchus kisutch</i>	5	0.3	27.5	Becker and Genoway (1979)
	15	0.3	29.7	
<i>Oncorhynchus pache</i>	10	0.02	28.5	Lee and Rinne (1980)
	20	0.02	29.4	
<i>Salvelinus confluentus</i>	8	0.17	26.4	Selong et al. (2001)
	12	0.17	27.1	
	16	0.17	28.3	
	20	0.17	28.9	
<i>Salvelinus namaycush</i>	8	0.17	26.0–26.2	Kelly et al. (2014)
	19	0.17	28.7–29.1	
<i>Salvelinus fontinalis</i>	10	0.02	28.7	Lee and Rinne (1980)
	20	0.02	29.8	
<i>Salvelinus alpinus</i>	0.5	0.033	22.6	Elliott and Klemetsen (2002)
	5	0.033	23.5	
	20	0.033	26.6	
<i>Thymallus arcticus</i>	8.4	0.4	26.4	Lohr et al. (1996)
	16	0.4	28.5	
	20	0.4	29.3	

CT<sub>max</sub> critical thermal maxima;  $\Delta T$  heating rate

resented by a bell-shaped curve (Zeng et al. 2009; Pang et al. 2013; Norin and Clark 2016). In the pre-experimental study, the *B. lenok tsinlingensis* were acclimated to 24 °C (the temperature that the fish occasionally encountered in the field). Unfortunately, 50% of fish died in that treatment and many fish became unhealthy and inactive. Based on the bell-shaped temperature curve, in consideration of no differences in  $U_{crit}$ ,  $rU_{crit}$  and MMR were observed between 18 and 12 °C, and 50% fish died in 24 °C. We then suspect that 12 and 18 °C are on either side of the thermal optimum, or on the flat region/peak region of the temperature curve (Norin and Clark 2016). On the other hand, some studies reported that fishes appear to exhibit a continuous increase in MMR until the point where temperature is at the upper end of the natural range and close to lethal levels (Norin and Clark 2016). For example, pink salmon *Oncorhynchus gorbuscha* increased MMR until c. 24 °C and MMR remained high until c. 28 °C (Clark et al. 2011). An acute temperature increase caused MMR increased until at a temperature extreme of 38 °C in the tropical barramundi *Lates calcarifer* (Norin et al. 2014). To gain a better understanding of the patterns in MMR of *B. lenok tsinlingensis* in response to tempera-

ture, it is worth considering including a plurality of temperature treatments (e.g., acute or chronic treatments with multiple temperature gradients) in a future study.

Is the thermal sensitivity of swimming performance in Salmonid fishes related to their migratory habits? A comparison of temperature coefficient ( $Q_{10}$ ) values for swimming performance in several Salmonid fishes is provided in Table 3. The  $Q_{10}$  values for  $U_{crit}$  or  $rU_{crit}$  indicate that the thermal sensitivity of the swimming performance of *B. lenok tsinlingensis* lies within the range reported for other salmonids (higher than that of *Oncorhynchus mykiss* and *Coregonus clupeaformis* but lower than that of *Oncorhynchus clarki* and *Oncorhynchus nerka*). This sensitivity level likely reflects the ecology of the fish. Compared with *B. lenok tsinlingensis*, the  $U_{crit}$  of *O. clarki* and *O. nerka* is more important to survival because these species are long-distance, anadromous migratory fish. We therefore hypothesize that the  $Q_{10}$  values for  $U_{crit}$  of long-distance migratory fish are generally higher than those of landlocked fishes.

In summary, although *B. lenok tsinlingensis* has relatively strong capacity to endure unfavorable thermal environments compared with other Salmonid fishes, in-

**Table 3** Comparison of the thermal sensitivity of the swimming performance in several Salmonidae fishes

Species	Acclimation temperature (°C)	$U_{crit}$ (cm s <sup>-1</sup> )	$Q_{10}$ of $U_{crit}$	$rU_{crit}$ (BL s <sup>-1</sup> )	$Q_{10}$ of $rU_{crit}$	Source
<i>Brachymystax lenok tsinlingensis</i>	6	37.4	1.60	3.25	1.57	Present study
	12	49.7	0.90	4.27	0.94	
	18	46.5		4.12		
<i>Oncorhynchus mykiss</i>	8.4	59.1	1.56	1.51	1.36	Jain and Farrell (2003)
	14.9	78.9		1.84		MacNutt et al. (2004)
<i>Oncorhynchus clarki</i>	7			2.96	2.00	Lee et al. (2003)
	14			4.81	0.80	
<i>Oncorhynchus nerka</i>	18			4.41		Bernatchez and Dodson (1985)
	12.2			1.41	1.71	
<i>Coregonus clupeaformis</i>	16			1.73		Bernatchez and Dodson (1985)
	5	63.0	1.28			
	12	75.0	0.81			
	17	67.4				

$Q_{10} = (U_{T2}/U_{T1})^{10/(T2-T1)}$ , where  $U_{T1}$  and  $U_{T2}$  represent the swimming speeds of fish at temperature 1 ( $T1$ ) and temperature 2 ( $T2$ ), respectively  
 $Q_{10}$  temperature coefficient,  $U_{crit}$  critical swimming speed,  $rU_{crit}$  relative critical swimming speed

creases in stream temperatures due to climate change are expected to have an adverse effect on the physiological functions of *B. lenok tsinlingensis* that may eventually result in the decreased fitness of the fish.

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