



Effects of prolonged exercise training and exhaustive chasing training on the swimming performance of an endangered bream *Megalobrama pellegrini*

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ABSTRACT: Swimming performance of fish is often improved following periods of moderate exercise training. To examine the effects of training regimes on swimming performance of juvenile bream *Megalobrama pellegrini* (5.10 ± 0.08 g), fish were held in slow-flowing water (control), or subjected to exercise training at 1 body length (BL) s⁻¹, 2 BL s⁻¹ or 4 BL s⁻¹, or were given exhaustive chasing training daily for 5 wk at 25°C. Aerobic swimming performance was assessed by measuring critical swimming speed (U_{crit}). Maximum metabolic rate (MMR), metabolic scope (MS), cost of transport (COT) and net cost of transport (COT_{net}) were assessed by measurement of oxygen consumption rates ($\dot{M}O_2$). U_{crit} was significantly higher in the 4 BL s⁻¹ training group than in the controls. The value of U_{crit} was 5% greater for the exhaustive training group compared with the control group, although exhaustive chasing training did not have a significant effect on U_{crit} . MMR and MS were not significantly different across treatments. $\dot{M}O_2$ and COT of fish in the 4 BL s⁻¹ and exhaustive training groups were significantly lower than in the control group in the swimming speed range of approximately 36–60 cm s⁻¹. This suggests that high-intensity prolonged exercise training and exhaustive chasing training have a positive influence on the swimming performance of juvenile *M. pellegrini*, which may be related to elevated anaerobic metabolism and improved swimming efficiency.

KEY WORDS: Aerobic exercise training · Anaerobic exercise training · Critical swimming speed · *Megalobrama pellegrini*

INTRODUCTION

Because fish can be prompted to swim against a water flow, they are ideal experimental animals for studies on the effects of exercise training (Davison 1997). The 2 main purposes of training research in fish are to improve the growth performance of fish and improve their survival rate once they are released into the wild (Jobling et al. 1993, Davison

1997). Many changes, such as morphological and histological characteristics (Davison & Goldspink 1977, Ibarz et al. 2011), flesh quality (Palstra & Planas 2011, Rasmussen et al. 2011, Song et al. 2012), physiological function (Bagatto et al. 2001, Liu et al. 2009, Castro et al. 2011) and behavior characteristics (Totland et al. 1987, van der Meulen et al. 2006), have been found after training in fish which are related to the species that was used and the training regime (Davi-

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son 1997). According to the demand for oxygen, the movement of fish can be divided into 2 types: aerobic and anaerobic. Therefore, exercise training protocols have been categorized as aerobic or anaerobic in fish (Pearson et al. 1990). So far, a large number of the studies investigating the effects of exercise training on fish species have studied cold-water salmonids (Davison & Goldspink 1977, Davison 1997, Castro et al. 2011). However, there has been some recent work on warm-water teleosts that has increased our knowledge of exercise training in these fish species (Li et al. 2013, 2016).

Survival, growth and reproduction in fish depend heavily on swimming ability (Brett 1964, Blake 2004, Graham et al. 2004, Kieffer 2010, Cai et al. 2014). The swimming ability of fish is complex and varied, and it is often evaluated through many different indicators, such as critical swimming speed (U_{crit}) and burst swimming speed (Colavecchia et al. 1998, Liu et al. 2009, Li et al. 2010a,b). U_{crit} is not only a widely accepted evaluation index of aerobic swimming performance, but also reflects an anaerobic metabolism from approximately 60% U_{crit} onward in fish (Nelson et al. 1996, Gregory & Wood 1998, Plaut 2001, Lee et al. 2003a,b). Many studies have found that U_{crit} is heavily influenced by factors such as species (Pang et al. 2011), gastrointestinal fullness (Li et al. 2010a,b), temperature (Pang et al. 2011, 2013), dissolved oxygen level (Fu et al. 2011, Zhao et al. 2012) and exercise training (Liu et al. 2009, Fu et al. 2011, Zhao et al. 2012, He et al. 2013) in fish species. Some research has shown that exercise training has a positive effect on U_{crit} in some fish, which is closely related to improvement of cardio-respiratory capacity (Farrell et al. 1991, Liu et al. 2009, Fu et al. 2011), increase in muscle fiber size, number of mitochondria (Davison & Goldspink 1977, Davie et al. 1986) and/or enhanced activity of mitochondrial enzymes (Johnston & Moon 1980). Improvement of swimming efficiency may also have a positive effect on U_{crit} and is often evaluated through the cost of transport (COT) and net cost of transport (COT_{net}) in fish (Claireaux et al. 2006, Pang et al. 2013). Additionally, the optimal water velocity of exercise training for improvement of swimming performance has been found to be different for different fish species. Despite a few studies reporting no positive response of U_{crit} to exercise training in some species (Farrell et al. 1991, Gruber & Dickson 1997, Gallagher et al. 2001), it is now widely accepted that optimal training water velocity is above 60% U_{crit} for improvement of U_{crit} in many fish species (Liu et al. 2009, Zhao et al. 2012, He et al. 2013). Therefore, whether exercise training shows

any effects on swimming performance has been suggested to be dependent on fish species, the type of training and environmental factors (Davison 1997, Pang et al. 2013).

In this study, we selected juvenile bream *Megalobrama pellegrini*, a warm-water cyprinid fish species, as the experimental model. *M. pellegrini* used to be found in the main streams and tributaries along the upper reaches of the Yangtze River of China (Duan et al. 2002). However, this species has become endangered due to the construction of dams and overfishing in the wild (Park et al. 2003, Li et al. 2007), and field surveys have revealed that populations of *M. pellegrini* have been decreasing rapidly across their original distribution (Li et al. 2007, Gao et al. 2010). Studies on reproductive biology and implementation of stock enhancement have contributed to the conservation of this endangered species (Wang et al. 2005, Li et al. 2007). Some studies have found that exercise training at a moderate water velocity can improve swimming ability and hence lead to better survival of released juvenile fish raised in hatcheries (Hoffnagle et al. 2006, Castro et al. 2011, Palstra & Planas 2011). Thus, investigation on how exercise affects swimming performance of this species will provide practical information for the protection of this species. The objectives of this study were to (1) investigate whether prolonged exercise training improves swimming performance in juvenile *M. pellegrini*, and, if it does, to determine the optimal water velocity for exercise training; (2) examine whether exhaustive chasing training could improve swimming performance in juvenile *M. pellegrini* and serve as an alternative training protocol to sustained exercise training; and (3) determine whether improved swimming performance is related to improved swimming efficiency. To achieve these aims, we assessed aerobic swimming performance by measuring U_{crit} , aerobic metabolic capacity by maximum metabolic rate (MMR) and metabolic scope (MS), and swimming efficiency by measuring oxygen consumption rates ($\dot{M}O_2$), COT and COT_{net} in juvenile *M. pellegrini* after prolonged exercise training and exhaustive chasing training.

MATERIALS AND METHODS

Experimental fish and acclimation

Juvenile *Megalobrama pellegrini* (Cypriniformes: Cyprinidae) were obtained from a local hatchery (Beibei, Chongqing, China) and kept in a cement

pond system (approximately 1200 l) with fully aerated recirculating water at Southwest University for 4 wk prior to the experiment. During this period, the temperature of the de-chlorinated tap water in the pond was maintained at $25.0 \pm 0.5^\circ\text{C}$, and the oxygen level was kept above 7 mg l^{-1} . The photoperiod was a 12 h light:12 h dark schedule, with the lights turned on and off at 08:00 and 20:00 h, respectively. Fish were fed twice daily (09:00 and 19:00 h) to apparent satiation with commercial floating pellets (Tongwei; dietary composition: $41.2 \pm 0.9\%$ protein, $8.5 \pm 0.5\%$ lipid, $25.7 \pm 1.2\%$ carbohydrate and $12.3 \pm 0.4\%$ ash).

Training protocol

After 4 wk of acclimatization, 150 fish with a similar size ($5.10 \pm 0.08 \text{ g}$, $6.52 \pm 0.10 \text{ cm}$) were slightly anesthetized (neutralized MS222, tricaine methanesulfonate, 50 mg l^{-1}) and then transferred into a self-made exercising system described by Li et al. (2016) for exercise training. Continuous water velocities in the experimental flume were achieved via the motors (30 W) with propeller. Different water velocities were produced by controlling the different voltages of transducer power. Fish were randomly divided into 5 groups of 30 individuals (control group, 1 body length [BL] s^{-1} training group, 2 BL s^{-1} training group, 4 BL s^{-1} training group and exhaustive training group) that were placed in flumes ($140 \times 15 \times 20 \text{ cm}$, L \times W \times D) with different water velocities within the exercising system. All 30 fish in each training group were trained as a unit.

The protocols of prolonged exercise training followed Li et al. (2013). Our pilot experiment found that a water velocity $>2 \text{ cm s}^{-1}$ can guarantee the full water exchange which is the prerequisite of the present study, but a water velocity $>5 \text{ cm s}^{-1}$ caused spontaneous swimming activities in juvenile *M. pellegrini*. Therefore, fish in the control group swam at an average water velocity of 3 cm s^{-1} . The water velocity was beneficial for water exchange and did not lead to spontaneous swimming activities in juvenile *M. pellegrini*. The fish in the 3 prolonged training groups were forced to swim against 3 different water velocities: 1, 2 and 4 BL s^{-1} (approximately 7, 14 and 28 cm s^{-1} , respectively), for a total of 18 h d^{-1} . After prolonged training for 9 h, the exercise was ceased and these fish were fed once to satiation at 09:00 h (or 19:00 h) with commercial floating pellets at a water velocity of 3 cm s^{-1} . To reduce physiological stress, water velocity was gradually increased over 4 d until

the desired water velocity was reached for the first round of training at the beginning of the experiment (Davison & Goldspink 1978). Water velocities were adjusted every other week after the BL of the fish had been measured.

The protocols for exhaustive chasing training followed the methods of Liu et al. (2009). The 30 fish in the exhaustive training group were transferred from the flume into a circular container with a water velocity of approximately 65 cm s^{-1} (diameter 104 cm) in the same time and chased with a hand net to an exhausted state for approximately 5 min. During chasing, any lagging individuals were occasionally touched by the hand net. The exhausted fish were immediately returned to the flume (with an average water velocity of 3 cm s^{-1}) when they lost their balance or did not respond to stimuli. Training was conducted once per day at 15:00 h.

The prolonged exercise training or exhaustive chasing training was carried out for 5 wk. The dissolved oxygen level was approximately 7 mg l^{-1} , and the feeding regime and holding conditions for the experimental period were consistent with those of the acclimation period.

Measurement of U_{crit} and swimming MO_2

U_{crit} was measured by a Blakza-type swimming tunnel respirometer (total volume 3.5 l; for details, see Li et al. 2010a, Pang et al. 2011). Eight fish from each group were individually transferred into the swim tunnel after 24 h of fasting and allowed to recover for 6 h at a water speed of 6 cm s^{-1} (Peng et al. 2014). Fish swam downstream of the propeller in a swimming chamber with a 19.9 cm^2 cross-sectional area. Water velocity was increased by 6 cm s^{-1} increments every 20 min until fish were fatigued. Fatigue was defined as failure to move away from the rear honeycomb screen of the swimming chamber for 20 s (Lee et al. 2003a,b). Water temperature in the swimming chamber was controlled within $\pm 0.2^\circ\text{C}$ using a water bath connected to a stainless-steel heat exchanger. U_{crit} was calculated for individual fish using Brett's equation (Brett 1964):

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

where v is the highest speed at which the fish swam for the full time period (cm s^{-1}), Δv is the velocity increment (6 cm s^{-1}), T is the constant period of swimming per speed (20 min), and t is the time that the fish swam at the final speed (min). U_{crit} was not corrected for the solid blocking effect because the cross-

sectional area of the fish did not exceed 10% of the area of the swimming chamber. After the measurement of U_{crit} , fish were taken out of the swimming chamber and euthanized with an overdose of MS-222. Measurements of body mass and BL were taken to the nearest 0.1 g and 0.1 cm.

$\dot{M}O_2$ values were measured during each U_{crit} test as a function of swimming speed. In open mode, the respirometer was supplied with fully aerated and thermoregulated water that circulated in a reservoir tank at an approximate flow rate of 500 ml min⁻¹. In closed mode, a small fraction of the water from the sealed respirometer was siphoned past the probe of an oximeter (HQ30, Hach) in a cuvette thermoregulated with a water bath. Water oxygen concentration (mg l⁻¹) was recorded once every 2 min. The standardized $\dot{M}O_2$ of an individual swimming fish was calculated from the depletion of oxygen according to the following equation (Li et al. 2010b):

$$\dot{M}O_2 = 60 \text{ slope VOL}/m \quad (2)$$

where slope is the decrease in the water's dissolved oxygen content per minute. The slope was obtained with linear regressions between time (min) and the water's dissolved oxygen content (mg O₂ l⁻¹). Only slopes with $r^2 > 0.95$ were considered in the analysis. VOL is the total volume of the respirometer (3.5 l) minus the volume of the fish, and m is the body mass (kg) of the fish. The water oxygen content in the respirometer was never allowed to fall below 85% oxygen saturation (Claireaux et al. 2006).

For the individual fish swimming trials, the relationship between the swimming speed (U , cm s⁻¹) and swimming $\dot{M}O_2$ (mg kg⁻¹ h⁻¹) was described with the following equation (Webb 1975):

$$\dot{M}O_2 = \alpha e^{\beta U} \quad (3)$$

where α and β are constants, and e is base of the natural logarithm. RMR (resting metabolic rate; mg kg⁻¹ h⁻¹) was determined by extrapolation of Eq. (3) to a swimming speed of 0 cm s⁻¹ (i.e. the U) (Priede 1985). The maximum $\dot{M}O_2$ was used as the value for MMR (mg kg⁻¹ h⁻¹) during the U_{crit} test. MS was calculated by subtracting RMR from MMR.

COT and COT_{net} were calculated according to the following equations (Claireaux et al. 2006):

$$COT = \dot{M}O_2 \times OE/v \quad (4)$$

$$COT_{net} = \dot{M}O_{2net} \times OE/v \quad (5)$$

where $\dot{M}O_2$ (mg O₂ kg⁻¹ h⁻¹) is the oxygen consumption rate of an individual swimming fish at a given water velocity. $\dot{M}O_{2net}$ (mg O₂ kg⁻¹ h⁻¹) was calculated by subtracting the corresponding RMR from

all $\dot{M}O_2$ measurements. OE is an oxycalorific equivalent of 13.54 J (mg O₂)⁻¹, and v (m h⁻¹) is the corresponding water velocity converted from cm s⁻¹.

Data analysis and statistics

SPSS 17.0 software was used for data analysis. The effects of prolonged exercise training (among different speeds: control, 1, 2 and 4 BL s⁻¹) on body mass, BL, U_{crit} , RMR, MMR and MS were determined using 1-way ANOVA. ANOVA was followed by a least significant difference multiple-comparison test if it was necessary to determine the difference between the values of the different prolonged training groups. The effects of exhaustive chasing training (between control and treatment groups) on body mass, BL, U_{crit} , RMR, MMR and MS were assessed using a t -test. The exponential function Eq. (3) translated to the linear equation $\dot{M}O_2 = \ln \alpha + \beta U$ via a double ln-transformation. The effects of swimming speed (U) and exercise training (prolonged training at a given speed vs. control or exhaustive training vs. control) on swimming $\dot{M}O_2$ were determined using ANCOVA with U values as covariates (see Fig. 1). Thus the effect of treatment (training) was the difference in intercept, and the effect of interaction was the difference in slope. The effects of swimming speed and exercise training on COT and COT_{net} were assessed using 2-way ANOVA. ANOVA was followed by a t -test if a statistical evaluation of a difference between the values was shown between the control and trained groups at the same swimming speed. A p -value < 0.05 was considered to be statistically significant, and all values are presented as mean \pm SE.

RESULTS

U_{crit}

Fish in the 4 BL s⁻¹ training group showed a significantly higher U_{crit} compared to the control group ($p < 0.05$), whereas there were no significant differences in U_{crit} among the 1 BL s⁻¹, 2 BL s⁻¹, exhaustive training and control groups (Table 1).

RMR, MMR and MS

There were no significant differences in RMR, MMR and MS among all of the trained and control groups (Table 1).

Table 1. Effects of exercise training on several variables related to critical swimming performance in juvenile *Megalobrama pellegrini* (mean \pm SE, N = 8). BL: body length, U_{crit} : critical swimming speed, RMR: resting metabolic rate, MMR: maximum metabolic rate, MS: metabolic scope. Among the 5 rows of groups, values in each column without a common lowercase letter indicate a significant difference. *Significant difference between exhaustive training and control group ($p < 0.05$)

	Body mass (g)	BL (cm)	U_{crit} (cm s ⁻¹)	RMR (mg O ₂ kg ⁻¹ h ⁻¹)	MMR (mg O ₂ kg ⁻¹ h ⁻¹)	MS (mg O ₂ kg ⁻¹ h ⁻¹)
Control group	5.61 \pm 0.18 ^a	6.98 \pm 0.08 ^a	63.86 \pm 0.84 ^b	448.47 \pm 39.49 ^a	1421.97 \pm 56.30 ^a	973.50 \pm 88.83 ^a
Prolonged training groups						
1 BL s ⁻¹	5.62 \pm 0.18 ^a	6.84 \pm 0.08 ^a	64.57 \pm 1.46 ^b	352.83 \pm 30.84 ^a	1537.73 \pm 60.28 ^a	1184.90 \pm 62.09 ^a
2 BL s ⁻¹	5.49 \pm 0.45 ^a	6.74 \pm 0.19 ^a	66.72 \pm 2.00 ^{ab}	386.22 \pm 35.51 ^a	1435.45 \pm 53.48 ^a	1049.23 \pm 58.53 ^a
4 BL s ⁻¹	5.50 \pm 0.21 ^a	6.78 \pm 0.11 ^a	69.87 \pm 1.67 ^a	359.01 \pm 36.31 ^a	1505.79 \pm 63.69 ^a	1070.27 \pm 80.69 ^a
Exhaustive training group	5.35 \pm 0.185	6.62 \pm 0.05*	67.02 \pm 1.95	393.23 \pm 42.54	1334.35 \pm 62.55	941.12 \pm 86.34
Significance						
Prolonged training	$F_{3,31} = 0.068$; $p = 0.977$	$F_{3,31} = 0.685$; $p = 0.569$	$F_{3,31} = 3.027$; $p = 0.046$	$F_{3,31} = 1.418$; $p = 0.258$	$F_{3,31} = 0.712$; $p = 0.553$	$F_{3,31} = 1.350$; $p = 0.278$
Exhaustive training	$t_{14} = -1.012$; $p = 0.329$	$t_{14} = -3.797$; $p = 0.002$	$t_{14} = 1.486$; $p = 0.159$	$t_{14} = -0.906$; $p = 0.357$	$t_{14} = -1.084$; $p = 0.315$	$t_{14} = -0.068$; $p = 0.798$

Swimming MO₂

Training and swimming speed had significant effects on MO₂ during the U_{crit} test for all of the training groups ($p = 0.001$, $p = 0.002$ or $p < 0.001$) (Table 2). $\dot{M}O_2$ significantly increased with an increase in swimming speed for all training and control groups (Fig. 1). The $\dot{M}O_2$ values of fish in the 4 BL s⁻¹ training group were significantly lower compared to fish in the control group for swimming speeds of 24, 36, 42, 48, 54 and 60 cm s⁻¹ ($p < 0.05$; Fig. 1). $\dot{M}O_2$ values of fish in the exhaustive training group were significantly lower than the control group at swimming speeds of 36, 42, 48 and 60 cm s⁻¹ ($p < 0.05$; Fig. 1).

COT

Training and swimming speed had significant effects on COT during the U_{crit} test for all training groups ($p = 0.002$ or $p < 0.001$) (Table 2). COT significantly decreased and then reached a plateau with an increase in the swimming speed for all training and control groups (Fig. 2). The COT values of fish in the 4 BL s⁻¹ training group were significantly lower compared to the control group at swimming speeds of 24, 36, 42, 48, 54 and 60 cm s⁻¹ ($p < 0.05$; Fig. 2). COT values of fish in the exhaustive training group were significantly lower than the control group at swimming speeds of 36, 42, 48 and 60 cm s⁻¹ ($p < 0.05$; Fig. 2).

COT_{net}

Training did not have a significant effect on COT_{net} in the 1 BL s⁻¹ and 2 BL s⁻¹ training groups, whereas training had significant effects on COT_{net} during the U_{crit} test for the 4 BL s⁻¹ and exhaustive training groups ($p = 0.002$ or $p = 0.008$) (Table 2). Swimming speed had significant effects on COT_{net} during the U_{crit} test for all training groups ($p < 0.001$) (Table 2). COT_{net} significantly increased with an increase in the swimming speed for all training and control groups (Fig. 3). COT_{net} in the exhaustive training group was significantly lower compared to the control group at a swimming speed of 12 cm s⁻¹ ($p < 0.05$), whereas COT_{net} in all 3 prolonged training groups had no significant difference compared to the control group at any given swimming speed (Fig. 3).

Table 2. Effects of exercise training and swimming speed on oxygen consumption rate ($\dot{M}O_2$), cost of transport (COT) and net cost of transport (COT_{net}) in juvenile *Megalobrama pellegrini* (mean \pm SE). $\dot{M}O_2$ based on ANCOVA; COT and COT_{net} based on 2-way ANOVA. BL: body length. *p < 0.05

Training group		$\dot{M}O_2$	COT	COT_{net}
1 BL s ⁻¹	Training effect	$F_{1,151} = 11.675$ p = 0.001*	$F_{1,133} = 10.491$ p = 0.002*	$F_{1,133} = 0.920$ p = 0.339
	Swimming speed effect	$F_{1,151} = 611.077$ p < 0.001*	$F_{10,133} = 19.497$ p < 0.001*	$F_{10,133} = 18.409$ p < 0.001*
	Interaction effect	$F_{1,150} = 5.411$ p = 0.021*	$F_{9,133} = 1.607$ p = 0.119	$F_{9,133} = 1.018$ p = 0.429
2 BL s ⁻¹	Training effect	$F_{1,153} = 9.915$ p = 0.002*	$F_{1,134} = 8.303$ p = 0.005*	$F_{1,134} = 0.450$ p = 0.503
	Swimming speed effect	$F_{1,153} = 572.852$ p < 0.001*	$F_{11,134} = 22.238$ p < 0.001*	$F_{11,134} = 11.434$ p < 0.001*
	Interaction effect	$F_{1,152} = 0.266$ p = 0.607	$F_{9,134} = 0.521$ p = 0.858	$F_{9,134} = 0.360$ p = 0.952
4 BL s ⁻¹	Training effect	$F_{1,158} = 33.104$ p < 0.001*	$F_{1,139} = 26.671$ p < 0.001*	$F_{1,139} = 7.154$ p = 0.008*
	Swimming speed effect	$F_{1,158} = 562.517$ p < 0.001*	$F_{11,139} = 21.536$ p < 0.001*	$F_{11,139} = 14.134$ p < 0.001*
	Interaction effect	$F_{1,157} = 1.015$ p = 0.315	$F_{9,139} = 0.577$ p = 0.814	$F_{9,139} = 0.853$ p = 0.569
Exhaustive	Training effect	$F_{1,153} = 20.601$ p < 0.001*	$F_{1,134} = 15.584$ p < 0.001*	$F_{1,134} = 10.315$ p = 0.002*
	Swimming speed effect	$F_{1,153} = 459.370$ p < 0.001*	$F_{11,134} = 18.845$ p < 0.001*	$F_{11,134} = 12.090$ p < 0.001*
	Interaction effect	$F_{1,152} = 0.779$ p = 0.379	$F_{9,134} = 0.504$ p = 0.870	$F_{9,134} = 0.890$ p = 0.536

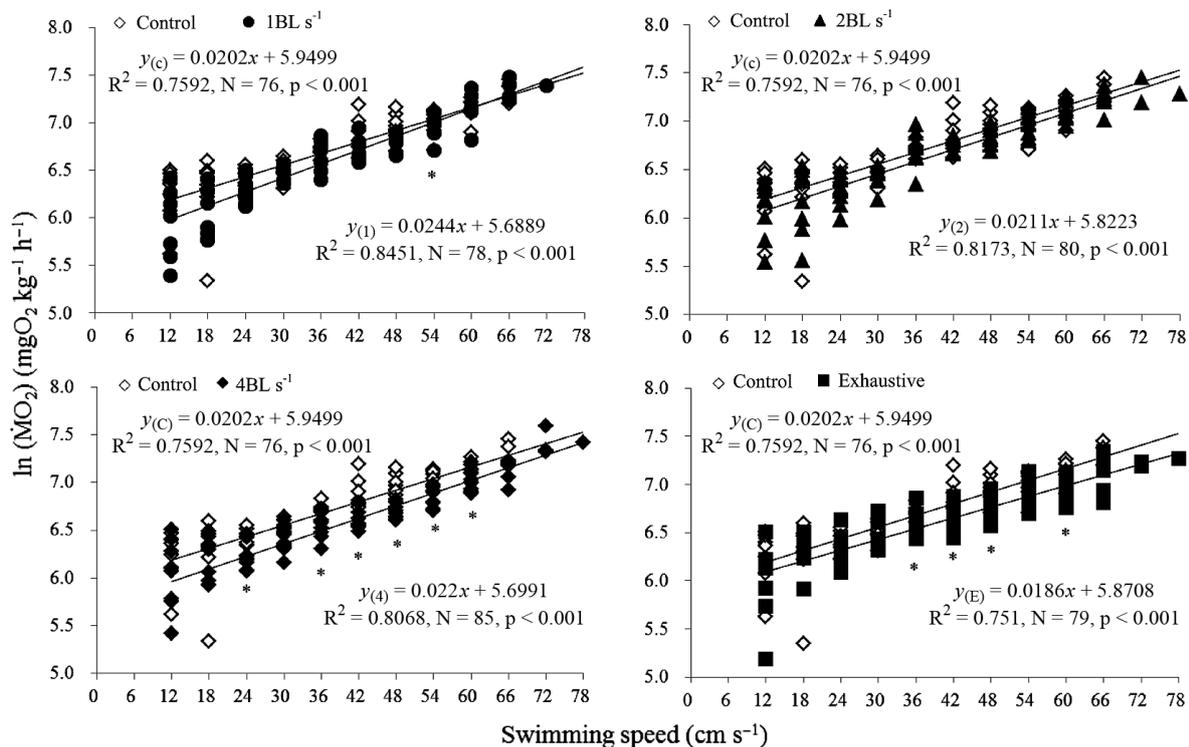


Fig. 1. Oxygen consumption rate ($\dot{M}O_2$) vs. swimming speed curves for juvenile *Megalobrama pellegrini* for different training regimes vs. the control group: 1 body length (BL) s⁻¹, 2 BL s⁻¹, 4 BL s⁻¹, and exhaustive training. *Significant difference between training and control group under the same swimming speed (p < 0.05)

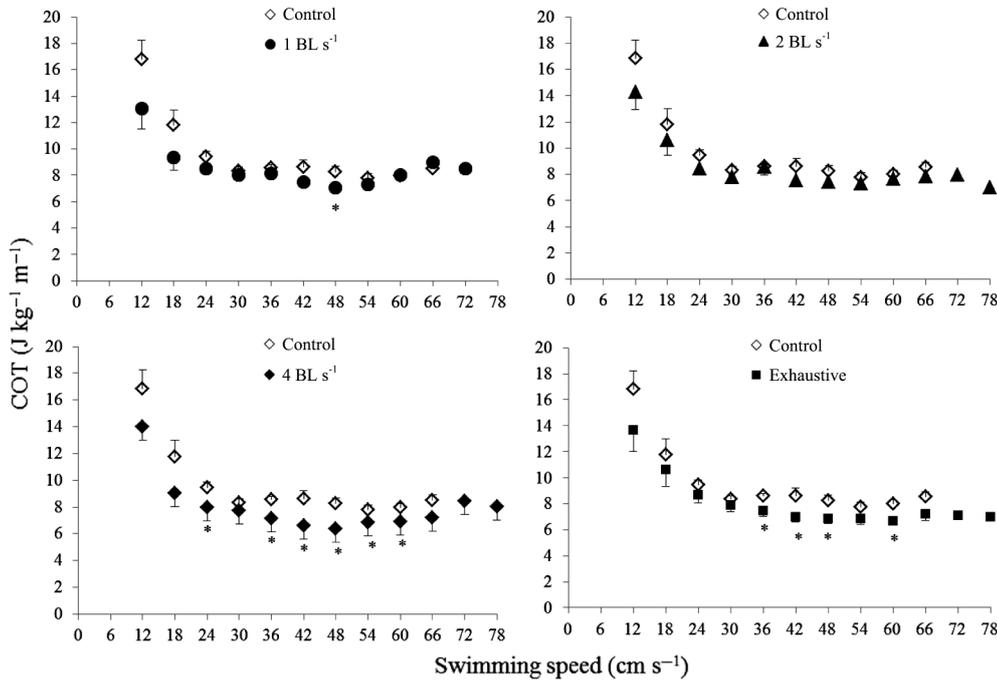


Fig. 2. Effects of swimming speed on cost of transport (COT) of juvenile *Megalobrama pellegrini* for different training regimes vs. the control group: 1 body length (BL) s^{-1} , 2 BL s^{-1} , 4 BL s^{-1} , and exhaustive training. *Significant difference between training and control group under the same swimming speed ($p < 0.05$). Data are mean \pm SE

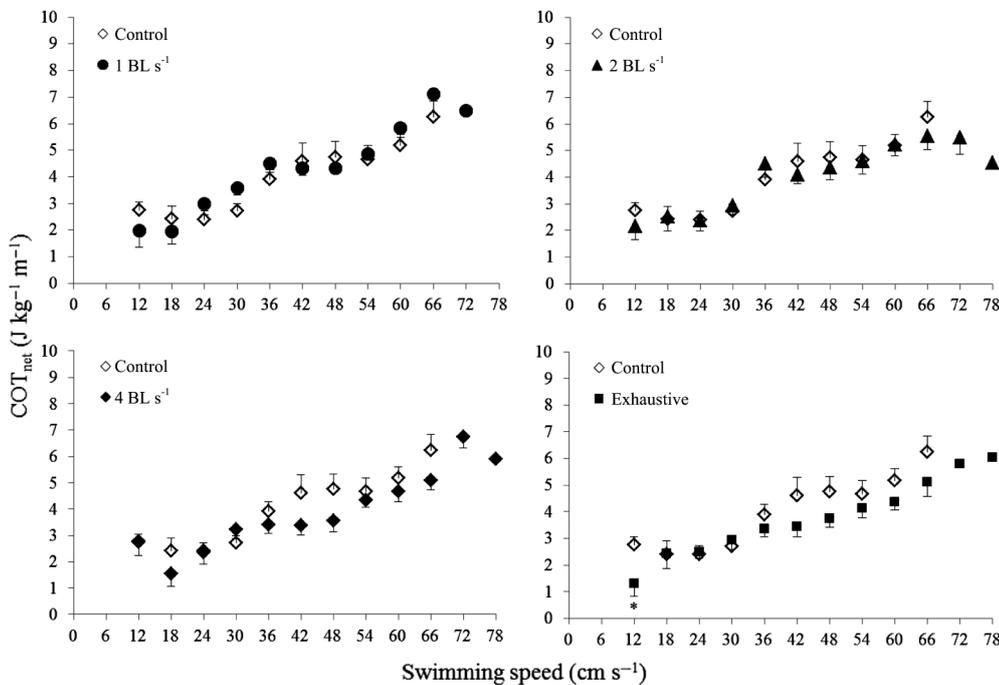


Fig. 3. Effects of swimming speed on net cost of transport (COT_{net}) of juvenile *Megalobrama pellegrini* for different training regimes vs. the control group: 1 body length (BL) s^{-1} , 2 BL s^{-1} , 4 BL s^{-1} , and exhaustive training. *Significant difference between training and control group under the same swimming speed ($p < 0.05$). Data are mean \pm SE

DISCUSSION

The objective of this study was to investigate the effects of different training regimes on swimming performance and the possible underlying physiological mechanisms in juvenile *Megalobrama pellegrini*.

This study showed that prolonged exercise training at a higher water velocity or exhaustive chasing training increased U_{crit} in this fish species, which was partly due to enhanced anaerobic metabolism and increased swimming efficiency compared to controls.

Effect on swimming performance

U_{crit} was not significantly different among the control and 1 and 2 BL s^{-1} groups. U_{crit} in the 4 BL s^{-1} group increased significantly (Table 1). These results suggest that the intensity of training had a significant effect on U_{crit} and that high-intensity swimming (4 BL s^{-1}) resulted in an improvement in swimming performance. Improved U_{crit} values have also been found in other trained fish species, such as the common carp *Cyprinus carpio* subjected to exercise training at 60% U_{crit} for 28 d (He et al. 2013), qingbo *Spinibarbus sinensis* subjected to exercise at 60% U_{crit} , 6 h d^{-1} for 14 d (Zhao et al. 2012) and striped bass *Morone saxatilis* subjected to exercise training at 1.2–2.4 BL s^{-1} for 60 d (Young & Cech 1993). However, U_{crit} values in trained leopard shark *Triakis semifasciata* (approximately 0.7 BL s^{-1} for 6 wk) and chinook salmon *Oncorhynchus tshawytscha* (U_{crit} swim test on alternate days for 4 mo) showed no significant differences compared to untrained fish (Gruber & Dickson 1997, Gallagher et al. 2001). The different effects of prolonged exercise training on the U_{crit} of fish species may be related to the differences in the fish species that are chosen and the intensity and duration of training that were set (Liu et al. 2009, Zhao et al. 2012, Pang et al. 2013).

Red muscle fibers power aerobic exercise using aerobic metabolic pathways, and white muscle fibers utilize anaerobic metabolism and are recruited for anaerobic exercise in fish species. Therefore, many researchers generally believe that aerobic exercise training will help improve aerobic swimming performance, whereas anaerobic exercise training is helpful to improve anaerobic locomotor capacity in fish species (Davison 1997). Most of the previous studies examining the effects of exercise training on the U_{crit} of fish have used aerobic rather than anaerobic exercise training (Young & Cech 1993, Gruber & Dickson 1997, Gallagher et al. 2001, Fu et al. 2011, He et al. 2013, Zhao et al. 2012). However, some studies found that anaerobic exercise training has a significant effect on the U_{crit} of fish species. For example, anaerobic exercise training (exhaustive chasing training daily for 14 d) resulted in significant increases in U_{crit} at 15°C in juvenile qingbo (Pang et al. 2013). Similar results were also documented in juvenile darkbarbel catfish *Peltebagrus vachelli* and southern catfish *Silurus meridionalis* (Liu et al. 2009, Li et al. 2010a,b). In the present study, exhaustive chasing training did not have a significant effect on U_{crit} , but did

lead to a 5% increase in the U_{crit} of juvenile *M. pellegrini* compared to the untrained fish (Table 1). These results suggest that anaerobic exercise training might have a positive effect on the improvement of critical swimming capacity in juvenile *M. pellegrini*.

The experimental fish were obtained from one of the local artificial hatcheries which provide juvenile fish for fishery enhancement in the Three Gorges Reservoir. The experimental fish used in the present study as well as those released in the field usually show poorer swimming performance compared to their wild conspecifics because of parental effects, i.e. many generations being grown in captivity. Therefore, improvement in critical swimming capacity may improve food-capture and predator-avoidance capability in juvenile *M. pellegrini* after prolonged exercise training at a higher water velocity and exhaustive chasing training (Fu 2015). It may be beneficial to the survival rate after releasing this fish species into the wild.

Effect on swimming efficiency

To explore the mechanism of the effect of exercise training on the swimming performance in fish species, fish morphology, physiology, biochemistry, molecular biology etc. have been extensively studied (Johnston & Moon 1980, Martin & Johnston 2005, 2006, Fu et al. 2011, He et al. 2013). Some studies have found that the improvement of U_{crit} was accompanied by the enhancement of aerobic metabolic capacity in trained fish (Liu et al. 2009, Li et al. 2010b). In the present study, prolonged exercise and exhaustive chasing training did not have significant effects on MMR and MS in juvenile *M. pellegrini* (Table 1). This suggests that neither training regime had any effect on aerobic metabolic capacity and that improvement of U_{crit} may not be attributed to changes in the aerobic metabolic capacity in juvenile *M. pellegrini* subjected to high-intensity prolonged exercise training (4 BL s^{-1} training) and exhaustive chasing training. This finding was similar to results previously documented for trained southern catfish and common carp (Li et al. 2010a, He et al. 2013).

Many studies have found that fish resort to anaerobic metabolism when swimming speed approaches approximately 60% U_{crit} or higher (Nelson et al. 1996, Lee et al. 2003a,b, Zhu et al. 2010). Therefore, the improved U_{crit} in trained fish species might be due to an improvement in anaerobic metabolic capacity (He et

al. 2013). In our study, the fish with 4 BL s^{-1} training and with exhaustive training showed a significantly lower $\dot{M}O_2$ at relatively high swimming speeds of 42–60 $cm s^{-1}$ ($>60\%$ U_{crit}) when anaerobic metabolism was recruited during the measurement of U_{crit} , which suggests that the trained fish may engage in more anaerobic metabolism at those speeds. This result may be a reason for the improvement in swimming performance in trained *M. pellegrini*. Furthermore, 4 BL s^{-1} training and exhaustive training had a significant effect on COT and COT_{net} of *M. pellegrini* (Table 2). Fish in the 4 BL s^{-1} and exhaustive training groups showed a significantly lower COT at a relatively low swimming speed of 24 and/or 36 $cm s^{-1}$ ($<60\%$ U_{crit}) (Fig. 2), suggesting that the increased swimming capacity may also result from improved swimming efficiency in juvenile *M. pellegrini* after training. A study of Atlantic salmon *Salmo salar* also found that continuous training at 0.8 BL s^{-1} for 6 wk resulted in more efficient swimming at the highest swimming speed (1.6 BL s^{-1}) (Castro et al. 2011).

CONCLUSIONS

The effect of exercise training on swimming performance was closely related to the intensity and type of training in juvenile *Megalobrama pellegrini*. Prolonged exercise training at water velocities of 1 or 2 BL s^{-1} had no effect on swimming performance. However, increased U_{crit} was found in the fish after undergoing aerobic exercise training at a higher water velocity (4 BL s^{-1}). Anaerobic exercise training (exhaustive chasing) also resulted in a 5% increase in U_{crit} compared to the control group. This enhancement may be related to an improvement in anaerobic metabolism and/or swimming efficiency in this bream, which may be beneficial to the survival rate after release into the wild. Further research is needed to determine swimming performance and survival rates in the field after undergoing training and detraining to provide more practical information for the protection of this fish species.

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