

## RESEARCH ARTICLE

# Predator-driven intra-species variation in locomotion, metabolism and water velocity preference in pale chub (*Zacco platypus*) along a river

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**ABSTRACT**

Fish inhabit environments that vary greatly in terms of predation intensity, and these predation regimes are generally expected to be a major driver of divergent natural selection. To test whether there is predator-driven intra-species variation in the locomotion, metabolism and water velocity preference of pale chub (*Zacco platypus*) along a river, we measured unsteady and steady swimming and water velocity preference among fish collected from both high- and low-predation habitats in the Wujiang River. We also measured the routine metabolic rate (RMR), maximum metabolic rate (MMR) and cost of transport (COT) and calculated the optimal swimming speed ( $U_{opt}$ ). The fish from the high-predation populations showed a shorter response latency, elevated routine metabolism, lower swimming efficiency at low swimming speed and lower water velocity preference compared with those from the low-predation populations. Neither of the kinematic parameters fast-start and critical swimming speed ( $U_{crit}$ ) showed a significant difference between the high- and low-predation populations. The fish from the high-predation populations may improve their predator avoidance capacity primarily through an elevated routine metabolism and shorter response latency to achieve advanced warning and escape, rather than an improved fast-start swimming speed or acceleration. Thus, the cost of this strategy is an elevated RMR, and no trade-off between unsteady and steady swimming performance was observed in the pale chub population under various predation stresses. It was interesting to find that the high-predation fish showed an unexpected lower velocity preference, which might represent a compromise between predation avoidance, foraging and energy saving.

**KEY WORDS:** Critical swimming speed, Divergent natural selection, Fast-start escape response, Predation stress, Steady and unsteady swimming performance, Swimming efficiency

**INTRODUCTION**

Inhabited environmental conditions vary greatly, and habitat conditions are generally believed to be of major evolutionary significance (Langerhans, 2009). Natural selection promotes the traits (e.g. locomotion, behaviour and life history) of populations from different habitats toward different adaptive peaks. Specifically, divergent natural selection (DNS) is generally believed to represent

the primary mechanism that generates and maintains phenotypic diversity (Rice and Hostert, 1993; Orr and Smith, 1998; Langerhans, 2009). As an important driver of DNS, predation has attracted considerable attention (Blumstein and Daniel, 2005; Domenici et al., 2008; Langerhans, 2009; Oufiero et al., 2011). The effects of predators have been documented to lead to phenotypic and (or) genetic changes in a wide array of traits at the whole- and sub-organismal levels (Langerhans, 2006; Domenici et al., 2008; Langerhans, 2009; Oufiero et al., 2011). For instance, Trinidadian guppies (*Poecilia reticulata*) from high-predation populations have evolved differences in coloration (Houde, 1997; Magurran, 2005), life histories (Reznick et al., 1990; Reznick and Bryga, 1996) and morphology (Hendry et al., 2006) that are closely connected to aspects of Darwinian fitness compared with low-predation populations. Locomotor performance is also tightly linked to Darwinian fitness and is expected to change as a consequence of predation (Djawdan and Garland, 1988; Irschick and Garland, 2001; Oufiero and Garland, 2007; Irschick et al., 2008). However, the DNS of locomotor performance driven by predation has received far less attention than aspects such as life history and behavioural traits.

Swimming performance can be classified as steady or unsteady. In nature, steady swimming is commonly employed during holding in place against a current, searching for food, seeking favourable abiotic conditions and migration (Plaut, 2001; Kieffer, 2010). In fish, the critical swimming speed ( $U_{crit}$ ) is widely used to evaluate steady swimming performance (Brett, 1964; Gregory and Wood, 1998; Plaut, 2001; Lee et al., 2003). Unsteady swimming refers to more-complex locomotor patterns in which fish change velocity or direction and is commonly used in social interactions, predator avoidance and the navigation of structurally complex environments (Blake, 1983; Domenici and Kapoor, 2010). Fast-start escape performance is widely used for the measurement of unsteady movement in fish (Webb, 1986; Law and Blake, 1996). In environments with a high predation intensity, the direct consumption of prey by predators should lead to natural selection that favours an increased escape ability, potentially through an increase in unsteady swimming performance (e.g. fast-start swimming performance) (Domenici et al., 2008). However, any potential increase in escape ability may come at a cost. In particular, performance functions may trade-off with one another because of biomechanical, biochemical or physiological inter-relationships (Clobert et al., 2000; Vanhooydonck et al., 2001; Blake, 2004; Dlugosz et al., 2009). For example, previous studies on fish have indicated that the body morphology characteristics that are necessary to maximise the steady swimming efficiency are an increased depth of the anterior body and head, a shallow caudal region and a streamlined body shape (Fisher and Hogan, 2007), whereas unsteady swimming is maximised by a long, deep caudal region and a relatively shallow anterior body and head region (Webb, 1986; Law and Blake, 1996;

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Walker, 1997). Consequently, populations subjected to high predation may exhibit diminished steady swimming performance (e.g. critical swimming performance) as a cost of improved unsteady swimming performance, which has been demonstrated in several fish species (Reidy et al., 2000; Langerhans, 2009; Oufiero et al., 2011). However, in other studies, predation was found to generate no effect on either unsteady or steady swimming performance (or on both types of swimming performance) (Chappell and Odell, 2004; Fu et al., 2012). These findings suggest that the effect of predation stress on swimming performance might be species specific. Thus, the first aim of the present study was to investigate the effect of predation stress on both unsteady and steady swimming performance in a small local cyprinid, the pale chub (*Zacco platypus* Temminck and Schlegel 1846).

The mechanisms underlying variation in swimming performance involve morphology, physiology, biochemistry and the neuroendocrine system (Woodward and Smith, 1985; Langerhans et al., 2004; Domenici et al., 2008; Fu et al., 2011b; He et al., 2013). Energy metabolism during swimming has been attracting considerable attention as a result of its close correlation with swimming performance (Webb, 1973; Dickson et al., 2002; Fu et al., 2011b). It has been found that metabolic adjustment might be involved in the variation of swimming performance driven by predation. For example, predation-driven morphological changes may decrease swimming efficiency because of increasing energy costs during swimming (Lagergren et al., 1997; Pettersson and Brönmark, 1999; Pettersson and Hedenström, 2000), and fish may enhance their oxygen-uptake capacity as a compensatory mechanism. Furthermore, compared with low-predation populations, fish from high-predation populations may maintain higher hormone levels and watchfulness to detect the predators in a timely fashion (Casillas and Smith, 1977; Czesny et al., 2003). As a result, these fish may exhibit higher routine energy expenditure. Thus, the second aim of the present study was to investigate the effect of predation stress on routine energy metabolism, respiratory capacity and swimming efficiency in the pale chub.

The habitat preference of fish is also greatly affected by predation because high-predation populations tend to seek shallow water (to exclude large fish) (Heithaus and Dill, 2002; Railsback and Harvey, 2002) and complex habitats (with more refuges) (Sih et al., 1992; Lehtiniemi, 2005). Furthermore, water velocity preference driven by predation has been demonstrated in larval blackfly (*Simulium ornatum*), which prefer a high-velocity current to reduce the risk of predation (Allan et al., 1987; Malmqvist and Sackmann, 1996). The pale chub is widely distributed in both high- and low-predation habitats. The most broadly distributed and common piscivorous fish species found in pale chub habitats is the southern catfish (*Silurus meridionalis*), an ambush predator with a weaker steady swimming performance (Yan et al., 2014) that mainly stays in areas of a river with low water velocity. Thus, the third aim of the present study was to test whether pale chub from high-predation populations are more likely to prefer a high water velocity to reduce the encounter rate with this predator.

The pale chub is a widely distributed, small Asiatic cyprinid, and previous studies have found that this species shows great morphological and locomotor divergence across different habitats (Johansson, 2006; Fu, S. J. et al., 2013). The predator intensity also varies profoundly among different natural habitats. However, it is difficult to determine whether morphological and locomotor variation is related to predation stress because the influence of predation is confounded by a number of other environmental factors that may interact with and weaken the impact of predation. Thus,

research on the swimming performance of pale chub from habitats with similar environmental characteristics with the exception of predation is still necessary. The Wujiang River is the largest tributary of the Upper Yangtze River and shows large differences in predation intensity among habitats. The pale chub is widely distributed in both high-predation (e.g. main streams) and low-predation (e.g. mountainous tributary streams) reaches of the Wujiang River. Thus, we selected the pale chub as our experimental animal. To fulfil our experimental objectives, we examined the fast-start swimming performance (as the unsteady swimming performance), critical swimming performance (as the steady swimming performance), oxygen consumption rates in both resting and swimming states and water velocity preferences of pale chub from high- and low-predation populations from habitats with similar environmental characteristics with the exception of predation intensity. We hypothesised that pale chub from high-predation populations would exhibit (1) a stronger fast-start swimming performance and weaker critical swimming performance; (2) a lower swimming efficiency and higher routine metabolism; and (3) a preference for a higher water velocity compared with the fish from low-predation populations. Furthermore, we examined the correlation between the main ecological parameters (i.e. temperature, dissolved oxygen level and water velocity) and swimming performance due to the very small difference between the selected high- and low-predation habitats to exclude this noise from other environmental factors.

## RESULTS

### Fast-start performance

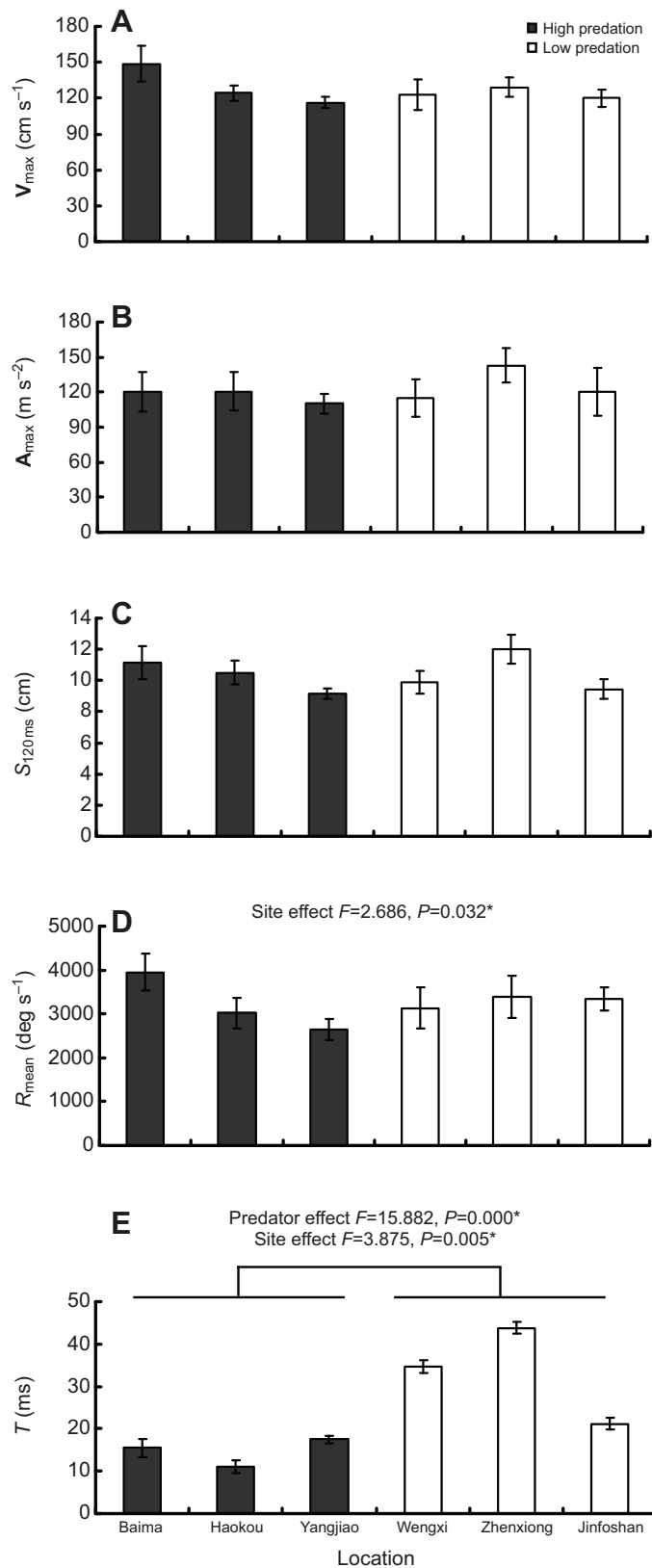
Maximum linear velocity ( $V_{\max}$ ), maximum linear acceleration ( $A_{\max}$ ), distance travelled during the first 120 ms ( $S_{120\text{ms}}$ ) and mean rotational velocity ( $R_{\text{mean}}$ ) did not significantly vary between the high- and low-predation populations (ANCOVA; Fig. 1A–D). Furthermore, only  $S_{120\text{ms}}$  showed a significant correlation with water velocity (multiple regression;  $r=0.292$ ,  $P=0.032$ ; Table 1). The high-predation populations exhibited a significantly shorter response latency ( $T$ ) compared with the low-predation populations ( $F=15.882$ ,  $P<0.001$ ; Fig. 1E).

### $U_{\text{crit}}$ and optimal swimming speed ( $U_{\text{opt}}$ )

There was no significant difference in  $U_{\text{crit}}$  between the high- and low-predation populations (Fig. 2A). Additionally,  $U_{\text{crit}}$  showed no significant correlation with any of the ecological parameters (Table 1).  $U_{\text{opt}}$  values of the high-predation populations were significantly higher than the values of the low-predation populations ( $F=8.066$ ,  $P=0.007$ ; Fig. 2B).

### Metabolic performance

The routine metabolic rate (RMR) of the high-predation populations was significantly higher than in the low-predation populations ( $F=11.103$ ,  $P=0.002$ ; Fig. 3A). Furthermore, RMR showed a negative correlation with dissolved oxygen ( $r=-0.349$ ,  $P=0.010$ ; Table 1). The maximum metabolic rate (MMR) exhibited no significant difference between the high- and low-predation populations (Fig. 3B). Moreover, MMR showed no significant correlation with any of the ecological parameters (Table 1). The metabolic scope (MS) of the high-predation populations was significantly lower than the value of the low-predation populations ( $F=6.956$ ,  $P=0.011$ ; Fig. 3C). The swimming metabolic rate ( $\dot{M}_{\text{O}_2}$ ) increased significantly with the swimming speed in all populations (two-way ANCOVA,  $F=84.706$ ,  $P=0.000$ ; Fig. 4A,C,E; Fig. 5A,C,E; Table 2). The predators had no significant effect on  $\dot{M}_{\text{O}_2}$ , but they



**Fig. 1.** Fast-start performance in high- and low-predation populations of pale chub along the Wujiang River, China. (A)  $V_{max}$ , (B)  $A_{max}$ , (C)  $S_{120ms}$ , (D)  $R_{mean}$  and (E)  $T$ . \* $P < 0.05$ , significant difference between high- and low-predation populations (ANCOVA). No significant differences were observed between populations for (A–C) predator, site or length effects, (D) predator or length effects and (E) mass effects (ANCOVA,  $P > 0.05$ ).

presented a significant interaction effect ( $F=2.535$ ,  $P=0.015$ ) with swimming speed (Table 2). The high-predation populations showed a higher  $\dot{M}_{O_2}$  at lower swimming speeds, and their  $\dot{M}_{O_2}$  increased more slowly as the speed increased compared with the low-predation populations (Fig. 4A,C,E; Fig. 5A,C,E; Table 2).

### Transport cost

The swimming speed had a significant effect on the transport cost (COT) ( $F=34.833$ ,  $P=0.000$ ; Fig. 4B,D,F; Fig. 5B,D,F; Table 2). In contrast, the presence of predators showed no significant effect on COT, although their presence showed a significant interaction effect ( $F=3.208$ ,  $P=0.003$ ) with the swimming speed (Table 2). The COT in both the high- and low-predation populations was higher at low swimming speeds and decreased more substantially with an increased swimming speed within the lower range of swimming speeds. COT remained unchanged within the high-speed ranges in the high-predation populations, while showing an increase in the low-predation populations. Furthermore, the COT in the high-predation populations was significantly higher than in the low-predation populations at lower speeds ( $P < 0.05$ ), whereas the COT showed no significant difference at higher speeds between the high- and low-predation populations (Fig. 4B,D,F; Fig. 5B,D,F; Table 2).

### Water velocity preference

The total distance travelled in 1 h ( $S_{1h}$ ) presented no significant difference between the high- and low-predation populations (Fig. 6A). The high-predation populations displayed a higher percentage of accumulated time in water with mean velocity of  $10 \text{ cm s}^{-1}$  ( $P_{zone 5}$ ;  $F=6.764$ ,  $P=0.012$ ) and a lower percentage of time in water with a mean velocity of  $24.5 \text{ cm}^{-1}$  ( $P_{zone 4}$ ;  $F=6.154$ ,  $P=0.016$ ) compared with the low-predation populations (Fig. 6B).

### DISCUSSION

In this study, we aimed to investigate the intra-species variation of locomotor and metabolic performance and water velocity preference driven by predation in the pale chub. We demonstrated that fish from high-predation populations showed a shorter response latency, elevated routine metabolism, lower swimming efficiency at low swimming speed and lower water velocity preference compared with fish from low-predation populations. Neither the fast-start nor  $U_{crit}$  kinematic parameter was significantly different between the high- and low-predation populations. The fish from the high-predation populations may improve their predator avoidance capacity primarily through a shorter response latency and hence a faster escape response, rather than an improved fast-start swimming speed or acceleration. However, the cost of this strategy is an elevated RMR and a reduced steady-swimming efficiency at low speeds, and no trade-off between unsteady and steady swimming performance was found in pale chub populations under different predation stress. Notably, the high-predation fish showed an unexpected lower velocity preference, which may represent a compromise between predation avoidance, foraging and energy saving.

### Swimming performance

The trade-off between steady and unsteady swimming performance has been hypothesised to be widespread and to play an important role in the ecology and evolution of fish (Webb, 1984; Langerhans et al., 2007). This trade-off has been confirmed in several fish species [e.g. killifish (*Rivulus hartii*) and mosquitofish (*Gambusia affinis*)], demonstrating divergence in both swimming performances among different populations driven by predation (Langerhans, 2009;

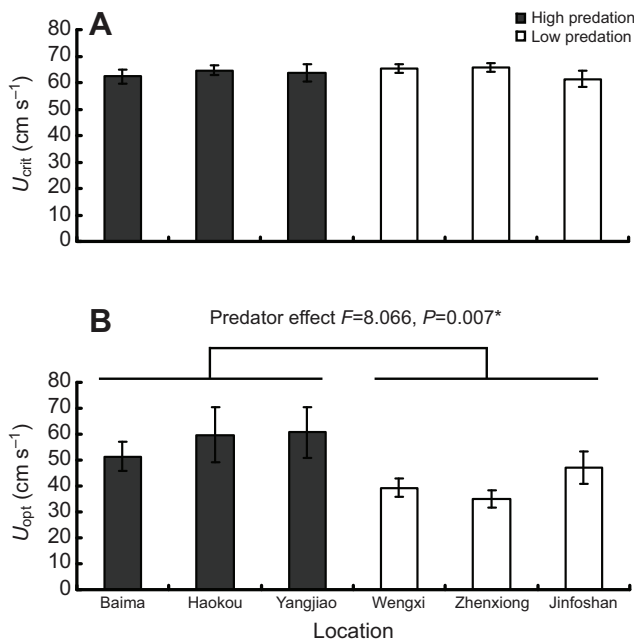
**Table 1. Results of multiple regression analyses of the effects of the main ecological parameters on swimming performance and metabolism**

Parameter		$U_{crit}$	$U_{opt}$	RMR	MMR	MS	$V_{max}$	$A_{max}$	$S_{120}$	$R_{mean}$	$T$
Water velocity	$r$	0.069	-0.105	-0.221	0.084	0.170	0.107	0.148	0.292	0.128	-0.167
	$P$	0.621	0.451	0.108	0.548	0.220	0.442	0.286	0.032*	0.356	0.228
Dissolved oxygen	$r$	0.020	-0.168	-0.349	-0.130	0.035	-0.098	0.089	0.053	0.017	0.201
	$P$	0.887	0.224	0.010*	0.348	0.800	0.481	0.522	0.702	0.901	0.145
Water temperature	$r$	-0.070	0.054	0.174	-0.183	-0.237	0.096	-0.159	-0.169	0.067	0.229
	$P$	0.614	0.701	0.208	0.185	0.084	0.490	0.251	0.221	0.628	0.096

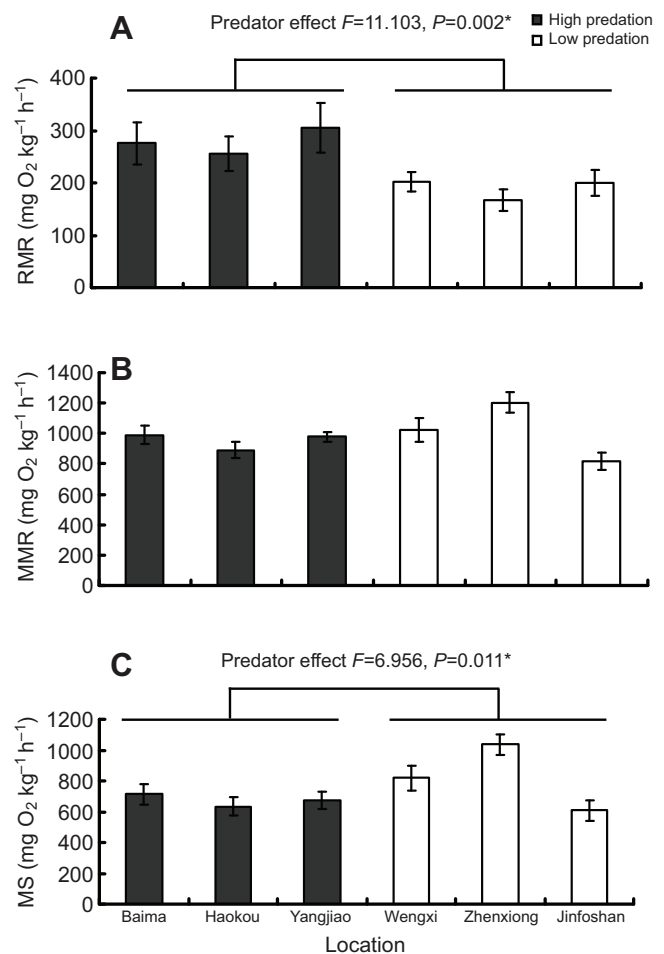
\*Significant correlation between swimming performance, metabolism and the ecological parameters ( $P < 0.05$ ).

Oufiero et al., 2011). In this study, we hypothesised that pale chub from high-predation populations would exhibit a greater fast-start performance but a lower critical swimming performance. However, neither fast-start nor  $U_{crit}$  showed significant variation between high- and low-predation fish. The fish from high-predation populations may improve their escape capacity by a shorter response latency rather than a faster escape speed and/or acceleration during their escape from predators. This strategy was completely different from previously demonstrated strategies such as improved velocity, acceleration and/or distance travelled during the fast-start process in crucian carp (*Carassius carassius*) (Domenici et al., 2008), mosquitofish (Langerhans et al., 2004) and the Trinidadian guppy (Ghalambor et al., 2004) from high-predation habitats. Notably, in these fish species, morphological changes such as a deeper body and a larger caudal fin with a lower aspect ratio are accompanied by an improved fast-start performance (Blake, 2004; Langerhans et al., 2004; Domenici et al., 2008), which eventually leads to a lower steady swimming performance (Webb, 1984; Langerhans, 2006). However, the improved anti-predator strategy of a shortened response latency exhibited by the pale chub in this study was not accompanied by a change in steady swimming performance. Thus,

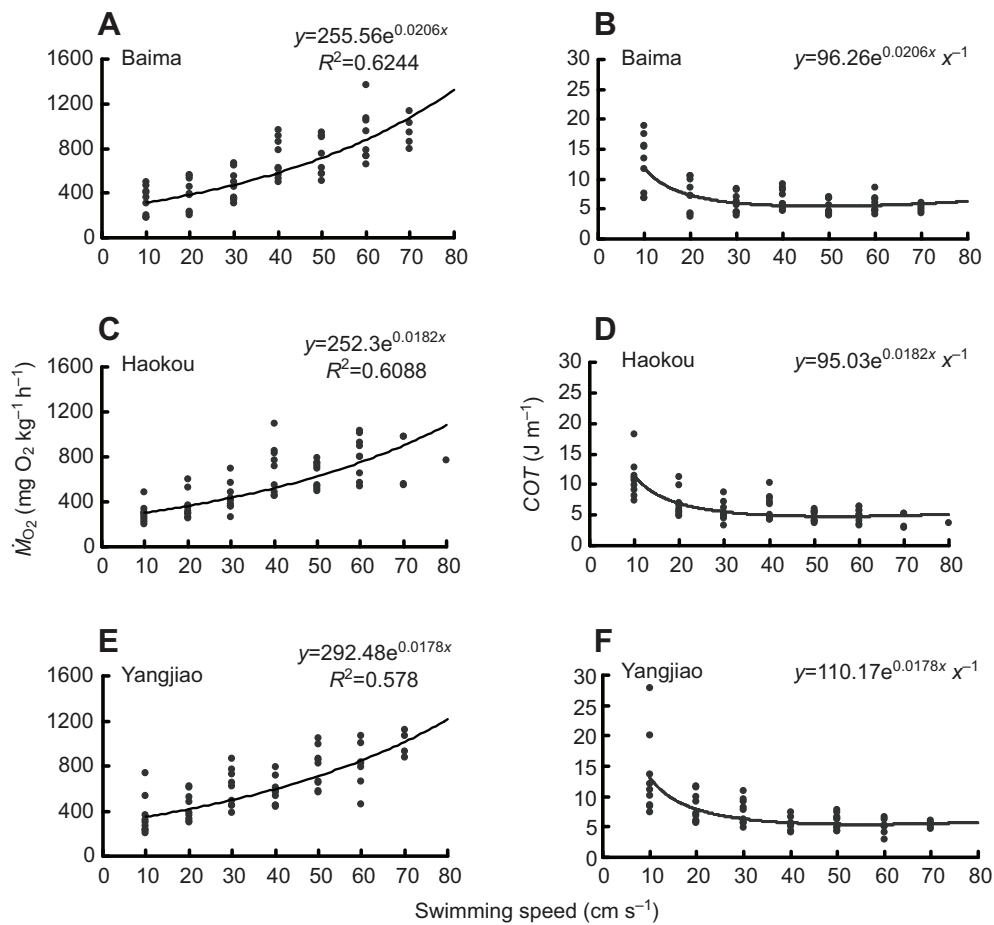
a trade-off between unsteady and steady swimming performance may not exist in pale chub populations under different predation stresses. However, a previous study found a negative relationship between unsteady and steady swimming performances in pale chub collected from different sites along the Wujiang River (Fu, S. J. et al., 2013). In this study, both water velocity and predator stress varied profoundly among different collecting sites; therefore, the possible trade-off between unsteady and steady swimming performance primarily resulted from the water velocity, rather than predators (i.e. the habitat with high water velocity favours



**Fig. 2. Critical and optimal swimming speed in high- and low-predation populations of pale chub.** (A)  $U_{crit}$  and (B)  $U_{opt}$  of pale chub along the Wujiang River, China. \* $P < 0.05$ , significant difference between the high- and low-predation populations (ANCOVA). No significant differences were observed between populations for (A) predator, site or length effects, (B) site or length effects (ANCOVA,  $P > 0.05$ ).



**Fig. 3. Metabolic performance in high- and low-predation populations of pale chub.** (A) RMR, (B) MMR and (C) MS of pale chub along the Wujiang River, China. \* $P < 0.05$ , significant difference between the high- and low-predation populations (ANCOVA). No significant differences in site or mass effects between populations were observed for A–C; no difference in predator effect was observed for B (ANCOVA).



**Fig. 4. Metabolic rate and transport cost in high-predation populations of the pale chub.** (A,C,E)  $\dot{M}_{O_2}$  and (B,D,F) COT measured in fish along the Wujiang River, China.

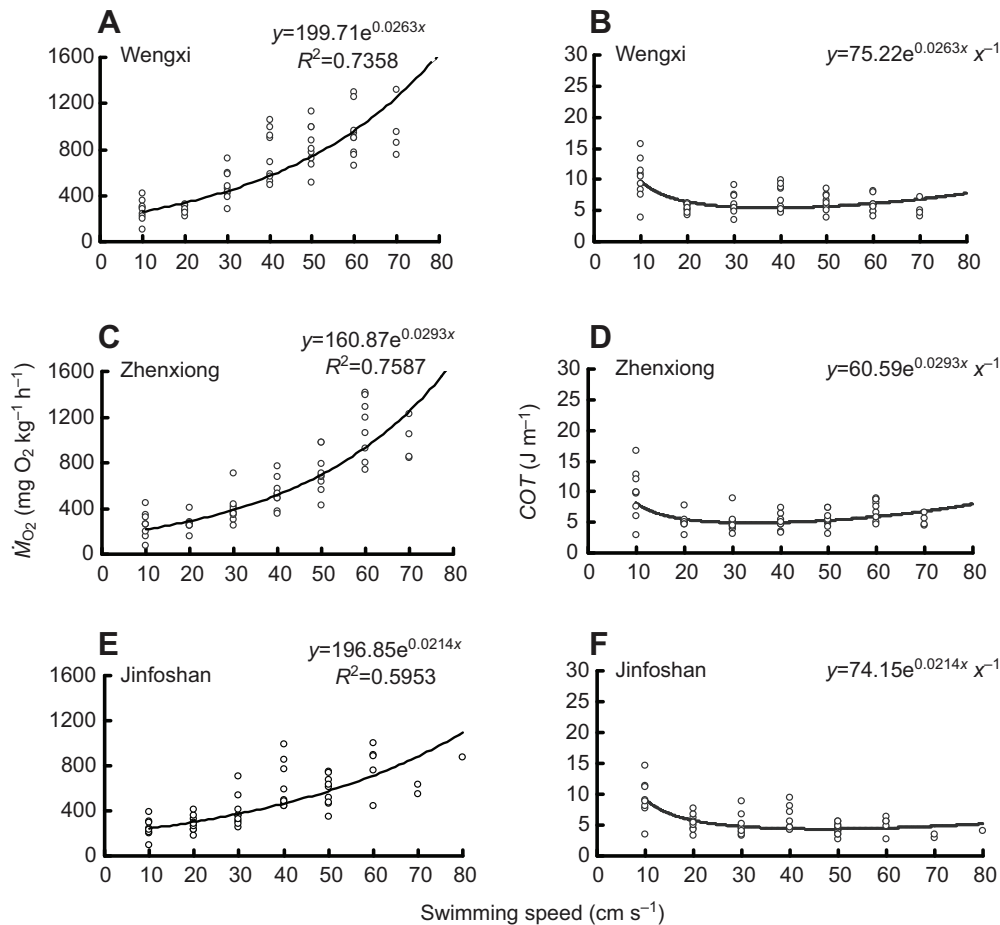
individuals with high steady swimming performance, causing a slender body shape instead of a higher unsteady swimming performance in a habitat with low water velocity) (Fu, S. J. et al., 2013). Furthermore, only six collection sites were selected in the present study, suggesting the possibility of a low sample size and large individual variation; therefore, a trade-off may exist that cannot be detected in the present study.

#### Metabolic performance

In this study, the high-predation fish showed a higher RMR compared with the low-predation fish. An elevation of maintenance metabolism in the presence of a predator has also been documented in other fish species (Brown et al., 2005; Millidine et al., 2006). This elevated energy expenditure may be primarily due to the higher catecholamine hormone levels required to maintain mental alertness for early predator detection (Millidine et al., 2006). Such increased hormone release and mental activity is metabolically demanding, and heightened vigilance may increase energy costs, with various knock-on consequences (Reid et al., 1998; Roulin, 2001). Furthermore, the fish from the high-predation populations may have to remain in a sustained state of physiological readiness for predator avoidance activities, which would also entail a greater metabolic investment in fuelling cardiac and respiratory pumps and maintaining the sensory apparatus (e.g. visual and auditory sense) at a sustained elevated state of acuity (Millidine et al., 2006). Finally, the reduced response latency of high-predation fish may be a result of improved nerve conduction velocity, and the involved morphological and physiological changes may also be energy consuming. It was interesting that a significant negative correlation

was found between RMR and dissolved oxygen, which may be because the pale chub in lower dissolved oxygen habitats need more maintenance energy for morphological and (or) physiological upregulation of cardio-respiratory organs as a compensation of low oxygen availability. Furthermore, food enrichment usually shows a negative correlation; however, a positive correlation with RMR might result from the indirect negative relationship between dissolved oxygen and RMR in the present study.

Fish from high-predation populations show a higher RMR than fish from low-predation populations (Brown et al., 2005; Millidine et al., 2006), which suggests that high-predation fish should upregulate their respiratory capacity (i.e. increase their MMR) to sustain the same metabolic scope and, hence, the same  $U_{crit}$  level as low-predation fish. However, neither  $U_{crit}$  nor MMR showed a significant difference between the high- and low-predation fish, which may be due to the significant difference in the  $O_2$  versus swimming speed curves between the high- and low-predation fish; i.e. the high-predation populations showed a higher  $\dot{M}_{O_2}$  at a lower swimming speed, whereas the  $\dot{M}_{O_2}$  increased more slowly with swimming speed compared with low-predation populations. Thus, the fish from the high-predation populations showed a higher COT and, thus, a lower swimming efficiency at lower swimming speeds, which is primarily due to their higher RMR, whereas at higher swimming speeds, the fish from high-predation populations showed a similar COT (i.e. swimming efficiency) to the fish from low-predation populations. This finding suggests that the net COT (without the effect of RMR) and, hence, the swimming efficiency of the high-predation fish was lower compared with the COT of the low-predation fish. However, determining the underlying



**Fig. 5. Metabolic rate and transport cost in low-predation populations of the pale chub.** (A,C,E)  $\dot{M}_{O_2}$  and (B,D,F) COT measured in fish along the Wujiang River, China.

mechanism will require further investigation. Perhaps the high-predation fish are in a sustained state of physiological readiness for locomotion (i.e. showing a ready-to-go status), and once swimming behaviour is initiated, physiological processes are recruited less intensively than in the case of low-predation fish.

### Water velocity preference

A previous study found that larval blackflies reduce predation risk by dwelling in microhabitats with higher current velocities to achieve a higher success rate of escaping from predators at the cost of a decreased feeding rate (Allan et al., 1987; Malmqvist and Sackmann, 1996). Thus, we hypothesised that pale chub from high-predation populations may prefer a higher water velocity compared with the fish from low-predation populations to avoid their primary predator, the southern catfish, which displays a weaker swimming performance and inhabits still or low-velocity water bodies. Notably, in this study, fish from both the high- and low-predation populations showed an obvious

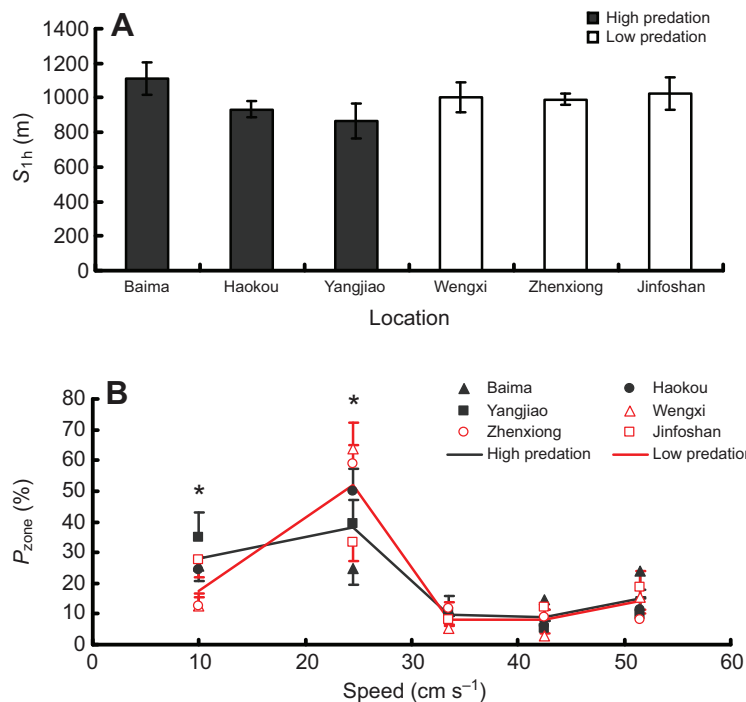
preference for remaining at an average velocity of 24.5 cm s<sup>-1</sup> (zone 4) and  $S_{1h}$  was not significantly different between the high- and low-predation populations. However, fish from the high-predation habitats spent a shorter time in zone 4 and a longer time at an average water velocity of 10 cm s<sup>-1</sup> (zone 5) compared with fish from the low-predation habitats. This finding suggests that the high-predation fish prefer a lower water velocity. This result is contrary to our expectations. The preference of the low-predation fish for a higher velocity may be due to the increasing intensity of intra-specific competition resulting from the lack of predators (Peacor, 2002). Thus, the fish may prefer relatively higher velocity water to achieve a greater foraging efficiency (Malmqvist and Sackmann, 1996). Furthermore, the high-predation fish exhibit higher routine energy expenditure (RMR) to cope with predation in their daily life; therefore, energy saving is more critical for high-predation fish because of its close correlation with survival (Post and Parkinson, 2001; Brown, 2004). Notably, the preferred water velocity of the pale chub was found to be much lower than both the common water velocity in its habitats and the calculated  $U_{opt}$ . Although pale chub preferred to remain in a rapid water current, in the wild they have a habit of hiding in cracks between stones. Thus, these fish can remain in a water area with high levels of dissolved oxygen, high food availability and low predation stress but incur a lower cost of energy expenditure against the water current. Therefore, the water velocity preference of the fish may not simply be determined by predation but may represent a compromise between predator avoidance, foraging behaviour and energy conservation.

Fish develop different adaptive strategies according to the demands of swimming performance under the pressure of natural

**Table 2. Effects of swimming speed and predators on metabolic rate ( $\dot{M}_{O_2}$ ) and cost of transport (COT) in pale chub, based on two-way ANCOVA with body mass as the covariate**

	df	$\dot{M}_{O_2}$		COT	
		F	P	F	P
Mass effect	1	0.073	0.788	0.385	0.535
Speed effect	7	84.706	0.000*	34.833	0.000*
Predators effect	1	0.038	0.845	1.541	0.215
Speed × predators	7	2.535	0.015*	3.208	0.003*

\*Significantly different ( $P < 0.05$ ).



**Fig. 6. Water velocity preference in high- and low-predation populations of pale chub.** (A)  $S_{1h}$  and (B) percentage of accumulated time in each zone (i.e. velocity;  $P_{zone}$ ) measured for fish from the Wujiang River, China. \* $P < 0.05$ , significant difference between the high- and low-predation populations (ANCOVA). No significant differences were observed between populations for predator, site or length effects in A (ANCOVA,  $P > 0.05$ ).

selection. The strategies may favour improved swimming performance or other specific behavioural and morphological adaptations. Most fish species improve their swimming performances (either steady or unsteady) as a response to increased water velocity or predation stress. However, several species such as Siluriformes: Sisoridae maintain their position in ripptide currents by attaching onto rocks through morphological specialisation (with the lips reflected and spreading to form a broad flat sucker) (Peng et al., 2004), rather than through better steady swimming performance. In addition, darkbarbel catfish (*Pelteobagrus vachelli*) deter predators primarily by using their spines rather than by making a speedy escape (Fu et al., 2009). In this study, the shorter response latency of high-predation pale chub may also be a specific adaptive anti-predator strategy, and this method needs further investigation.

## MATERIALS AND METHODS

### Collection sites and the fish collection process

Individuals from six populations of pale chub (three populations from habitats with predators and three without predators) were collected from the Wujiang River in China, in August 2013. The populations were classified 'high predation' or 'low predation' based on the presence or

absence of the southern catfish and other piscivorous fish. Geographical information and ecological parameters for each collecting site are listed in Table 3. The water temperature, water velocity and dissolved oxygen level were measured three times per day for 3 days in the morning (07:00–08:00 h), at noon (12:00–13:00 h) and in the late afternoon (17:00–18:00 h) (Fu, S. J. et al., 2013). Predatory fish were surveyed by reviewing the most recent 5 days of local fishery catch data (Fu, S. J. et al., 2013).

While piscivorous fish serve as major predators of the pale chub, avian predators pose an additional potential threat (Langerhans et al., 2007). However, because the habitats of pale chub are dominated by rapid currents, the avian threat is limited (Railsback and Harvey, 2002). In addition, even though an avian threat to pale chub may exist, it is not expected to differ among the six examined populations with and without piscivorous fish (Langerhans et al., 2007). Therefore, the effects of avian predators were ignored in this study.

Female pale chub were collected by hook-and-line angling and seine fishing at each site. Females were chosen because in males, the development and presence of an enlarged sexually selected anal fin that may alter thrust is a potential confounding factor. Moreover, females are much more easily caught in the field (Fu, S. J. et al., 2013).

After being collected from the above-mentioned sampling sites, the fish were maintained in fully aerated and thermoregulated tanks (100 l) at a water

**Table 3. Summary of pale chub collection sites and differences in ecological parameters between high- and low-predation populations based on an independent samples test**

Collecting site	High predation <sup>a</sup>			Low predation			Significance
	Baima	Haokou	Yangjiao	Wengxi	Zhenxiiong	Jinfoshan	
Coordinates	N29°24'37" E107°31'55"	N29°02'32" E107°50'43"	N29°23'48" E107°36'49"	N27°37'26" E108°01'34"	N27°23'29" E104°53'52"	N29°06'52" E107°13'19"	
Water velocity (m s <sup>-1</sup> )	1.43	1.7	1.1	1.35	1.62	1.4	$t = -0.243$ , $P = 0.820$
Dissolved oxygen (%)	94.2	105.2	91.6	101.7	103	106.6	$t = -1.532$ , $P = 0.200$
Water temperature (°C)	26.1	22.9	23.8	26.7	20.4	23.5	$t = 0.357$ , $P = 0.739$
River width (m)	100–200	30–50	100–200	5–15	20–30	20–50	$t = 2.408$ , $P = 0.074$
Sample size <sup>b</sup>	9 or 12	9 or 12	9 or 12	9 or 12	9 or 12	9 or 12	
Body mass (g)	4.62±0.51	4.09±0.20	5.19±0.61	4.29±0.18	4.56±0.45	4.77±0.47	$t = 0.261$ , $P = 0.795$
Body length (cm)	6.67±0.21	6.62±0.15	7.08±0.23	6.71±0.09	6.88±0.19	6.80±0.22	$t = -0.048$ , $P = 0.962$

<sup>a</sup>*Silurus meridionalis* and *Siniperca kneri* are the predator species in areas of high predation.

<sup>b</sup>Swimming performance was assessed in 9 fish and water velocity preference in 12 different fish.

temperature of 23.9°C ( $\pm 1^\circ\text{C}$ , the average water temperature at the six collection sites, Table 3). After 48 h of fasting, nine fish from each population were selected (with a similar body mass and length, Table 3) for the measurement of fast-start swimming performance. Then, after 8 h of recovery, the fish were transferred to a Brett-type swim tunnel (Brett, 1964) for measurements of  $U_{\text{crit}}$  and oxygen consumption. The same fish were subjected to the two performance measurements to test whether there was a trade-off between fast-start and  $U_{\text{crit}}$  performance at the individual level. The pilot experiment revealed no relationship between the fast-start measurements and subsequent  $U_{\text{crit}}$  measurements. Twelve other fish from each population were selected to measure water velocity preference individually. All of the tests were performed at a water temperature of 24°C.

### Measurement of fast-start performance

After 48 h of fasting, each fish was covered with a wet towel and placed into a square container (42×31×3.5 cm) with water, and the water depth was maintained at ~1 cm to ensure that the gills of each fish were submerged while the dorsal side remained exposed to attach a plastic ball. A small white polystyrene foam ball (diameter 1 mm, mass <0.001 g) was then attached to its dorsal side with glue at the centre of mass (CM) position (Webb, 1977; Yan et al., 2013). The procedure lasted for less than 1 min, and then the fish were individually transferred to an experimental glass tank (40×40×15 cm) for 1 h of acclimation, prior to being startled (Domenici et al., 2008; Yan et al., 2013). Square reference grids (1×1 cm) were attached to the floor of the tank. The sides of the experimental tank were covered with black paper so that the fish could not see the approaching stimulus. The depth of the water in the tank was 10 cm. After 1 h of habituation, an escape response was elicited with an electrical impulse (0.5 V cm<sup>-1</sup>; 20 ms, as determined in a previous study (He et al., 2011) that was manually triggered when the fish was in a holding position at the centre of the filming zone. Meanwhile, a high-speed camera (A504K, Basler, Germany; 500 frames s<sup>-1</sup>) was used to record the entire duration of the response (time span, 2 s). The recording was initiated as soon as the light-emitting diode (LED; synchronised with the electrical stimulus) was illuminated (0 ms delay). The resulting sequence images then digitised with TpsUtil and TpsDig software (<http://life.bio.sunysb.edu/morph>) to define the track of the CM in the locomotion performed by the fish during its escape response. The following parameters were calculated:  $V_{\text{max}}$  (cm s<sup>-1</sup>),  $A_{\text{max}}$  (m s<sup>-2</sup>),  $S_{120\text{ ms}}$  (cm),  $R_{\text{mean}}$  (deg s<sup>-1</sup>) and  $T$  (ms). The  $V_{\text{max}}$  and  $A_{\text{max}}$  parameters are the maximum velocity and acceleration of the CM during the escape response, respectively.  $S_{120\text{ ms}}$  was defined as the total escape distance travelled by the CM during the first 120 ms after the stimulus, and  $V_{\text{max}}$ ,  $A_{\text{max}}$  and  $S_{120\text{ ms}}$  were computed using a five-point smoothing regression to smooth the CM displacement data (Lanczos, 1956).  $R_{\text{mean}}$  is average rotational velocity of the head during stage 1 (stage 1 rotation angle divided by stage 1 duration). Parameter  $T$  was defined as the time elapsed between the initiation of the stimulus (LED light) and the time when escape behaviour was observed (Fu et al., 2012; Yan et al., 2012; Fu, S. J. et al., 2013).

### Measurement of $U_{\text{crit}}$ and oxygen consumption

After the measurement of fast-start performance, the fish were transferred to a Brett-type swim tunnel respirometer for measurement of  $U_{\text{crit}}$ . The swim respirometer was previously described (Fu et al., 2011a; Fu, C. et al., 2013). Briefly, the respirometer was constructed from clear plastic polymethylmethacrylate. A circulating water flow was generated in the tunnel (total volume 3 l) by an acrylic propeller attached to a variable speed pump. The variable speed pump was connected to a stepper motor driver controlled by a computer. A written program was used to regulate the speed of the pump by putting out a specified electric pulse, thus determining the water velocity. A high-speed digital camera (EX-ZR200, Casio, Japan) in video mode was employed to calibrate the water velocity from the pump controller by tracking small black agar balls (with the same density as water) in the current. Then, the relationship between water velocity and the number of electric pulses could be represented as a linear equation. Thus, the water velocity could be determined by setting a specific electric pulse output. A honeycomb screen was secured at both ends of the swimming chamber to reduce turbulence and to ensure a uniform water velocity across the swimming chamber. Each individual fish was placed downstream of the propeller in a swimming

chamber with a 19.9 cm<sup>2</sup> cross-sectional area. The water temperature in the swimming chamber was controlled to within  $\pm 0.2^\circ\text{C}$  using a water bath connected to a stainless steel heat exchanger. The fish were individually transferred to the swim tunnel and allowed to recover for 8 h. During this time, aerated water flowed continually through the respirometer.

After 8 h of acclimation, the water velocity was increased in 10 cm s<sup>-1</sup> increments starting from 10 cm s<sup>-1</sup> every 30 min until the fish became fatigued. Fatigue was defined as the time at which the fish failed to move from the rear honeycomb screen of the swimming chamber for 20 s (Lee et al., 2003).  $U_{\text{crit}}$  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 velocity at which the fish swam for the full time period (cm s<sup>-1</sup>);  $\Delta V$  is the velocity increment (10 cm s<sup>-1</sup>);  $T$  is the prescribed period of swimming at each speed (30 min); and  $t$  is the time the fish swam at the final speed (min).

The swim tunnel was designed to switch between a closed mode and an open mode, with the former being used for respirometry and the latter to replenish oxygen levels. In the closed mode, a small volume of water was drawn from the sealed respirometer with a peristaltic pump, forced past a dissolved oxygen probe (HQ30; Hach, CO) housed in a sealed temperature-controlled chamber and then returned to the respirometer. The oxygen concentration (mg l<sup>-1</sup>) was recorded once every 2 min. The  $\dot{M}_{\text{O}_2}$  (mg kg<sup>-1</sup> h<sup>-1</sup>) of individual fish while swimming was calculated based on the depletion of oxygen according to the equation:

$$\dot{M}_{\text{O}_2} = 60(S_t - S_0)v / m, \quad (2)$$

where  $S_t$  and  $S_0$  (slope, mg l<sup>-1</sup> min<sup>-1</sup>) represent the decrease in the dissolved oxygen content of the water per minute with and without fish, respectively. These values were obtained from the linear regressions between time (min) and the dissolved oxygen content (mg l<sup>-1</sup>);  $v$  is the total volume of the respirometer (3 l) minus the volume of the fish; and  $m$  is the body mass (kg) of the fish.

The maximal  $\dot{M}_{\text{O}_2}$  during the  $U_{\text{crit}}$  test was defined as the MMR and was used as an indicator of respiratory capacity. For each population, the relationship between  $\dot{M}_{\text{O}_2}$  ( $y$ ) and swimming speed ( $x$ ) can generally be described by the equation:

$$y = a \cdot e^{bx}, \quad (3)$$

where  $a$  is the corresponding  $\dot{M}_{\text{O}_2}$  at a zero swimming speed calculated from Eqn 2, i.e. the RMR (Yan et al., 2013).  $b$  is a constant that can be used as an index of the swimming efficiency; that is, the higher the value of  $b$ , the more marked the increase in the swimming  $\dot{M}_{\text{O}_2}$  with an increased swimming speed. The MS was calculated from MMR minus RMR. The  $U_{\text{opt}}$  was calculated according to the following equation (Weihs, 1973):

$$U_{\text{opt}} = b^{-1}. \quad (4)$$

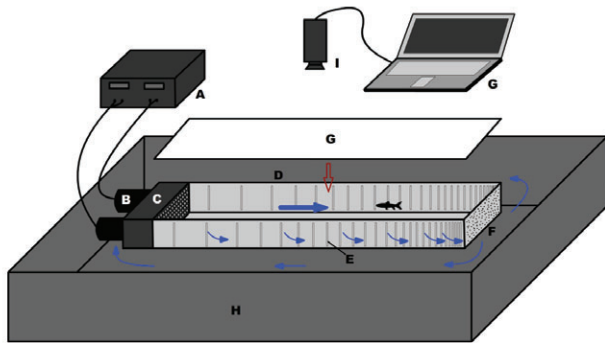
### Swimming efficiency (cost of transport)

The COT (J m<sup>-1</sup> g<sup>-1</sup>) provides an index of the overall swimming efficiency; the lower the COT value, the more efficient locomotion is. At each swimming speed, the obtained  $\dot{M}_{\text{O}_2}$  values were converted to J m<sup>-1</sup> g<sup>-1</sup> using an oxycaloric equivalent, 13.54 J mg O<sub>2</sub> (Claireaux et al., 2006). The values were then divided by the corresponding speed to obtain the COT.

### Water velocity preference

The water velocity preference of individual fish was measured with a device constructed as shown in Fig. 7. This consisted of a variable voltage power source, two variable speed pumps and a square water tunnel (1.3×0.2×0.1 m) immersed in a large water tank (1.5×0.8×0.5 m). The water tunnel was constructed from clear acrylonitrile-butadiene-styrene plastic. The water current was created by two speed pumps controlled by a variable voltage power source. A honeycomb screen was secured upstream of the tunnel to reduce turbulence and to ensure a uniform water velocity across the section. The two sides and the bottom of the tunnel were covered with square incisions (8×1 cm) for drainage; the square incisions were distributed increasingly intensively along an upstream-to-downstream gradient to generate greater drainage and, thus, slow the water velocity along the water





**Fig. 7. Device used to determine water velocity preference of pale chub.** (A) 20 V power source, (B) speed pump, (C) honeycomb screen, (D) velocity preference channel, (E) draining incisions, (F) multihole dam-board, (G) transparent polymethylmethacrylate, (H) water tank, (I) internet protocol camera, and (J) computer. The blue arrows indicate direction of flow, while red arrow indicates position of transparent panel installed on top of the velocity preference channel (D) during measurement.

tunnel. The end of the tunnel was closed with a removable multihole dam board to slow the water velocity approximately to  $0 \text{ cm s}^{-1}$  and to prevent the fish from escaping. The top of the square was covered with transparent polymethyl methacrylate to permit observation of the velocity preference of individual fish.

The water tunnel was divided into five zones, designated zone 1 to zone 5 from upstream to downstream, and the average velocities in the five zones were  $\sim 51.5, 42.5, 33.5, 24.5$  and  $10 \text{ cm s}^{-1}$ , respectively (The method for calculating the water velocity was similar to that employed in the  $U_{\text{crit}}$  swim tunnel respirometer). Individual fish were transferred downstream of the water tunnel for 1 h of acclimation to experience the different water velocities along the tunnel, and preferential behaviour was then recorded using an internet protocol camera connected to a computer for an additional 1 h. The videos of individual fish were then analysed using Nodus software (EthoVision XT 9, Nodus, Netherlands) and  $P_{\text{zone}}$  (for zones 1–5, %) and  $S_{1\text{h}}$  (m) were calculated.  $P_{\text{zone}}$  was the percentage of accumulated time the fish stayed in a given zone (zone 1 to zone 5).  $S_{1\text{h}}$  was the total distance the fish travelled in 1 h of observation, which was calculated using the following equation:

$$S_{1\text{h}} = t_1v_1 + t_2v_2 + t_3v_3 + t_4v_4 + t_5v_5, \quad (5)$$

where  $t_1$  to  $t_5$  (h) represent the accumulated time the fish stayed in zone 1 to zone 5, respectively, and  $v_1$  to  $v_5$  ( $\text{m h}^{-1}$ ) are the average speeds of zone 1 to zone 5, respectively.

### Statistical analysis

SPSS 17 was used for data analysis. All values are presented as the mean  $\pm$  s.e., and  $P < 0.05$  was taken as the level of statistical significance. The differences in swimming ( $U_{\text{crit}}$ ,  $U_{\text{opt}}$ ), metabolism (RMR, MMR, MS), fast-start performance ( $V_{\text{max}}$ ,  $A_{\text{max}}$ ,  $S_{120 \text{ ms}}$ ,  $R_{\text{mean}}$ ,  $T$ ) and velocity preference ( $S_{1\text{h}}$ ,  $P_{\text{zone}}$ ) between the high- and low-predation populations were analysed with an ANCOVA, with body mass (used in RMR, MMR, MS and  $T$  parameters) or length (used in  $U_{\text{crit}}$ ,  $U_{\text{opt}}$ ,  $V_{\text{max}}$ ,  $A_{\text{max}}$ ,  $S_{120 \text{ ms}}$ ,  $R_{\text{mean}}$ ,  $S_{1\text{h}}$ ,  $P_{\text{zone}}$  parameters) as the covariate and population as the random factor. A multiple regression of steady swimming ( $U_{\text{crit}}$  and  $U_{\text{opt}}$ ), metabolism (RMR, MMR, MS) and fast-start performance ( $V_{\text{max}}$ ,  $A_{\text{max}}$ ,  $S_{120 \text{ ms}}$ ,  $R_{\text{mean}}$ ,  $T$ ) on ecological parameters (water velocity, dissolved oxygen, and water temperature) was then performed at individual levels to determine the extent to which each ecological parameter explained the observed differences. The effects of the swimming speed and predation on swimming  $\dot{M}_{\text{O}_2}$  and COT were analysed with a two-way ANCOVA (with body mass used as the covariate).

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### Competing interests

The authors declare no competing or financial interests.

### Author contributions

S.-J.F. conceived and designed the experiments. C.F., Z.-D.C., X.-Z.Y. and S.-J.F. performed the experiments. C.F., Z.-D.C. and S.-J.F. analysed the data. C.F. and S.-J.F. wrote the paper.

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