

Research



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Is repeatability of metabolic rate influenced by social separation? A test with a teleost fish

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Metabolic rates are typically thought to have important influences on fitness and more broadly be relevant to the ecology and evolution of animals. Previous studies demonstrate that metabolic rates are repeatable to a certain extent under constant conditions, but how social conditions influence the repeatability of metabolic rate remains largely unknown. In this study, we investigated the repeatability of resting metabolic rate (RMR) in the highly social crucian carp (*Carassius auratus*) after being socially separated for different time periods relative to control fish that were not socially separated. We found that RMR was repeatable in fish in the control group, while the repeatability of RMR disappeared quickly (even within 7 days) when fish were exposed to social separation. This study is the first to our knowledge to examine the role of social separation for different time periods on the repeatability of intra-individual physiological variation in fish. We highlight that the inter-individual repeatability of metabolic rate can be substantial over time but was eliminated by social separation. The findings indicate that the repeatability of metabolic rate in fish is condition dependent, and that the change in repeatability of metabolic rate should not be overlooked when considering the ecological and evolutionary effects of environmental change.

1. Introduction

Metabolic rate is an integrative measure of the energetic cost of living and is one of the most widely measured key physiological traits [1,2]. Recently, individual differences in the energy cost of self-maintenance (i.e. resting metabolic rate, RMR, which is closely related to basal and standard metabolic rate, SMR) have become the focus of an emerging research area at the interface of ecology, evolution and physiology [3–6]. Intraspecific RMRs can vary greatly, by as much as threefold among individuals within a population, even after correcting for factors such as body mass, age, sex and reproductive state [3,4,6]. Variation in RMR has been linked to several components of fitness and can play an important role in the evolutionary trajectories of species since RMRs usually constitute up to 50% of an individual's energy expenditure [7] and are positively correlated with a range of fitness-related physiological functions and behavioural performance [1,8]. However, from both an ecological and evolutionary perspective, variation in RMR must be consistent (i.e. repeatable) over at least some portion of the lifetime in order to predict its longer-term effects on population dynamics and how it will respond to selection [6]. Interestingly, although metabolic rates may appear to be (and are often treated as) consistent and repeatable traits of an individual [6], the repeatability of metabolic rates may decline over long time periods and is typically lower for animals living under field versus laboratory conditions [9–12].

Table 1. The effects of social separation on body morphological parameters, RMR and specific growth rate (SGR).

		day post-social separation			
		0	7	14	28
body length (cm)	control	6.67 ± 0.044	6.71 ± 0.044	6.83 ± 0.046	7.00 ± 0.048
	separation	6.64 ± 0.043	6.74 ± 0.042	6.86 ± 0.041	6.97 ± 0.043
body mass (g)	control	8.61 ± 0.172	8.82 ± 0.178	9.25 ± 0.193	9.70 ± 0.218
	separation	8.40 ± 0.160	8.85 ± 0.146	9.24 ± 0.143	9.68 ± 0.161
condition factor (100 g cm ⁻³)	control	2.89 ± 0.025	2.89 ± 0.028	2.88 ± 0.024	2.80 ± 0.025
	separation	2.87 ± 0.037	2.89 ± 0.031	2.85 ± 0.031	2.86 ± 0.028
RMR (mg O ₂ h ⁻¹)	control	2.77 ± 0.059	2.73 ± 0.051	2.74 ± 0.042	2.67 ± 0.047
	separation	2.87 ± 0.059	2.76 ± 0.059	2.73 ± 0.068	2.41 ± 0.045 ^a
SGR (% d ⁻¹)	control	—	0.329 ± 0.047	0.506 ± 0.038	0.414 ± 0.034
	separation	—	0.789 ± 0.073 ^a	0.701 ± 0.065 ^a	0.515 ± 0.040 ^a

^aIndicates a significant difference between the social separation group and the control group at a given time period ($p < 0.05$).

Table 2. Correlation analyses on RMR of *Carassius auratus* before and after being/not being socially separated for different time periods. * indicates a significant correlation ($p < 0.05$).

	day	7	14	28
control	0	$r = 0.368$; $p = 0.006^*$	$r = 0.291$; $p = 0.031^*$	$r = 0.404$; $p = 0.002^*$
	7		$r = 0.363$; $p = 0.006^*$	$r = 0.471$; $p < 0.001^*$
	14			$r = 0.428$; $p = 0.001^*$
separation	0	$r = 0.205$; $p = 0.133$	$r = 0.263$; $p = 0.052$	$r = 0.172$; $p = 0.209$
	7		$r = 0.389$; $p = 0.003^*$	$r = 0.061$; $p = 0.660$
	14			$r = -0.003$; $p = 0.982$

The social environment is one of the major modulating factors of individual physiology and behaviour [13] despite rarely being considered in metabolic studies. The exception is research on dominant–subordinate hierarchies in salmonids which have been well studied (e.g. *Oncorhynchus mykiss* and *Salmo trutta*), where dominant fish typically experience down-regulated metabolic rate while subordinate fish experience up-regulated metabolic rate [14,15]. Although the majority of fish are group-living animals, the members within a group are often not fixed, and diverse factors (e.g. courtship, foraging, environmental changes in habitats, etc.) may cause individuals in a group to be separated or rejoin other groups [13,16,17]. To date, whether social context outside of fish species that establish dominant–subordinate relationships influences repeatability of RMR remains largely unknown. From an ecological perspective, we hypothesized that the repeatability of RMR was condition dependent for a social fish. We predicted that the repeatability of metabolic rate in social fish would be reduced or even disappear when exposed to social separation.

2. Material and methods

We used crucian carp (*Carassius auratus*), a freshwater teleost fish, widely distributed in the rivers, lakes and other water bodies in China, as the experimental fish in this study. The crucian carp is a highly social fish [18] that typically exhibits remarkable individual

variation in metabolic rate, maintains loose groups or shoals, and forms significant dominance hierarchies [18,19]. Juvenile crucian carp (5.06–7.84 g, $N = 110$) were obtained from a local hatchery (Hechuan, Chongqing, China). The fish were temporarily housed in a 1000 l recirculating water tank system for one week and were then randomly selected and divided into two identical recirculating tanks (500 l), with 55 fish in each tank ($n = 55$). Fish were marked for identification using passive integrated transponder (PIT) tags (0.03 g, HT-157, Hongteng Corporation, Guangdong, China) three weeks prior to the start of the experiments. No fish became infected or died as a result of tagging during the experiment. The water temperature was maintained at $26 \pm 0.5^\circ\text{C}$; the photoperiod was 14 L : 10 D, and oxygen tension was maintained above 85%. Fish were fed to satiation once daily with Cyprinid fish diet (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) (Tongwei, Sichuan, China), which was used for the entire experiment.

Individual fish were transferred to continuous flow respirometer chambers for the first RMR (RMR₀) determination after being fasted for 24 h [20]. The total volume of the respirometer chamber was 100 ml. Throughout the acclimation period and during all measurements, two sides of each chamber were covered by a darkened blind to minimize disturbance to the fish. The water temperature was $26 \pm 0.1^\circ\text{C}$. Fish were allowed to habituate for at least 15 h. After that the RMR, in terms of the oxygen consumption rate, (M_{O_2}) (mg O₂ h⁻¹) of individual fish was measured using a respirometer. The following formula was used to calculate RMR (mg O₂ h⁻¹):

$$\text{RMR} = \Delta\text{O}_2 \times v,$$

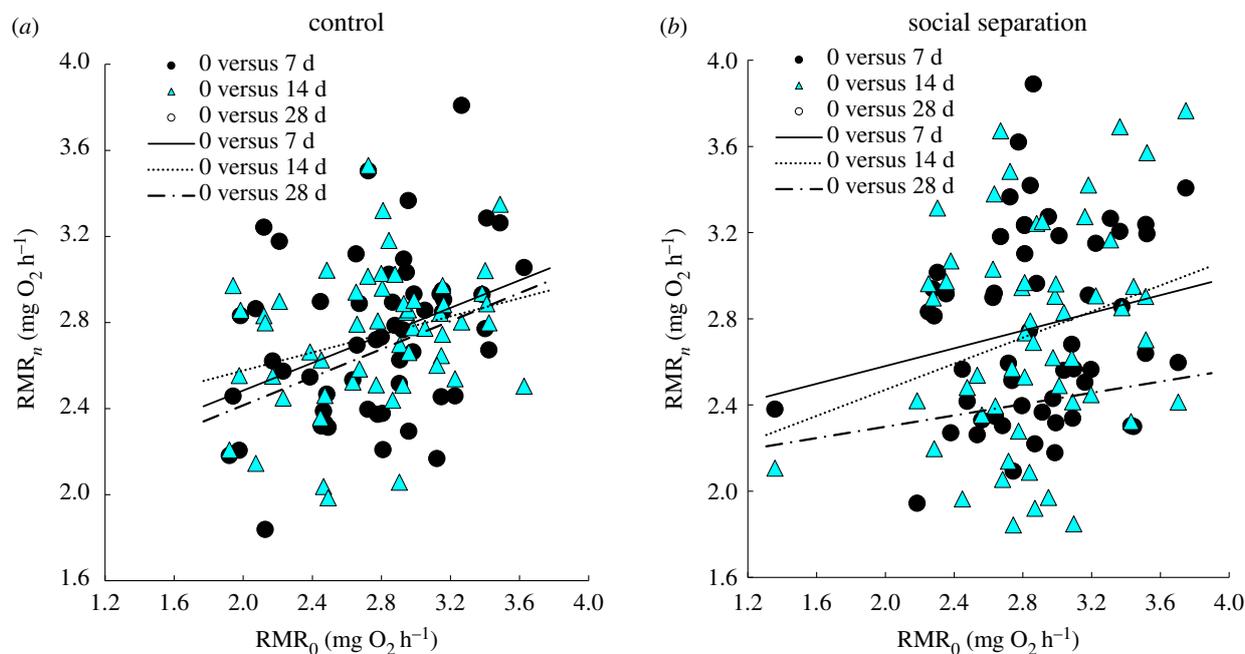


Figure 1. Relationships between RMR in *Carassius auratus* before and after being/not being socially separated for different time periods. RMR, resting metabolic rate. RMR_0 and RMR_n are the metabolic rate of fish before and after being/not being socially separated for different time periods (n days), respectively. (Online version in colour.)

where ΔO_2 is the difference in oxygen concentration (mg l^{-1}) in the outflow water between the blank chamber (an empty chamber run in parallel with the chamber where the fish is) and fish chamber; v is the water flow rate through the fish chamber (l h^{-1}). The dissolved oxygen concentration was measured at the outlet of the chamber by an oximeter (HQ40d, Hach Company, Loveland, CO, USA). The flow rate of water through each respirometer was determined by measuring the time taken for 100 ml of water to flow from the respirometer outlet ($5.15 \pm 0.02 \text{ l h}^{-1}$).

For each fish, the RMR was determined at six time points (9.00, 11.00, 13.00, 15.00, 17.00 and 19.00) and repeated three times at each time point. Variability across time points was low (intraclass correlation coefficient (ICC) = 0.881, $p < 0.001$), and the six measurements were averaged to provide a value for RMR. After the measurement of RMR_0 , fish were put back into their original rearing tanks, where fish in the control group were held with conspecifics while those that were separated socially were held alone in compartments measuring 17 cm length \times 10 cm width \times 60 cm height (40 cm water depth) (the body length of the fish was approximately 7 cm, table 1), under cycling tank systems. The RMRs of the fish from each treatment were re-assayed on the 7th (RMR_{7d}), 14th (RMR_{14d}) and 28th (RMR_{28d}) day.

Statistical analyses were performed using the software program SPSS (v. 19.0). All values are presented as the means \pm s.e. and $p < 0.05$ was considered statistically significant. Because metabolic rates can be affected by body mass (M), the metabolic rates and M were both \log_{10} -transformed prior to examining the relationship between double-log-transformed metabolic rates and M . M -independent estimates of individual metabolic rate (i.e. the residuals from the regression) were used in subsequent analyses. The RMRs were then standardized to a mean body mass of 9.068 g, by calculating the residuals of the least squares regression using the following equation [20]:

$$\log_{10} \text{RMR} = a \log_{10} M + b + \varepsilon,$$

where a and b are constants and ε is the residual variation. The residuals of individuals from this regression were added to the fitted value for an animal of 9.068 g to obtain adjusted estimates of RMR.

The growth performance was estimated in terms of specific growth rate (SGR, $\% \text{ d}^{-1}$), which was calculated according to

the equation:

$$\text{SGR} = \frac{\ln M_x - \ln M_0}{d} \times 100,$$

where M_0 and M_x are the body masses (g) of a given fish at the beginning and end of a specific experimental period and were measured during RMR measurements; d is the time elapsed (in days).

The effects of social separation on body morphological parameters (i.e. body length, body mass and condition factor), RMR and SGR were determined using a t -test when data were parametric or Mann-Whitney U -test when the data were non-parametric. Pearson's correlation was used to analyse the repeatability of individual RMR measurements before and after treatment for different time periods [21].

3. Results

Social separation had no significant effect on body size parameters of the experimental fish ($p > 0.05$), but led to an increase in SGR ($p < 0.05$) (e.g. SGR increased by 139.8, 38.6 and 24.3%, respectively, after 7, 14 and 28 days of social separation compared with the control group). No difference in RMR was observed except at 28 days, when RMR decreased by 9.47% after 28 days of social separation compared with the control group ($p < 0.05$) (table 1). The repeatability of RMR in fish was condition dependent. In the control group, RMRs were significantly correlated across all time periods ($p < 0.05$). However, in the social separation group, RMRs were not correlated for all time periods ($p > 0.05$) except between day 7 and day 14 (table 2 and figure 1).

4. Discussion

What causes individual variation in RMRs? Although several intrinsic causes of individual variation in RMRs have been thoroughly investigated, the role of social conditions in shaping the variation of metabolic phenotype is poorly understood. Social interactions in schooling fish lead to the

formation of dominance hierarchies [13]. The behavioural characteristics associated with social status may be due in part to divergent changes in RMRs [17], which, interestingly, is also likely to be affected by the social conditions [14,15,22]. Grobler & Wood [14] demonstrated that dominant *O. mykiss* had higher growth rate and lower routine metabolic rate compared with subordinate individuals after hierarchy formation. Similarly, Sloman *et al.* [15] suggested that the social stress experienced by subordinate *S. trutta* when confined with a dominant increases the SMR of the subordinate fish, and therefore imposes a metabolic disadvantage. In this study, we demonstrated that the RMR of individuals within a population after long-term (28 days) social separation significantly decreased. Moreover, social separation led to an increase in SGR, suggesting that desocialization leads to savings in individual energy expenditure (e.g. maintenance of social hierarchy, stress in response to aggression, etc.). To our knowledge, this study is the first to examine the role of social separation for different time periods on the intra-individual physiological variation of fish. The findings provide insight on the ecological basis of individual variation in fish and also inform future research given that social conditions prior to testing are often not considered [13].

How stable is the individual variation of physiological traits in fish? This is a key issue in understanding physiological plasticity, as repeatability is a prerequisite for heritable variation, and exploring the sources of within-individual variation in plasticity can help to inform understanding of the proximate constraints and limitations to the heritability or evolvability of phenotypic plasticity [23,24]. Metabolic rate is heritable to a certain extent, meaning that a proportion of the phenotypic variance among individuals can be attributed to additive genetic effects [2,9,25]. Nespolo & Franco [26] suggested that the metabolic rate was a repeatable trait and that repeatability was not affected by time between measurements. Similarly, the RMR of spined loaches (*Cobitis taenia*) was highly repeatable and was consistent with heritable variation of RMRs [27]. However, some recent studies have reported that the repeatability of

metabolic rate declined over time (e.g. [10,12]). In this study, we confirmed that the repeatability of metabolic rate in crucian carp is condition dependent. Inter-individual repeatability of metabolic rate in crucian carp was substantial over time in the control group but was eliminated in the social separation group, where the repeatability of RMR disappeared quickly (even within 7 days of separation). We speculate that social separation influences an individual's energy budget and, therefore, results in alterations in RMR. Since each hierarchy consists of different fish, each hierarchy will inevitably create a different behavioural environment. Each fish will then presumably adjust its physiology and behaviour based on its unique social environment [14]. This study is the first to examine the role of social separation on the repeatability of intra-individual physiological variation in fish. The results indicated that metabolic phenotype was highly ecologically plastic. Moreover, our findings suggest the need to consider the functional associations between the RMR and other fitness-related phenotypic traits (e.g. growth, competition and survival) under different social contexts. The extent to which this phenomenon is observed in other vertebrates remains unknown and represents a fascinating topic for future research.

Ethics. Experimental procedures were conducted in accordance with the ethical requirements and recommendations for animal care of the Key Laboratory of Animal Biology of Chongqing, China (Permit no. Zhao-20171022-01).

Data accessibility. Data are available from the Dryad Digital Repository at <https://doi.org/10.5061/dryad.n2z34tmsr> [28].

Authors' contributions. J.X. designed the research with input from S.F. and S.J.C.; Y.H. collected the data; J.X. performed statistical analyses and wrote the original manuscript; all authors contributed to the revisions and gave final approval for publication. All authors agree to be held accountable for the content of the article.

Competing interests. We declare we have no competing interests.

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