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Predator stress decreases standard metabolic rate and growth in juvenile crucian carp under changing food availability

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ABSTRACT

Animals adapt to the challenges of fluctuations in predator risk and food availability in their natural habitats. Phenotypic plasticity allows animals to handle environmental changes. However, the patterns of flexibility in metabolic rates and its ecological consequences under different predator stress and food availability conditions are poorly understood. Here, we used crucian carp (*Carassius auratus*) as a prey species and northern snakehead (*Channa argus*) as a predator to test whether predator stress influences metabolism and growth, and alters the link between flexibility in metabolic rate and its ecological consequences (e.g., growth) in crucian carp. The experiment was carried out under the conditions of predator stress (with or without a predator) and three food availabilities (satiation feeding 1 time per day, low food availability; 2 times per day, intermediate food availability; and 3 times per day, high food availability) for 3 weeks. After 21 days of feeding, the final body mass and body length in the two treatments increased compared to the initial values in all three food availabilities. The feeding intake (FI) and specific growth rate (SGR) of the two treatments increased with increasing food availability. The control treatment had a higher FI and SGR than the predator stress treatment in all three food availabilities. The feeding efficiency (FE) of the two treatments was higher at the high and intermediate food availabilities than at the low food availability. However, no effect of predator stress on FE was detected. The final values of original or standardized SMR were higher in the control treatment than the predator stress treatment at the intermediate and high food availabilities. The changes in SMR (Δ SMR) were higher in the control treatment than in the predator stress treatment. The positive correlation between the Δ SMR and SGR was found in the intermediate food availability in the predator stress treatment, suggesting that individuals with a higher flexibility in SMR had a larger growth rate and vice versa, but this relationship was dependent on food availability. Our results suggest that predator stress decreased maintenance metabolism, feeding and growth of juvenile crucian carp irrespective of food availability. Predator stress does not alter the growth advantages conferred by the metabolic plasticity of the fish under changing food availability.

1. Introduction

In nature, the habitats of animals are always variable due to seasonal changes, human activities and climate changes. Environments often impose directional selection pressures for the adaptive optimum phenotypes of animals when phenotypic flexibility and selection are in parallel (Handelsman et al., 2013). Phenotypic flexibility is a type of ability in which animals can adjust their physiological, morphological and behavioral traits, which allows animals to address environmental changes (e.g., variations in predator pressure and food availability) (Bolnick et al., 2011; Auer et al., 2015). Moreover, phenotypic flexibility may lead to different ecological consequences and hence increase

animal survival and growth fitness in the face of fluctuations in living conditions (Piersma and Drent, 2003; Hofmann and Todgham, 2010; Fu et al., 2017). Since global climate change and habitat modification have increasing potential negative effects on the daily life of animals (Pörtner and Farrell, 2008), it is urgent to quantify how the phenotypic flexibility of individuals varies within populations and what the ecological consequences for this ability under changing environments are.

Predation is a key natural selection pressure that can regulate prey population dynamics and maintain the diversity of ecological species (Daniels et al., 2017; Elbroch et al., 2017). Prey species detect high predator stress through visual (Kelley and Magurran, 2003), olfactory (Dixson et al., 2010; Kondoh et al., 2016) or chemical alarm signaling

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(Chivers and Smith, 1998; Wisenden, 2000). Previous studies have documented that predators have long-term effects on the morphology, physiology, behavior and life history of prey species (Brown et al., 2005; Chivers et al., 2008; Fu, 2015). Strong predator stress could lead to an increase in adrenalin and cortisone in plasma (Barton, 2002), an increase in metabolic rates (Bell et al., 2010) and vigilance (Roulin, 2001) of prey species, suggesting that predator stress can be metabolically costly (Millidine et al., 2010). Because of finite energy budgets, the increased proportion of energy allocated to high maintenance metabolism, vigilance and hormone levels could decrease the energy allocation to other competing physiological functions, resulting in a decreased growth (Van Buskirk and Schmidt, 2000). Additionally, prey may become more shy and hence decrease their foraging activities when they are living with predators (Thomson et al., 2012; Liu et al., 2016). Hence, in the trade-off between foraging and predation risk, prey may decrease the encounter rates with predators, which could reduce food intake and energy allocation for growth.

Metabolic rate is a crucial physiological trait and is usually represented by the oxygen consumption rate, which is related to other physiological and life history traits, such as growth, survival, immunity, predator avoidance and reproduction (Careau et al., 2008). Standard metabolic rate (SMR) is the minimum energy required to sustain life in ectotherms (e.g., fish) and is one of the key physiological traits of energy metabolism (Fry, 1971). SMR can vary extensively by 2–3-fold among individuals within a population even after controlling for differences in body mass, sex or age (Burton et al., 2011). The cost of self-maintenance is thought to have important influences on individual fitness, but its ecological consequences (e.g., growth) are diverse and conflicting; SMR is positively related to growth (Mccarthy, 2010; Álvarez and Nicieza, 2005), though negative relationships between SMR and growth have also been reported (Norin and Malte, 2011). SMR exhibits high flexibility to cope with changing environments, such as variations in food availability, which can confer a growth advantage in animals that experience changes in food availability (Auer et al., 2015; Zeng et al., 2017, 2018). However, how animals adjust their metabolic rates and growth trajectories when they are living with different predator pressures under changing food availability remains largely unknown.

Here, we examined the effects of predator stress on the metabolism, growth, and their relationships of cyprinid fish with three food availabilities to test whether predator stress alters the links between flexibility in metabolic rate and its ecological consequences (e.g., growth). The crucian carp (*Carassius auratus*), a freshwater omnivorous cyprinid, is widely distributed in rivers, lakes and other water bodies in China (Ding, 1994). The northern snakehead (*Channa argus*), a carnivorous fish, is a natural predator of the crucian carp (Ding, 1994). The crucian carp were housed individually and indirectly with the northern snakehead (nonlethal predator stress) during an experimental period of three weeks. The morphology (e.g., body mass and length) and metabolic rate (e.g., SMR) were determined at the beginning and end of the experiment.

2. Materials and methods

2.1. Fish

Fish were obtained from a local aquaculture farm in Chongqing, China, and housed in three laboratory tanks (1.5 m in length × 0.8 m in width × 0.5 m in height) at Chongqing Normal University for at least four weeks. During the acclimation period, dechlorinated fresh water was used to replace approximately 20% of the tanks every two days. The water temperature was maintained at 25.4 ± 0.2 °C, and the photoperiod was 14 h light: 10 h dark. Fish were fed to satiation twice daily with a commercial diet (composition: 28.0% protein, 3.0% lipids, 13.0% crude fiber, and 15% ash; Tongwei Ltd., Jiangsu, China). The diet was in the form of spheres and could float on the water surface

without dissolving for at least 4 h. Uniform pellet sizes (approximately 2 mm in diameter) acquired by filtering them through a mesh screen were used for the experiment.

All animal handling and experiments were conducted in strict accordance with both the ethical requirements and the recommendations for animal care of the Key Laboratory of Animal Biology of Chongqing, China (Permit No. Zhao-20,180,116-02) and the requirements for environmental and housing facilities for laboratory animals in China (GB/T14925–2001). All of the experiments also complied with the local animal welfare laws (e.g., Measures of Chongqing Municipality for the administration of experimental animals) of Chongqing City, China.

2.2. Experimental overview

After acclimation, fish of similar morphologies (body mass = 3.69 ± 0.03 g; body length = 5.12 ± 0.01 cm; $n = 240$) were selected for the experiment. In nature, fish often encounter a high heterogeneity in food availability, which induces different nutritional statuses. Variations in such physiological conditions might result in different responses by individuals to predation stress. Flexibility in metabolic rate could confer individual growth advantages with changing food availability (Auer et al., 2015; Zeng et al., 2017). Hence, this study was designed to have two predator stress treatments (without and with the predator) under three food availabilities. High, intermediate and low food availability was represented by three satiation feedings per day (9:00 h, 15:00 h and 21:00 h), two satiation feedings per day (9:00 h and 21:00 h), and one satiation feeding per day (9:00 h), respectively. To reduce the potential influence of dominance hierarchies and group competition for food, each fish was individually raised in one of 240 independent compartments during the experimental period in four independent cycling tank systems. Each of the cycling tank systems consisted of a large tank with 60 independent compartments, a temperature controller (500w, Shanghai Jingcun, China), a UV sterilizer (Atman-18w, Chuangxing Ltd., Zhongshan, China), an air pump and a water filtration system. In each of the two cycling tank systems, three northern snakehead (body mass = 93.50 ± 14.75 g; body length = 19.33 ± 1.01 cm) and 25 individuals (body mass = 5.94 ± 0.31 g; body length = 6.2 ± 0.1 cm) of crucian carp as a companion prey were housed together under the compartments where the focal crucian carp were raised in the predator stress treatment. In this environment, the fish could experience visual and olfactory stress from the predator through the epidermal damage-released chemical cues of the crucian carp, 90% of which were hunted by the snakehead during the experiment. In each of the other two cycling tank systems, however, only 25 individuals (body mass = 6.28 ± 0.36 g; body length = 6.1 ± 0.1 cm) of crucian carp as a companion prey were housed under the compartments in the control treatment.

All the fish were fed to satiation within 1 h at each feeding. Fish were fasted for 36 h and measured for their morphologies (body mass and body length) and SMR at the beginning and end of the experiment. The SMR of individual fish was determined using a continuous-flow respirometer (Fu et al., 2009a; Killen et al., 2016). After the initial SMR and morphology measurements, fish from each of the two treatments were randomly assigned to one of three food availability treatments and were placed into corresponding compartments for a feeding period of 21 days.

2.3. Measurements of growth performance

The fish from both the control and the predator stress treatments were raised in four independently cycling tank systems. All of the environmental conditions (e.g., temperature, oxygen concentration and photoperiod) in the cycling tank system were controlled using the same conditions as those during the acclimation. Each compartment was cycled with water saturated with oxygen pumped from the bottom of the tank. This water could flow through the bottom of the

compartment, which was composed of nontoxic mesh. However, the food pellets were too large to pass through the mesh, ensuring that each fish consumed its own food and that the exact quantity of food consumed by fish at each feeding could be calculated. Each compartment was covered with mesh to prevent fish from escaping. After 1 h of feeding, uneaten food pellets and feces in each compartment were collected using a siphon. The body mass (0.01 g, using a digital scale) and body length (0.1 cm, using a Vernier caliper) of fish were measured after the fish were slightly anesthetized with neutralized tricaine methanesulfonate (MS-222, 50 mg L⁻¹) at the beginning and end of the experiment. The following formulas were used to calculate the growth parameters:

$$\text{food intake (FI, g kg}^{-1}\text{d}^{-1}) = I / [(M_1 + M_2) / 2 / 1000] / T \quad (1)$$

$$\text{special growth rate (SGR, \%d}^{-1}) = (\ln M_2 - \ln M_1) / T \times 100 \quad (2)$$

$$\text{food efficiency (FE, \%)} = (M_2 - M_1) / I \times 100 \quad (3)$$

where M_1 and M_2 are the body mass (g) of individual fish at the beginning and end of the experiment, respectively; I (g) represents the total amount of food consumed by individual fish during the experiment; and T represents the experimental period of 21 days.

2.4. Measurement of SMR

This study used the oxygen consumption rate to represent the metabolic rate of the fish, which was determined by four continuous-flow respirometers at 25.0 ± 0.1 °C. The details for the structure of the continuous-flow respirometer were described in a previous study (Zeng et al., 2017). To eliminate the influence of digestion on the measurements of SMR, all fish were fasted for 36 h before the SMR determination, which allowed the fish to evacuate their digestive tract contents. Fish were placed into the respiratory chambers and allowed to acclimate overnight. The water oxygen level from the outlet of the experimental chamber (with fish) and blank chamber (without fish) was measured 17 times during the following day (once per hour from 08:00 to 24:00). The SMR was determined by the mean of the three lowest values measured during this period. Because visual communication may trigger individuals to show minor social activity that would affect the SMR measurements, two sides of each chamber were covered with black tape to minimize potential disturbance caused by seeing each other. Additionally, each respirometer was covered with an opaque board to decrease disturbances from the laboratory environment or from the experimenter to the fish. The dissolved oxygen concentration was measured to the nearest 0.01 mg L⁻¹ at the outlet of each chamber using an oxygen meter (HQ30d, Hach Company, Loveland, CO, USA). The water flow rate through each chamber was determined by measuring the time required to fill a 50-mL volumetric flask from the outlet of the chambers. The water flow rate through the chambers was controlled at 1.0 L h⁻¹ at the beginning of the experiment and then increased to 1.2 L h⁻¹ at the end of the experiment due to the substantial growth of the fish, which ensured that the oxygen concentrations remained above 80% saturation. The SMR ($\dot{M}O_2$, mg O₂ h⁻¹) of the fish was determined using the following formula:

$$\dot{M}O_2 = (C_{O_2\text{blank}} - C_{O_2\text{fish}}) \times v \quad (4)$$

where $C_{O_2\text{blank}}$ and $C_{O_2\text{fish}}$ are the oxygen concentrations (mg L⁻¹) in the outflow water of the blank chamber and the fish chamber, respectively, and v (L h⁻¹) is the flow rate of water through the chamber.

2.5. Statistical analysis

Since body mass can affect both metabolic and growth rates, the metabolic rates and body mass were both log₁₀-transformed prior to examining the relationship between the double-log-transformed body mass and metabolic rate. Mass-independent estimates of individual

metabolic rate, i.e., the residuals from the regression, were used in subsequent analyses. For tables and figures, SMR was standardized to a mean body mass of 4.24 g by calculating the residuals of the least squares regression using the following equation:

$$\text{Log}_{10}\text{SMR} = \text{Log}_{10}b + a\text{Log}_{10}M + \varepsilon \quad (5)$$

where letters a and b are constants; M is body mass (g); and ε is the residual variation. The residuals of each individual from this regression were added to the fitted value for an animal of 4.24 g (the mean body mass of all fish used in this study based on two measurements of metabolic rates, $n = 432$) to obtain the adjusted estimates of metabolic rate for each fish.

We then examined how the morphology and SMR of fish changed over the experimental period under the different predator stress treatments and food availability based on the General Linear Model (GLM). This model included predator stress and food availability as fixed categorical effects and morphology and SMR as the dependent variables. We used the same approach to test the effects of the predator stress treatment and food availability on the growth parameters (FI, SGR and FE) and changes in morphology and SMR (100 × (final value – initial value)/initial value) with the model, including the predator stress treatment and food availability as fixed categorical effects, and the growth parameters (FI, SGR and FE) and changes in morphology and SMR as the dependent variables. A Duncan's multiple range test or an independent Student's t -test was performed after the models reported significant differences among the treatments. We used partial correlation to examine the relationships among growth parameters (FI, SGR and FE) since both FI and FE could influence SGR. Finally, we used Pearson product-moment correlation to examine the relationship between SGR and changes in SMR under different predator stresses and food availability.

Since most fish grew substantially during the experiment and their spontaneous activity might be limited by the space of the compartments, some individuals jumped out of the compartments and died or consistently consumed less than one-third of their mean daily ration during the experiment; these fish were excluded from data analysis. The final total fish numbers were 216 for the two treatments. All correlation and linear model analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Effects were considered statistically significant when the P -value was < 0.05, and all data are presented as the means ± 1 S.E.

3. Results

3.1. Changes in morphology and SMR

Fish in the control and the predator stress treatment increased in body mass, body length and condition factor with increases in food availability (Table 1, Fig. 1A-C). In the control treatment, the changes in body mass and body length were larger with high food availability than those with intermediate and low food availability (Table 2, Fig. 1D-E). Likewise, in the predator stress treatment, the changes in body mass and body length were larger with high food availability than with intermediate and low food availability (Table 2, Fig. 1D-E). Both the control and the predator stress treatments exhibited a decrease in the condition factor with low food availability (Table 2, Fig. 1F). However, these two treatments increased the condition factor of fish with both the high and intermediate food availability during the experiment (Fig. 1F). The change in the condition factor was only found to be higher in the control treatment than in the predator stress treatment with high food availability (Fig. 1F).

Difference in initial individual SMR was found among three food availabilities in the control treatment due to the difference in body mass (Fig. 2A). The final values of individual SMR were higher in the control treatment than in the predator stress treatment at three food availabilities (Fig. 2A). After standardization, however, difference in the

Table 1
The effects of predator stress, food availability and measurement time on the morphology of juvenile crucian carp based on the general linear model.

Parameters	Predator stress	Food availability	Measurement time	Predator stress × food availability	Predator stress × measurement time	Food availability × measurement time
Body mass (g)	F = 22.892, P < .001	F = 35.239, P < .001	F = 289.495, P < .001	F = 1.870, P = .155	F = 7.303, P = .007	F = 37.671, P < .001
Body length (cm)	F = 37.000, P < .001	F = 13.806, P < .001	F = 250.419, P < .001	F = 0.378, P = .686	F = 5.459, P = .020	F = 12.639, P < .001
Condition factor (g cm ⁻³)	F = 19.278, P < .001	F = 40.111, P < .001	F = 24.211, P < .001	F = 5.724, P = .004	F = 1.996, P = .158	F = 36.978, P < .001
SMR (mg O ₂ h ⁻¹)	F = 13.966, P < .001	F = 51.799, P < .001	F = 35.898, P < .001	F = 5.840, P = .003	F = 43.893, P < .001	F = 21.451, P < .001
Standardized SMR (mg O ₂ h ⁻¹)	F = 0.218, P = .641	F = 17.167, P < .001	F = 19.282, P < .001	F = 2.840, P = .060	F = 31.587, P < .001	F = 2.147, P = .118

final value of SMR was still found between the control and predator treatment with the intermediate and high food availabilities, not with the low food availability (Fig. 2B). The changes in SMR (Δ SMR) were higher in the control treatment than in the predator stress treatment at the intermediate food availability (Table 2, Fig. 2C). Difference in Δ SMR was found among the three food availabilities in the predator stress treatment, not in the control treatment (Fig. 2C).

3.2. Growth performance

Both the fish in the control and the predator stress treatments increased their FI as food availability increased (Table 2, Fig. 3A). FI was smaller in the predator stress treatment than in the control treatment in all three food availabilities (Table 2, Fig. 3A). Similar to FI, the SGR increased with increasing food availability in both the control and the predator stress treatments, and was larger in the control treatment than in the predator stress treatment in all three food availabilities (Table 2, Fig. 3B). However, FE first increased from low food availability to intermediate food availability and then leveled off between the intermediate and high food availability (Table 2, Fig. 3C). No difference in FE was found between the control and the predator stress treatment irrespective of food availability (Table 2, Fig. 3C).

Except for low food availability in the control treatment, SGR positively correlated with FI and FE in both the control and predator stress treatment at three food availabilities (Table 3). Similarly, the FE was negatively related to FI in the control and predator stress treatment with three food availabilities with the exception of low food availability in the control treatment (Table 3).

3.3. Relationships between change in SMR and growth

No correlation was found between Δ SMR (presented in % or mg O₂ h⁻¹) and SGR in the control treatment under the three food availabilities (Fig. 4A, Fig. 4C). However, the Δ SMR (presented in % or mg O₂ h⁻¹) correlated positively with SGR in the predator stress treatment at the intermediate food availability, not with either low or high food availability (Fig. 4B, Fig. 4D).

4. Discussion

During the juvenile period, food availability plays a key role in the growth and life history of fish (Nicieza and Metcalfe, 1999; Saikkonen et al., 2011). When food availability increases, juvenile fish can obtain more food and thus have high growth performance, by which they can have competitive and ecological advantages in locomotion, position within a swimming school, predator avoidance and mate choice (Werner and Gillam, 1984; Nilsson and Brönmark, 2000). In the present study, we found body mass, body length and condition factor increased with increasing food availability in both the predator stress and control treatments, which has been documented in previous studies (Auer et al., 2015; Zeng et al., 2017; Zeng et al., 2018), suggesting that change in morphology is a function of food availability. The increase in body mass was much smaller in the predator stress treatment than in the control treatment with all three food availabilities, which was consistent with previous studies on the Trinidadian guppy *Poecilia reticulata* (Handelsman et al., 2013) and Chinese bream *Parabramis pekinensis* (Fu et al., 2017). Individual crucian carp in the predator stress treatment ate less food at the three food availabilities, resulting in a smaller increase in the body mass of these fish. Individuals could detect and perceive strong predator stress by visual and olfactory cues or chemical alarm signaling released by conspecifics and thus increased energy expenditure on vigilance, spontaneous activity and antipredation behavior (Cooke et al., 2003; Liu et al., 2016).

Consistent with previous study (Daudpota et al., 2016), we also found FI increased with the increase in food availability in both the predator stress and control treatment, indicating that food intake is

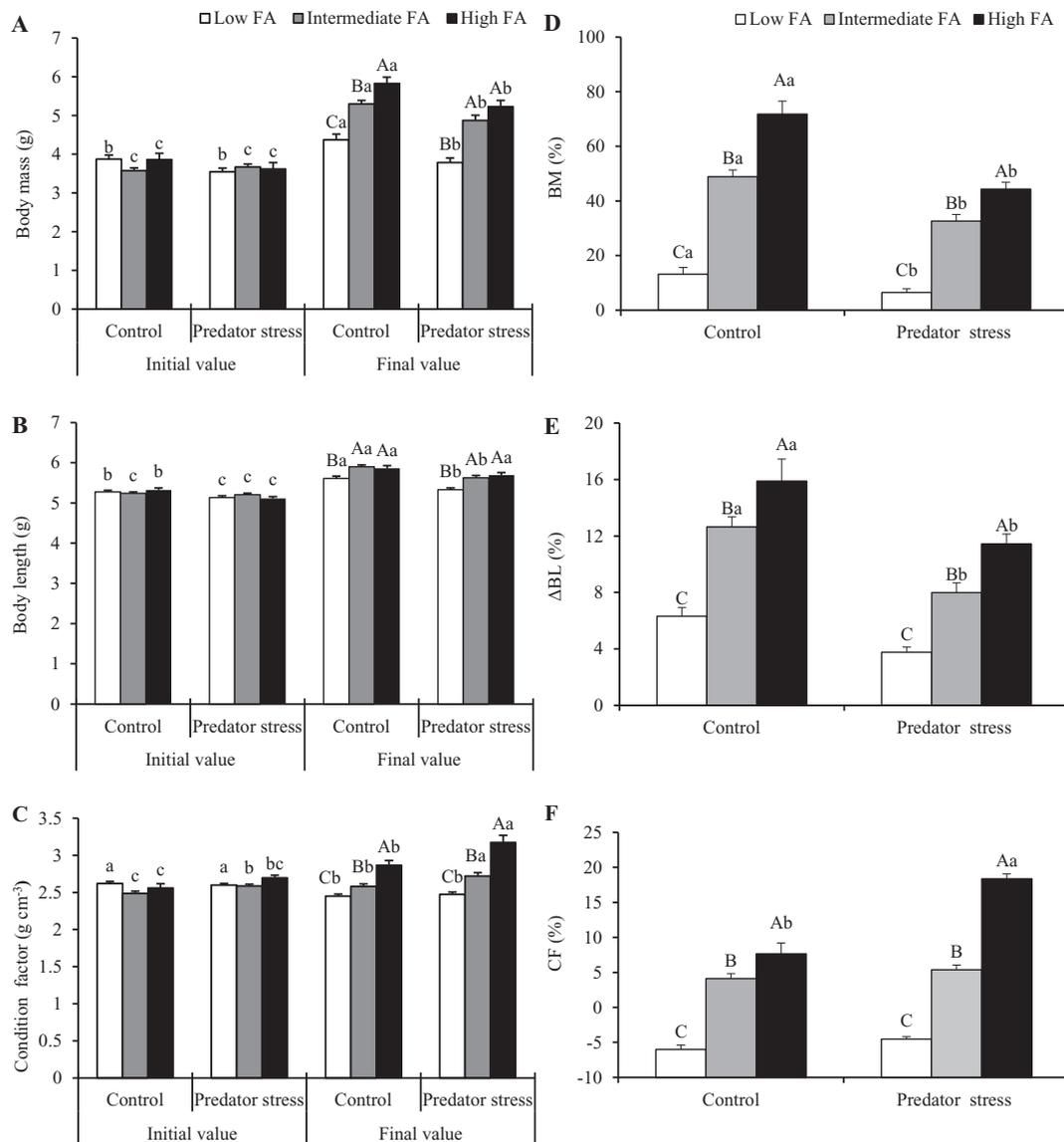


Fig. 1. The morphology and its changes in juvenile crucian carp under the conditions of different predator stress and food availabilities. Δ BM = change in body mass; Δ BL = change in body length; Δ CF = change in condition factor; FA = food availability. Note that bars that do not share common lowercase letters (a, b, c or d) are significantly different within a given food availability ($P < .05$), whereas bars that do not share common capital letters (A, B or C) are significantly different within a given predator stress treatment ($P < .05$).

close related to food availability irrespective of the predator threat effect. However, the changes in FI decreased with the increase of food availability. The increase proportions (47.5% in the predator stress treatment and 48.9% in the control treatment) of the FI between the low and intermediate food availability were higher than those (19.5% in the predator stress and 17.9% in the control treatment) between the intermediate and high food availability, suggesting that there was an

allometric relationship between the change in FI and food availability. As a crucial physiological process, feeding is associated with an increase in metabolic rate due to the energetic cost of the mechanical and biochemical digestion of food and the uptake of nutrients, the specific dynamic action (SDA) response (Secor, 2009). The SDA costs could superimpose and overlap in fishes that were fed frequently within a given feeding period (Fu et al., 2005). The SDA duration of the crucian

Table 2

The effects of food availability and predator stress on the morphology and growth performance of juvenile crucian carp based on the general linear model.

Parameters	Predator stress	Food availability	Predator stress \times food availability
Δ BM (%)	$F = 251.08, P < .001$	$F = 90.49, P < .001$	$F = 46.46, P < .001$
Δ BL (%)	$F = 234.49, P < .001$	$F = 103.99, P < .001$	$F = 47.09, P < .001$
Δ CF (%)	$F = 5.331, P = .022$	$F = 29.50, P < .001$	$F = 2.545, P = .081$
Δ SMR (%)	$F = 21.713, P < .001$	$F = 2.655, P = .073$	$F = 2.462, P = .088$
FI ($\text{g kg}^{-1}\text{d}^{-1}$)	$F = 233.806, P < .001$	$F = 289.797, P < .001$	$F = 9.240, P < .001$
SGR ($\% \text{d}^{-1}$)	$F = 46.110, P < .001$	$F = 161.801, P < .001$	$F = 3.645, P = .028$
FE (%)	$F = 0.371, P = .543$	$F = 43.200, P < .001$	$F = 0.980, P = .377$

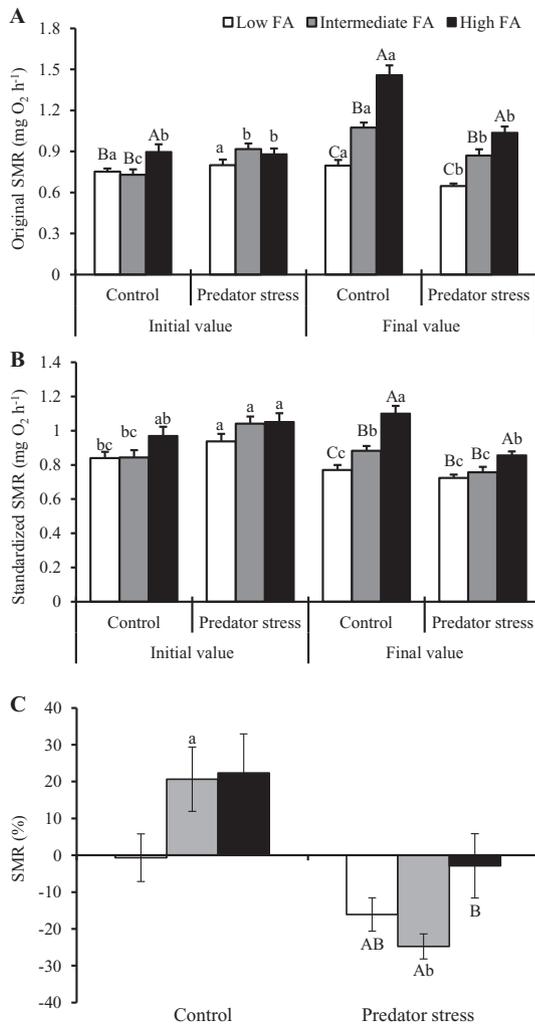


Fig. 2. Standard metabolic rate and its changes of juvenile crucian carp. Δ SMR = change in standard metabolic rate; FA = food availability. Note that bars that do not share common lowercase letters (a, b or c) are significant among different food availabilities within a given treatment ($P < .05$), whereas bars that do not share common capital letters (A, B or C) are significantly different within a given predator stress treatment ($P < .05$).

carp on satiation feeding was > 10 h (Fu et al., 2009b), but this duration was longer than the feeding interval (6 h) of the high food availability in the present study. This would contribute to the digestive tract remaining at a given state of fullness between two consecutive feeding. It is therefore possible that short interval feeding reduces the appetite of fish and hence the total amount of food intake at each satiation feeding event in the high food availability.

Our study found that the FI was lower in the predator stress treatment than in the control treatment at each of three food availabilities. In the present study, fish reared in the predator stress treatment were individually housed in independent compartments by which food consumption of individual fish at each feeding could be precisely determined. However, the predator, northern snakehead, could not directly hunt the crucian carp that were living within the compartments but ate 90% of the companion prey that were reared with the snakehead during the experiment. Hence, chemical alarm cues from the crucian carp, the companion prey, were released when they were attacked, captured or ingested. Like other fishes (Chivers and Smith, 1998; Wisenden, 2000), individual crucian carp reared within the compartments could be aware of predator danger by detecting these cues due to the effective transmission of chemical information within

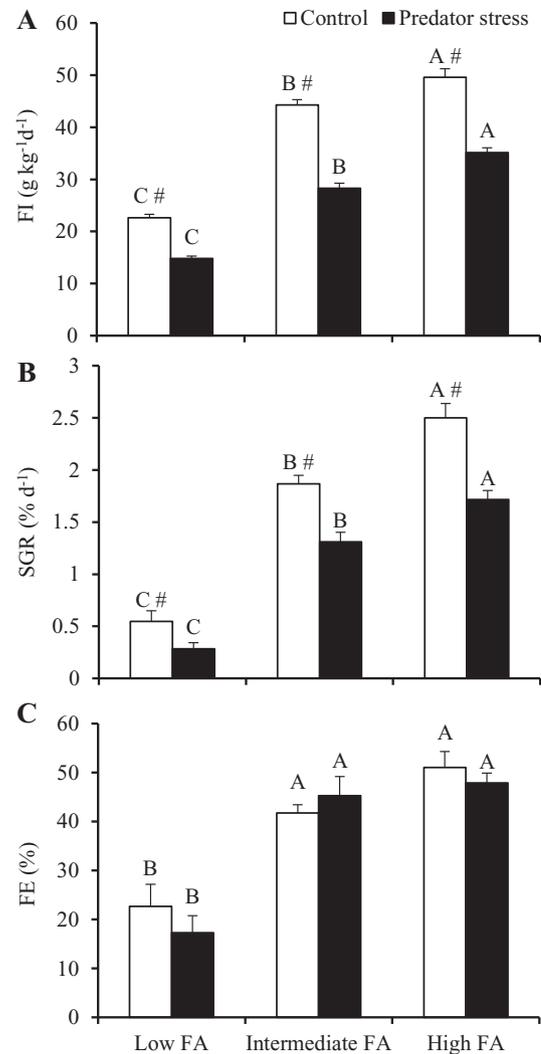


Fig. 3. Comparisons of the growth parameters of juvenile crucian carp in two predator stress treatments. SGR = specific growth rate; FE = feeding efficiency; FI = feeding intake. Note that bars that do not share common lowercase letters (a, b or c) are significantly different among different food availabilities with a given treatment ($P < .05$). The pound sign (#) indicates that a significant difference exists between the control and the predator stress treatments within food availability ($P < .05$).

the independent-cycling tank systems. It is possible, therefore, that fish under predator threat may increase the utilization of various shelters or refuges and reduce their routine foraging activity and hence their food intake (Tigeros et al., 2018).

The present study found that predator stress did not decrease the FE of the fish in any of the three food availabilities when compared to those in the control treatment, suggesting that individuals could retain the approximate function of their digestive tracts even though these fish were reared with “nonlethal” predator threat and lower food intake. Additionally, food availability influenced the FE of fish, as the FE (average of 20.0%) of fish reared with low food availability was smaller than that of fish reared with both intermediate (average in 43.5%) and high food availabilities (average of 49.5%), which was similar to the study on fingerlings *Cirrhinus mrigala* (Garg and Kalla, 2017). However, no difference in FE could be found between the intermediate and high food availabilities. A decrease in FE at low food availability may be related to the fact that food eaten by fish could not maintain the balance between energy intake and expenditure in low food availability conditions. The proportion of the daily energy expenditure accounted for by the maintenance metabolism of fish may be approximate

Table 3
Correlations between SGR, FE and FI in juvenile crucian carp under three food availabilities.

Food availability		Control		Predator stress	
		FI	FE	FI	FE
SGR	Low	$r = -0.024, P = .887$	$r = 0.984, P < .001$	$r = 0.704, P < .001$	$r = 0.985, P < .001$
	Intermediate	$r = 0.954, P < .001$	$r = 0.990, P < .001$	$r = 0.811, P < .001$	$r = 0.939, P < .001$
	High	$r = 0.829, P < .001$	$r = 0.972, P < .001$	$r = 0.969, P < .001$	$r = 0.988, P < .001$
FI	Low		$r = 0.072, P = .665$		$r = -0.641, P < .001$
	Intermediate		$r = -0.951, P < .001$		$r = -0.738, P < .001$
	High		$r = -0.872, P < .001$		$r = -0.953, P < .001$

irrespective of food availability because the fish were reared under the same temperature conditions. Therefore, the proportion of energy allocated to growth in low food availability was lower than that in high food availability.

Animals living under the risk of predation often handle the balance between foraging to fulfill energy requirements and avoiding predators (Ball and Baker, 1996; Handelsman et al., 2013). Prey species must respond to different levels of predation risk by adaptive strategies to regulate their daily energy expenditures. For example, routine metabolic rate increased when fish were reared directly with a predator (lethal stress) (Liu et al., 2016; Fu et al., 2017) or was maintained when fish were reared indirectly with a predator (nonlethal stress) (Liu et al., 2016), or even decreased when fish were reared directly with a predator (lethal stress) (Ball and Baker, 1996; Beckerman et al., 2007; Handelsman et al., 2013). Similar to these flexible patterns of metabolic rate, we found that the final adjusted SMR increased by average 14.1% compared to their initial values without predator stress. Although the final adjusted SMR of fish reared with a predator decreased in the three food availabilities, the decreased proportion of the SMR were only lower in the predator threat treatment than in those without predator at

intermediate food availability, indicating that predator stress decreased the self-maintenance cost in juvenile crucian carp. It is possible that fish living under the risk of predation reduced their daily foraging activity, as indicated by a decrease in their food intake, and hence had a smaller body mass, which could affect their maintenance metabolism compared to fish living without a predator. To decrease the frequency encountered by predators, prey species decrease their spontaneous activity (Lima, 1998; Fu et al., 2015) or risk-taking behavior (Herczeg and Válimäki, 2011), which might potentially decrease the maintenance metabolism of prey species.

Flexibility in SMR may be crucial since SMR reflects the energetic cost of self-maintenance and closely correlates with other organismal performance. The ecological consequences of flexibility in SMR for growth (e.g., SGR) have been documented in environmental changes in food availability and temperature, which showed that metabolic flexibility can maximize individual growth performance under changing food availability (Auer et al., 2015). The degree of change in the SMR was approximate at different food availabilities, but links between the shifts in the SMR and its consequence varied greatly with food availability (Auer et al., 2015; Zeng et al., 2017). Individuals who increased

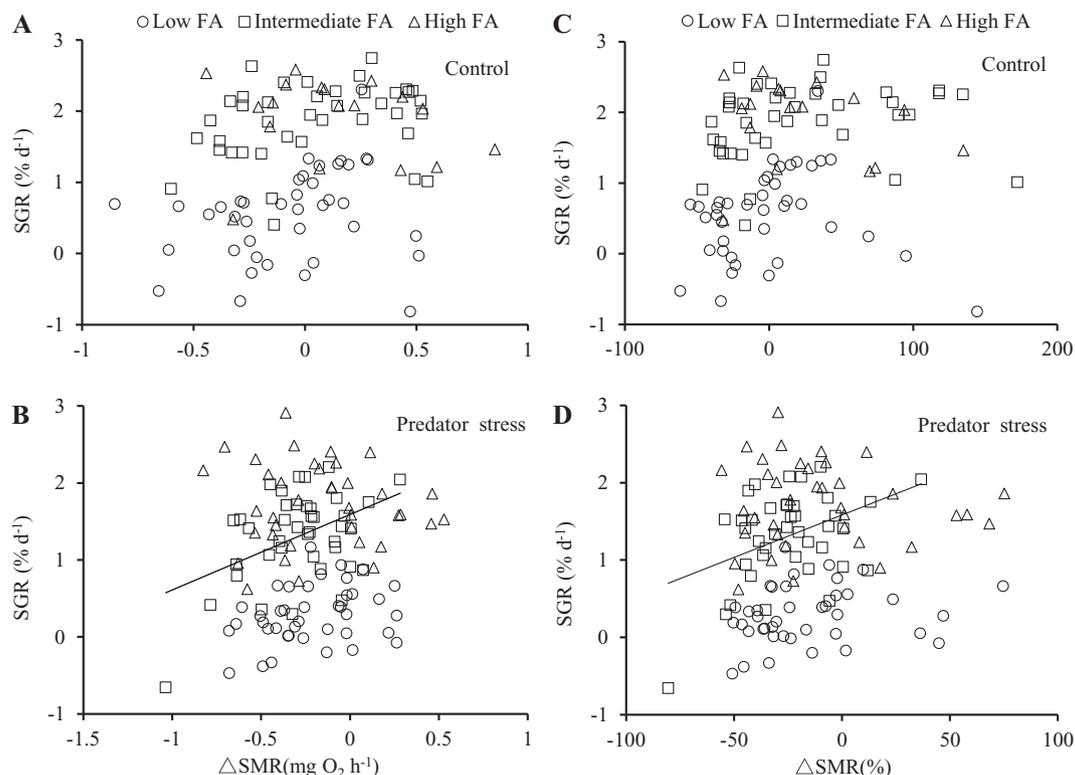


Fig. 4. Correlations between changes in SMR (Δ SMR) and specific growth rate (SGR) of juvenile crucian carp under different predator stress and food availabilities. Solid line = intermediate food availability. The positive correlations existed between SGR and Δ SMR in the predator stress treatment at intermediate food availability (Fig. 4B, $r = 0.413, P = .008$; Fig. 4D, $r = 0.442, P = .004$). Plotted in figures A–B are residuals of growth rate evaluated at mean rSMR = 0 as a function of back-transformed values for the change in SMR standardized for the mean body mass of 4.24 g.

their SMR more in response to high food availability but not to intermediate or low food availability, had higher growth rates (Auer et al., 2015; Zeng et al., 2017), indicating that single 'optimal' living conditions could favor individuals with a high degree of metabolic flexibility to have a selective advantage in individual fitness. However, animals are often undergoing interactions with variable environmental factors. In the present study, we found that the SGR of the crucian carp in the two treatments was positively related to changes in the SMR when intermediate food availability was present but not when low or high food availability was present, suggesting that predator stress does not alter the relationship between flexibility in SMR and growth performance under changing food availability.

Fish can address environmental changes by integrating adaptive strategies. They can adjust their morphology, physiology and behavior to cope with predator stress. As we showed here, juvenile crucian carp living with predators reduce their food intake, which leads to low growth performance. However, predator stress does not affect food efficiency in any food availability condition. Given that SMR is a crucial physiological trait and has an important influence on individual fitness, flexibility in SMR as a fundamental ability could endow a growth advantage to individuals living in variable environments (Auer et al., 2015; Zeng et al., 2017). Our study found that predator stress decreased SMR of juvenile crucian carp under changing food availability, but not altered the links between flexibility in SMR and its ecological consequences (e.g., growth). More attention should be paid to the interaction effects of flexibility in the SMR and growth performance under the selection of different environmental factors, such as temperature, hypoxia and ocean acidification. This would not only be beneficial for understanding the mechanisms underlying these interactions and effects but also for predicting what the ecological consequences are for the fitness and dynamics of populations in the face of a rapidly changing environment.

Conflict of interest

The authors declared that they have no conflicts of interest to this work.

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