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Effect of water temperature on reproductive performance and offspring quality of rare minnow, *Gobiocypris rarus*

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ABSTRACT

Water temperature plays a significant role in the reproductive processes of temperate fishes. In the present study, the effects of water temperature on the reproductive performance and offspring quality of rare minnow (*Gobiocypris rarus*) were evaluated by cultured parent fish at different temperature (18 ~ 30 °C) in a 2-month trial. The results revealed that rare minnows could spawn continuously within the range from 18 °C to 30 °C, and these at 24 °C and 27 °C spawned every 3–4 days. Batch size of rare minnow increased with increasing water temperature, while egg production increased with increasing water temperature and then decreased at 30 °C. High water temperature (30 °C) had significantly adverse effects on fertilization rate and hatching rate ($P < 0.05$). It was found that the oocyte growth at 18 °C, 21 °C, and 30 °C were slower than those at 24 °C and 27 °C. Histologic analysis further showed that low temperature (18 °C and 21 °C) slowed down vitellogenesis and oocyte maturation, while high temperature (30 °C) had suppressive effects on oocyte maturation and ovulation. Based on present results, it was concluded that 24–27 °C was optimal breeding temperature for rare minnows and water temperature higher than 30 °C resulting from climate change would pose a threat to its wild populations.

1. Introduction

Water temperature plays a significant role in the reproductive processes of temperate fishes (Peter and Yu, 1997; Wang et al., 2010). It modulates the physiological processes (e.g. energetic and material allocation) and endocrine regulations (Taranger et al., 2010), and then affects induction (initiation of oogenesis), vitellogenesis and the final stages (including maturation, ovulation and oviposition) (Wang et al., 2010). Inappropriate water temperature has been associated with slow gonadal development (Brian et al., 2011; Hermelink et al., 2011), small body size (Tobin and Wright, 2011), failed artificial reproduction (Mazzeo et al., 2014; Nowosad et al., 2014), low egg production (Brown et al., 2006; Dzikowski et al., 2001; Koger et al., 1999), and poor offspring quality (Jobling et al., 1995; King et al., 2003; Lahnsteiner et al., 2013). For cyprinids, water temperature is not only a modulated factor but also the main environmental cue controlling reproduction (Peter and Yu, 1997; Wootton, 1990). Defining optimal temperatures regime is of pivotal importance for their aquaculture. On the other hand, with climate warming, weather anomalies such as high temperature are clearly increasing in frequency and intensity in recent years (IPCC, 2007), which poses a serious threat to global biodiversity (Sala et al., 2000). It was reported that 24% of fish species were in the danger of extinction due to climate warming (McGrath, 2012). The effect of water temperature on the effectiveness of fish reproduction has increasingly aroused general concern (Donelson et al., 2010; Nowosad et al., 2014; Pankhurst and Munday, 2011; Soria et al., 2008). In this sense, information on the effect of water temperature on reproduction of those endangered fishes is essential for the maintenance and conservation of these species.

Rare minnow (*Gobiocypris rarus*) is an eurythermal small cyprinid fish in the southwest of China, which could survive at temperatures ranged from 0 °C to 35 °C (Wang, 1996). According to Wang (1992), reproduction of rare minnow took place from March to November when temperature was between 14 °C and 30 °C. Rare minnows could reach maturity in 4 months and spawn throughout the year under the suitable conditions (Cao and Wang, 2003), which spawned 96–655 eggs every 2–9 days at 21.3 ± 3 °C, with a mode of 4 days (Wang, 1999). These features make it possible to provide abundant samples for scientific

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research and then place them in an attractive position in toxicological researches (State Environmental Protection Administration, 2003). To date, rare minnows have been widely applied to assess the toxicity of chemicals and its ecological risk. To accomplish its standardization of laboratory animal and optimize egg production techniques, systematic methods for the breeding (Wang, 1992), hatching (Chang et al., 1995), and larval culture (Wang et al., 1998a), tolerance to physical-chemical factors such as water temperature (Wang, 1996), hypoxia (Wang, 1995), and dissolved CO_2 (Wang, 1995), requirements for nutrients in artificial diet including protein, fat, and protein-energy ratio (Wu et al., 2015a, 2016), and control standard for physical-chemical factors such as nitrogenous wastes (Luo et al., 2016a), water hardness (Luo et al., 2016b) were established. However, the effects of water temperature regimes on the reproductive performances and reproductive parameters (i.e., spawning interval, egg production, fertilization, and hatching rates) have never been accurately evaluated. It still remains unknown whether rare minnow could spawn continuously within a wide range of water temperature or not. In general, a water temperature of 25 ℃ is recommended for its breeding (State Environmental Protection Administration, 2003). Whether 25 ℃ is the optimal temperature for breeding for this promising laboratory fish also requires further study. On the other hand, although rare minnows have a long breeding season and show a high fecundity in the laboratory, it is ranked as endangered species by China Species Red List due to its small and declining wild populations (Wang et al., 1998b). It is unclear whether the deteriorating condition of natural environment, climate or other changes would exert adverse influences on their reproductive performance and offspring quality and then reduce the abundance of this endangered species.

In the present study, a 2-month trial was conducted to evaluate the effect of temperature on the reproductive performance by cultured parent fish at different temperature (18 ~ 30 ℃). The objectives of the present study were to 1) determine the effect of water temperature on reproductive performance; 2) define the optimal water temperature for breeding of the rare minnow; and 3) test the hypothesis whether climate change poses a threat to wild populations of the rare minnow.

2. Materials and methods

2.1. Fish, facilities and water quality

Adult rare minnows of closed colony (Ihb: IHB) were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, with the body weight (g) of 0.863 ± 0.128 for males, 1.305 ± 0.193 for females (data are presented as mean values ± SD). Paired parent fish were maintained in an aquarium (16 L) respectively. To maintain water quality, approximate 1/3 rearing water were renewed by fresh water with same temperature daily. Water-quality parameters were determined weekly and maintained as follows: dissolved oxygen, 7.0–8.5 mg/L; pH, 7.80–8.55; ammonia, less than 0.5 mg/L $N\text{-}NH_4^+$; nitrite, less than 0.1 mg/L $N\text{-}NO_2^-$; nitrate, less than 1.0 mg/L $N\text{-}NO_3^-$; conductivity, 420–480 µS/cm; alkalinity, 140–174 mg/L $CaCO_3$; and total hardness, 160–172 mg/L $CaCO_3$. The photoperiod and light intensity was maintained as a 12 L:12D and 150 lx, respectively. Rare minnows were fed to satiation with ozone-disinfected frozen blood worm larvae (Yuerle, Tianjin, China) twice daily.

2.2. Temperature regimes

Prior to the trial, rare minnows were cultured at a water temperature of 24 ± 1 °C to check whether the pairing (♀: ♂ = 1:1) successed. Thereafter, 30 paired parent fish with six replicates were randomly subjected to 18 ℃, 21 ℃, 24 ℃, 27 ℃, and 30 ℃ within a week acclimation at a rate of 1–2 °C/day. The trial lasted for 60 days, maintained from mid October 2014 to mid December 2014 when room temperature was between 14 ℃ and 21 ℃. Water temperature were obtained by heaters and a water cooler (RESUN, CW-1000A, Guangzhou, China) and monitored by sophisticated mercurial thermometer twice daily.

2.3. Reproductive performance and offspring quality

After a week acclimation, the spawning events were recorded at 11:30 p.m. daily. Once spawning, the parent fish were separated from the eggs by a net cage (1 L) immersed in the same aquarium. On the following day, eggs were siphoned into cylindrical glass containers (600 mL; diameter, 12 cm; Huaou Industry, Yancheng, China) and parent fish were put back to the aquarium for further observation. Unfertilized and fertilized eggs were counted to calculate batch size and the fertilization rate. Later, fertilized eggs from all temperature conditions were transferred to a hatching tank with a temperature of 25 ℃ for incubation. Upon hatching, the number of newly hatched larva was recorded to calculate the hatching rate and the number of abnormality (deformities, edema, and yolk-sac necrosis) was counted to calculate the malformation rate according to a previous study (Luo et al., 2016a).

2.4. Organ sampling and morphometry

At the end of the experiment, all fish were anesthetized with 200 mg/L tricaine methanesulfonate (MS 222, Sigma-Aldrich, St Louis, MO) solution. Body weight, net body weight, total length, and body length were measured and the gonads and visceral mass were removed and weighed to calculate the condition factor (CF), the gonadosomatic index (GSI) and visceral-somatic index (VSI) according to previous studies (Brian et al., 2011; Wu et al., 2015b). Afterwards, the left and right ovaries were preserved in Bouin's solution for histological observations and 10% formalin (Sinopharm Chemical Reagent, China) for oocyte measurement respectively. According to the method reported by Wang (1999), oocyte was measured for oocyte diameter under the dissecting microscope (model SMZ 168, Motic Inc. Ltd., Hong Kong) to analyze the frequency distribution of oocyte sizes. Experiments were conducted according to the Chinese Ministry of Science & Technology Guiding Directives for Humane Treatment of Laboratory Animal (Chinese Ministry of Science and Technology, 2007). All animals were treated humanely and with the aim of alleviating any suffering.

2.5. Statistical analysis

Parameters including initial body weight, spawning interval, egg production, fertilization rate, hatching rate, malformation rate, CF, GSI, and VSI, log-trans-formed if necessary, were checked for assumptions of normality and assumptions of homogeneity of variance using the Levene's test. Wherever the assumption was met, data were analyzed by analysis of variance, followed by Waller-Duncan's multiple-comparison tests. If the assumption was not met, data were analyzed using the nonparametric Kruskal-Wallis test followed by the Mann-Whitney *U*-test. The significance level was set at $P < 0.05$. Except when stated otherwise, the software SPSS 19.0 was used. All figures were created by Microsoft Excel 2007 and Adobe Photoshop CS 7.0.

3. Results

3.1. Water temperature

Water temperatures during the whole experiment were maintained at a relatively stable level. Measured water temperature (mean ± SD) in the treatments was 18.51 ± 0.40 ℃, 21.76 ± 0.85 ℃, 24.24 ± 0.68 ℃, 26.77 ± 0.65 ℃, and 29.55 ± 0.71 ℃, respectively. These values were close to the nominal one and fell within an expected range of 90–110% of nominal values.