

# The relationship between metabolic rate and sociability is altered by food deprivation

Shaun S. Killen<sup>\*1</sup>, Cheng Fu<sup>2</sup>, Qingyi Wu<sup>2</sup>, Yu-Xiang Wang<sup>2,3</sup> and Shi-Jian Fu<sup>\*2</sup>

<sup>1</sup>Institute of Biodiversity, Animal Health, and Comparative Medicine, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Glasgow, G12 8QQ UK; <sup>2</sup>Laboratory of Evolutionary Physiology and Behaviour, College of Life Sciences, Chongqing Normal University, Chongqing, 400047 China; and <sup>3</sup>Department of Biology, Queen's University, Kingston, Ontario, Canada, K7L 3N6

## Summary

1. Individuals vary in the extent to which they associate with conspecifics, but little is known about the energetic underpinnings of this variation in sociability. Group-living allows individuals to find food more consistently, but within groups, there can be competition for food items. Individuals with an increased metabolic rate could display decreased sociability to reduce competition. Long-term food deprivation (FD) may alter any links between sociability and metabolic rate by affecting motivation to find food.

2. We examined these issues in juvenile qingbo carp *Spinibarbus sinensis*, to understand how FD and metabolic rate affect sociability. Like many aquatic ectotherms, this species experiences seasonal bouts of FD.

3. Individuals were either: (i) food-deprived for 21 days; or (ii) fed a maintenance ration (control). Fish from each treatment were measured for standard metabolic rate (SMR) and tested for sociability twice: once in the presence of a control stimulus shoal and once with a food-deprived stimulus shoal.

4. Control individuals ventured further from stimulus shoals over a 30-min trial, while food-deprived fish did not change their distance from stimulus shoals as trials progressed. Control fish with a higher SMR were least sociable. Well-fed controls showed decreased sociability when exposed to food-deprived stimulus shoals, but there was evidence of consistency in relative sociability between exposures to different shoal types.

5. Results contrast with previous findings that several days of fasting causes individuals to decrease associations with conspecifics. Prolonged FD may cause individuals to highly prioritize food acquisition, and the decreased vigilance that would accompany continuous foraging may heighten the need for the antipredator benefits of shoaling. Conversely, decreased sociability in well-fed fish with a high SMR probably minimizes intraspecific competition, allowing them to satisfy an increased energetic demand while foraging. Together, these results suggest that FD – a challenge common for many ectothermic species – can affect individual sociability as well as the attractiveness of groups towards conspecifics. In addition, the lack of a link between SMR and sociability in food-deprived fish suggests that, in situations where group membership is linked to fitness, the extent of correlated selection on metabolic traits may be context-dependent.

**Key-words:** ecophysiology, energetics, foraging, metabolism, social behaviour, teleost fish

## Introduction

Group-living reduces the risk of predation for group members and provides a number of foraging advantages (Krause & Ruxton 2002). Animals foraging in groups, for example, find food patches more consistently than those

foraging alone and experience less variable times between periods of food intake (Ekman & Hake 1988; Ruxton, Hall & Gurney 1995). A trade-off is that there can be competition among group members for discovered food items, reducing food intake for some individuals and potentially promoting intraspecific aggression (Webster & Hart 2006). Individual animals must weigh the balance of these trade-offs, and there is consistent interindividual variation in

\*Correspondence authors. E-mails: shaun.killen@glasgow.ac.uk; shijianfu9@hotmail.com

sociability (Mills & Faure 2000; Cote, Fogarty & Sih 2012; Webster & Laland 2015), defined as the extent to which an animal seeks to associate with conspecifics for non-aggressive interactions (Réale *et al.* 2007; Careau & Garland 2012).

The mechanistic and energetic basis for variation in sociability remains poorly understood. Metabolic traits, such as metabolic rate or maximum aerobic capacity, show wide intraspecific variation and are often associated with various aspects of individual behaviour (Biro & Stamps 2010; Burton *et al.* 2011; Killen *et al.* 2013). For example, the minimum metabolic rate needed to sustain life (i.e. standard metabolic rate (SMR) in ectotherms; basal metabolic rate in endotherms), is often linked to risk-taking behaviours and dominance. To date, however, no study has examined covariation in metabolic demand and sociability among individuals (Careau & Garland 2012). Increased hunger after short periods of fasting has been shown to decrease sociability by altering the trade-offs associated with foraging and intragroup competition (Krause 1993a), and in fish, hungry individuals form less cohesive shoals (Morgan 1988). The decrease in sociability with hunger likely decreases competition with groupmates in the event that food becomes available. It is plausible that interindividual variation in baseline metabolic demand may have a similar effect, with individuals with a relatively high metabolic rate being less sociable because they are more motivated to acquire food.

Although previous studies have examined the effect of recent feeding and hunger on sociability and the behaviour of animal groups (Krause 1993a; Reeb & Saulnier 1997; Krause, Hartmann & Pritchard 1999; Frommen, Luz & Bakker 2007), no study has examined the effects of more prolonged food deprivation (FD) on sociability. Many wild ectotherms experience FD for several weeks at a time (Martinez *et al.* 2002, 2004; Gingerich, Philipp & Suski 2010; Killen, Marras & McKenzie 2014). Many fishes, for example, undergo extended periods of FD while overwintering and also experiencing sporadic decreases in prey availability as a result of eutrophication during the summer (Marchini, Gauzer & Occhipinti-Ambrogi 2004). The effects of longer-term FD on sociability could differ drastically from the effects of short-term reductions in feeding. For example, longer-term FD can decrease swimming ability in fish (Martinez *et al.* 2002; Zhao *et al.* 2012; Killen, Marras & McKenzie 2014), potentially decreasing the ability to escape predation and increasing the need for the relative safety provided by group membership. Therefore, although hunger can decrease sociability, it is possible that there is a threshold of FD beyond which sociability may actually increase. Any link between sociability and metabolic rate could also be exacerbated by FD, if individuals with a higher metabolic rate lose biomass more quickly during fasting, therefore amplifying their existing motivation to find and secure food (Killen *et al.* 2013).

The nutritional status or competitive ability of a group may also influence whether individual fish decide whether

or not to join that group (Metcalf & Thomson 1995). Individuals are less likely to join groups comprised of hungry individuals (Krause 1993a; Krause, Hartmann & Pritchard 1999), presumably because those animals would be more voracious competitors for discovered food items. However, the effects of long-term FD in potential groupmates remain unknown. Again, FD could actually decrease competitive ability if locomotory ability is compromised (Killen, Marras & McKenzie 2014), therefore making an individual more likely to join a group of food-deprived individuals. It is also possible that either the competitive or nutritional status of groupmates will interact with an individual's own metabolic demand and nutritional history to influence sociability in relation to that particular group.

We studied these issues in juvenile qingbo carp *Spinibarbus sinensis*. This is a highly gregarious cyprinid species and one of the most common fish species in the Yangtze River system. It is a relatively active, omnivorous species that prefers to occupy clear, flowing waters while living in shoals. Studies of gut contents have shown that this species experiences large seasonal fluctuations in food availability and intake that can include nearly complete FD for 2–4 weeks at a time (Ding 1994). As occurs in many other fishes, this reduction in food intake can reduce locomotor capacity in this species (Zhao *et al.* 2012; Pang *et al.* 2014) but the effects on behaviours such as sociability are unknown. We tested three main questions: (i) Among individuals, is there a link between metabolic rate and sociability, measured as the tendency to associate with a stimulus shoal? (ii) Does FD alter sociability or the relationship between sociability and metabolic rate? and (iii) How do the effects of individual metabolic rate and FD interact with the feeding history of groupmates to affect sociability?

## Materials and methods

### EXPERIMENTAL ANIMALS

Approximately 300 juvenile qingbo carp *Spinibarbus sinensis* were obtained from a local supplier and acclimated to the laboratory for 3 weeks before beginning experiments ( $8.9 \pm 0.15$  cm TL;  $7.3 \pm 0.31$  g body mass). The dechlorinated fresh water was constantly aerated and temperature maintained at  $25.0 \pm 0.5$  °C. During holding before experiments, fish were fed to satiation with a commercial diet once daily. The project was approved by the National Natural Science Foundation of China (No. 31172096) and Chongqing Normal University. Experimental procedures were non-invasive, and every effort was made to minimize suffering. The period of FD, though prolonged, is common feature of the natural life-history of *S. sinensis* and many other fish species (Ding 1994; Wang, Hung & Randall 2006; Killen, Marras & McKenzie 2014).

### DIET TREATMENTS

Fish were randomly designated to one of two treatments: (i) the FD treatment; and (ii) the control treatment. Food-deprived fish were not fed for the duration of the experiment while the control fish were fed a maintenance ration throughout (1.2% body mass

per day). After 21 days of FD in the food-deprived treatment, 18 fish from each treatment were randomly removed from their common holding tank, measured for total length and wet mass and tagged for identification using elastomer. The remaining untagged fish were held separately and later used to populate the shoals for the sociability behavioural trials. Trials were conducted over an 8-day period with final measurements of standard length, and body mass taken after all sociability trials were completed (with a total of 15 days between measurements used for estimates of growth rate). Mortality was low among fish exposed to both treatments (three fish out of approximately 300 fish in total).

#### MEASUREMENT OF SMR

The 18 tagged fish from each treatment were measured for SMR at 25 °C. The SMR of individual fish was determined using continuous-flow respirometry (Fu *et al.* 2011; Auer *et al.* 2015). Fish in the control group were fasted for 24 h prior to being placed into individual acrylic respirometers (300 mL volume), and fish from both groups were left in the respirometer chambers to acclimatize overnight. Rates of oxygen uptake were measured 10 times during the following day (approximately once per hour between 08:00 and 18:00). The mean of the lowest three measurements throughout this period was taken as SMR. Throughout the acclimation period and during all measurements, respirometers were covered by a darkened blind to minimize disturbance to the fish. Dissolved oxygen concentration was measured at the outlet of each respirometer using an oxygen meter (HQ20; 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 (approximately 60 mL min<sup>-1</sup>). The following formula was used to calculate the oxygen uptake (MO<sub>2</sub>; mg O<sub>2</sub> h<sup>-1</sup>) of individual fish:

$$MO_2 = \Delta O_2 \times v \quad \text{eqn 1}$$

where  $\Delta O_2$  is the difference (mg O<sub>2</sub> L<sup>-1</sup>) in oxygen concentration between an experimental respirometer and the control respirometer without fish, and  $v$  is the water flow rate in the experimental chamber (L h<sup>-1</sup>).

#### SOCIABILITY TRIALS

The sociability of individual fish was tested using a binary choice test (Krause, Hartmann & Pritchard 1999; Jones *et al.* 2010), in which the focal individual could choose to spend time near an arena section containing a stimulus shoal, or range closer to another arena section which was empty (Video S1, Supporting information). The experimental tank consisted of a rectangular white PVC tank (85 cm L × 30 W × 30 H), filled with water to a depth of 8 cm. The tank was divided into three sections: a larger central chamber (40 cm × 30 cm), and two smaller chambers at each end (25 cm × 30 cm). These sections were divided by transparent partition such that fish within the arena could easily see the contents of all other chambers. Water did not flow between tank sections and so visual cues were assessed by focal individuals in trials.

To begin a test, a group of eight fish were randomly selected from the pool of untagged fish. The group was randomly determined to contain either food-deprived or control individuals (control individuals had been fasted for at least 24 h prior to shoaling trials). The group of fish was then transferred carefully and without air exposure into a randomly selected end section of the experimental arena. The other end section was left empty. One of the tagged individuals for which SMR had previously been assessed then was introduced to the central chamber. The entire tank was then filmed from above for the following 30 min at 25 frames s<sup>-1</sup>

(Sony Handycam, HDR XR260, Tokyo, Japan). All tagged fish were tested using this protocol once over the course of a 4-day period. The entire process was then repeated again for each fish, again over a 4-day period, to complete a reciprocal design in which each food-deprived and control fish was tested in the presence of both a food-deprived and control shoal (with the treatment order randomized for each fish). Fish used to populate the stimulus shoals were only used once per day.

Videos were later analysed using the automated tracking software idTracker (Pérez-Escudero *et al.* 2014). This software logged the  $x$ - $y$  coordinates of the focal fish for every frame of the video recording. For each focal individual in each trial, this information was used to calculate mean speed and distance from the chamber containing the group over 5-min intervals throughout the 30-min trial. To estimate consistency of sociability, and to quantify relationships between SMR and sociability, a sociability score was calculated for each fish in each trial as the mean distance from the stimulus shoal over the final 15 min of the trial. Note that these analyses were also performed using the mean distance over the course of the entire 30-min trial, and identical trends were observed.

#### STATISTICAL ANALYSIS

All analyses were performed with SPSS statistics v20.0 (SPSS Inc., and IBM, Chicago, IL, USA). The level of significance for all tests was  $\alpha = 0.05$ . The consistency of sociability within diet treatments for focal fish and between stimulus shoal treatments (i.e. when exposed to control vs. food-deprived shoal) was quantified by calculating the intraclass correlation coefficient (ICC), using the within- and among-groups sums of squares from a single-factor ANOVA (Lessells & Boag 1987). Factors influencing sociability were examined using linear mixed-effects models (LMEs) with a compound symmetry covariance structure, because variables were on similar scales and showed similar covariances. Estimation was performed using restricted maximum likelihood. An initial model examined the effect of time on distance from the stimulus shoal using log-transformed mean distance (cm) during each 5-min interval as an explanatory variable, and focal treatment, stimulus shoal treatment and time interval as categorical explanatory variables, and fish ID as a random effect. This model revealed a significant effect of time for some treatment combinations, and so a subsequent model was produced which only examined mean distance from the stimulus shoal during the final 15 min of each trial (to minimize the effect of acclimation to the arena as a confounding factor). This latter model included log-transformed mean distance (cm) from the stimulus shoal (over the final 15 min of the trial) as the dependent variable, focal treatment and stimulus shoal treatment as categorical explanatory variables, log body mass (g) and log SMR (mg O<sub>2</sub> h<sup>-1</sup>) as continuous explanatory variables and fish ID as a random effect.

Differences in SMR among focal fish from each treatment were analysed using general linear models with log SMR as the dependent variable, and log body mass and treatment (control vs. food-deprived) as explanatory variables. For use in figures, SMR was standardized to a common body mass of 7.65 g, by first calculating the residuals of an ordinary least squares regression of the following form:

$$\log_{10}SMR = b + a \log_{10}M + \epsilon \quad \text{eqn 2}$$

where  $M$  is body mass (g),  $a$  and  $b$  are constants and  $\epsilon$  is the residual variation. The residuals of this regression were added to the fitted value for an animal of 7.65 g (the mean of all fish used in the study at the time of SMR measurement) to obtain adjusted estimates of SMR.

Rates of mass loss or growth were estimated in terms of body mass ( $G_M$ ) and standard length ( $G_L$ ), and were calculated between measurement periods according to the equation:

$$G = 100 * (\ln(s_t) - \ln(s_i)) / d \quad \text{eqn 3}$$

where  $s_t$  is the body mass or standard length at time  $t$ ,  $s_i$  is the initial body mass or standard length and  $d$  is the time elapsed (in days) (Hopkins 1992). Differences in growth rate ( $G_M$  and  $G_L$ ) between treatments were examined using general linear models with treatment as a categorical factor. Because growth rate is size-dependent (Nicieza & Álvarez 2009; Killen *et al.* 2014), the initial standard length during each measurement period was also included in models as a covariate.

## Results

### EFFECTS OF DIET ON GROWTH/MASS LOSS AND METABOLIC RATE

Control fish had higher values for both  $G_L$  and  $G_M$  as compared to FD fish (Table 1). SMR varied with body mass ( $\log_{10} \text{SMR} = -0.4802 + 0.559 \log_{10} M$ ,  $r^2 = 0.15$ ,  $P = 0.019$ ), but there were no differences in mean SMR between control and FD individuals after accounting for body mass (Table 1). In the control treatment, individuals with a higher SMR had a higher  $G_L$  (GLM, effect of SMR,  $F_{1,36} = 5.308$ ,  $P = 0.028$ ) and  $G_M$  (GLM, effect of SMR,  $F_{1,36} = 5.057$ ,  $P = 0.032$ ). In the FD treatment, there was no association between SMR and  $G_M$ .

### FACTORS AFFECTING SOCIABILITY

As a trial progressed, control fish increased their distance from the stimulus shoal, particularly when exposed to shoals from the FD treatment (Figs 1, 2 and 3; LME, focal  $\times$  time interaction,  $F_{1,384} = 3.411$ ,  $P = 0.005$ ; focal  $\times$  shoal interaction,  $F_{1,384} = 11.230$ ,  $P = 0.001$ ). In contrast, FD fish did not venture further from stimulus shoals with time (Fig. 2). Control focal fish exposed to an FD shoal kept the greatest distance from the shoal compared to all other groups (Figs 2 and 3). Control focal fish with a higher SMR were less sociable, ranging further away from the stimulus shoal (Fig. 4, LME, focal  $\times$  SMR interaction,  $F_{1,31.51} = 4.196$ ,  $P = 0.049$ ). There was no association between SMR and distance from the shoal among FD individuals.

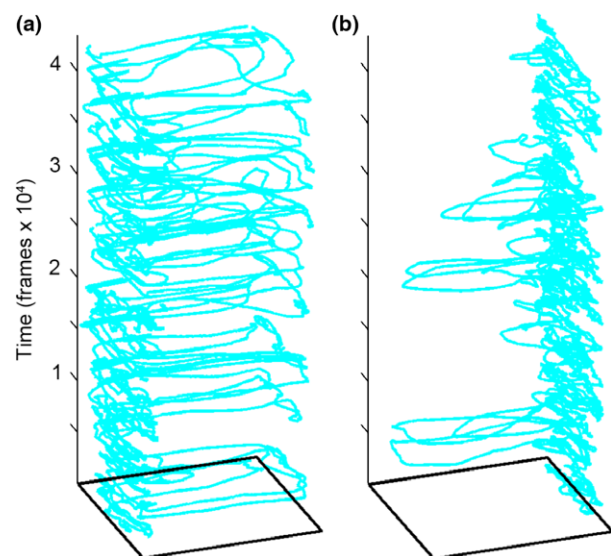
**Table 1.** Comparisons of length-specific growth rate ( $G_L$ ), mass-specific growth rate ( $G_m$ ) and standard metabolic rate (SMR) in juvenile qingbo carp that were either food-deprived or fed a maintenance ration for 21 days (control). Also shown are  $F$  and  $P$  values from general linear model analyses (see Methods). Sample size is  $n = 18$  per treatment

	Control	Food-deprived	$F$	$P$
$G_L$ (% day <sup>-1</sup> )	0.25 $\pm$ 0.08	-0.016 $\pm$ 0.02	7.747	0.009
$G_m$ (% day <sup>-1</sup> )	0.57 $\pm$ 0.17	-0.56 $\pm$ 0.04	35.587	<0.001
SMR (mg O <sub>2</sub> h <sup>-1</sup> )	1.17 $\pm$ 0.19	1.01 $\pm$ 0.19	1.323	0.258

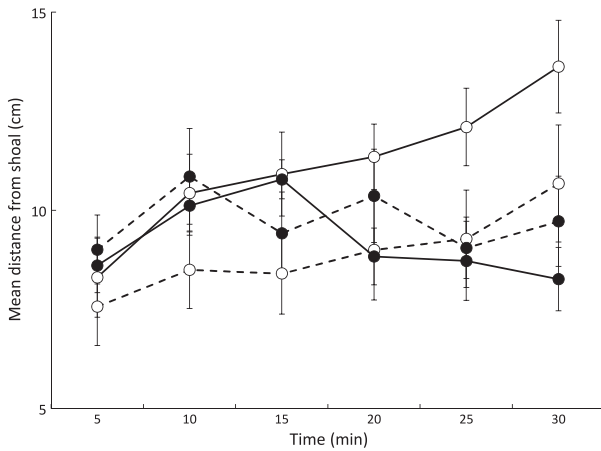
Sociability showed variability among individuals and between shoal exposures (Fig. 5). Interindividual variation in sociability was greater when fish were exposed to control shoals (approximately 11-fold range between minimum and maximum values) as compared to when fish were exposed to FD shoals (approximately fourfold variation). However, although sociability changed depending on whether fish were exposed to a control or food-deprived shoal, relative sociability among individuals showed consistency between exposures to each type of shoal. Consistency was especially high for FD focal fish (ICC = 0.60;  $P = 0.003$ ), but was reduced between shoal exposures for control focal fish (ICC = 0.26,  $P = 0.135$ ). Across both diet treatments for focal fish, ICC was equal to 0.40 ( $P = 0.007$ ).

## Discussion

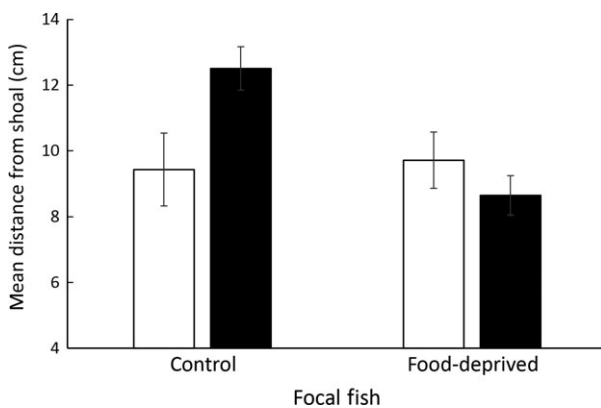
This study provides evidence that sociability is associated with metabolic rate but that the strength of this relationship is decreased by FD. Furthermore, the results here show that although relative sociability is a consistent characteristic of individual fish, FD can modulate the tendency to associate with conspecifics. Control fish ranged



**Fig. 1.** Examples of space use by the same individual juvenile *Spinibarbus sinensis* during two separate 30-min sociability trials. The individual was from the control treatment, having been fed a maintenance ration throughout the experiment. In panel a, the individual was exposed to a food-deprived shoal and displayed relatively low sociability (mean distance from stimulus shoal = 12.80 cm), spending more time away from the stimulus shoal. In panel b, the individual was exposed to a control shoal and displayed higher sociability (mean distance from stimulus shoal = 2.78 cm), spending more time close to the stimulus shoal. The black square represents the central section of the experimental arena; the blue lines trace the movements of the focal fish in two separate scenarios. In panel a, the stimulus shoal was located to the left of the central section; in panel b the stimulus shoal was located to the right of the central section.



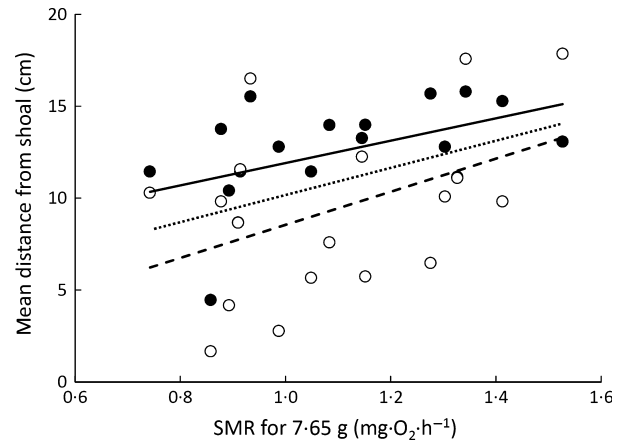
**Fig. 2.** Changes in mean distance from the stimulus shoal over 30-min sociability trials in juvenile *Spinibarbus sinensis*. White circles represent control focal fish; black circles represent food-deprived focal fish. Dashed lines represent trials with a control stimulus shoal; solid lines represent trials with a food-deprived stimulus shoal. Error bars = SEM.



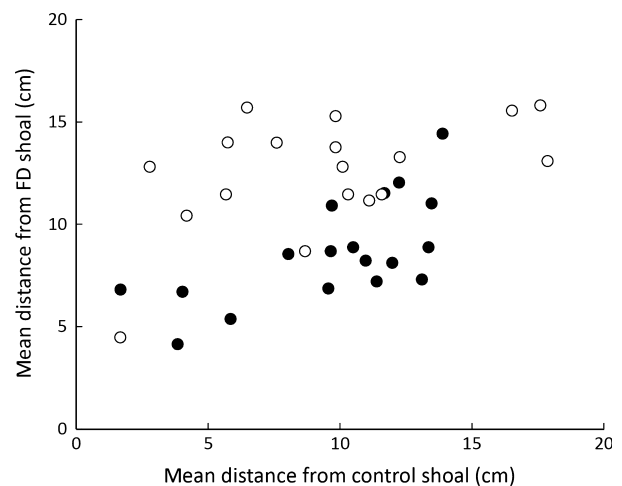
**Fig. 3.** Sociability (mean distance from the stimulus shoal during the final 15 min of each trial) in juvenile *Spinibarbus sinensis*. Black bars represent focal fish exposed to food-deprived stimulus shoals; white bars represent focal fish exposed to control stimulus shoals. Error bars = SEM.

further from shoals than food-deprived fish, especially when exposed to a shoal of fish that had been food-deprived.

Fish with a higher SMR in the control treatment showed decreased sociability. It is possible these fish were less attracted to the shoal or were even avoiding the shoal to some degree. Increased metabolic costs may increase motivation to find food first and avoid competition with groupmates, in a manner analogous to the effects of short-term FD on sociability (Krause 1993a; Krause, Hartmann & Pritchard 1999). An alternative, but not mutually exclusive, explanation is that these fish were generally more exploratory, covering more of the arena during the trial and thus having a higher mean distance away from the shoal. If the shoal had been able to interact with the focal fish, it is possible that such increased exploratory



**Fig. 4.** Relationships between standard metabolic rate (SMR; normalized to 7.65 g, the mean mass of all fish used in the study) and sociability (mean distance from the stimulus shoal over the final 15 min of the trial) for control focal *Spinibarbus sinensis* in the present study. White points represent trials when fish were exposed to control stimulus shoals (dashed black line: distance =  $0.25 + 8.34 \cdot \text{SMR}$ ,  $r^2 = 0.16$ ,  $P = 0.098$ ); black points represent trials when fish were exposed to food-deprived shoals (dashed black line: distance =  $5.84 + 6.91 \cdot \text{SMR}$ ,  $r^2 = 0.24$ ,  $P = 0.039$ ). For reference, the dotted black line represents the ordinary least squares regression through all points (distance =  $3.04 + 7.24 \cdot \text{SMR}$ ,  $r^2 = 0.15$ ,  $P = 0.018$ ; note that the linear mixed-effects model (LME) examining the effect of SMR on sociability included fish ID as a random effect). There were no links between SMR and sociability for food-deprived focal fish.



**Fig. 5.** Relative consistency of sociability (mean distance from the stimulus shoal over the final 15 min of the trial) of individual juvenile *Spinibarbus sinensis* across two trials, one in which they were exposed to a control stimulus shoal and another in which they were exposed to a food-deprived stimulus shoal. Each data point represents one individual. White points are control focal fish; black points are food-deprived focal fish. See Results for intraclass correlation coefficients. Pearson correlations for sociability between the two trials are as follows: control:  $r = 0.50$ ,  $P = 0.035$ ; food-deprived:  $r = 0.67$ ,  $P = 0.003$ ; overall:  $r = 0.43$ ,  $P = 0.008$ .

behaviour would have resulted in the high SMR fish leading the movements of the entire group (Burns *et al.* 2012; Nakayama *et al.* 2012; Jolles *et al.* 2015). A final possibil-

ity is that these fish were generally more active and therefore have more skeletal muscle and other metabolic machinery to support an increased capacity for activity that also needs to be maintained at rest. In this regard, however, it is notable that the average speed of movement of fish during trials did not differ among feeding treatments or shoal exposures and was not related to SMR in any treatment combination (Table S1). It is also interesting that individual FD fish showed strong consistency in sociability between shoal exposures despite there being no relationship between sociability and SMR among FD individuals. This could suggest that proximate mechanisms influencing sociability may shift depending on context (e.g. fed conditions vs. conditions of FD; Killen *et al.* 2013; Mathot *et al.* 2015). In support of this conjecture, SMR can itself be affected by nutritional history, perhaps altering the degree to which SMR drives particular types of behaviour (Van Leeuwen, Rosenfeld & Richards 2012; Killen 2014; Rosenfeld *et al.* 2015). In the current study, there were no significant differences in SMR between the control and FD fish, but subtle changes in metabolic rates or effects on a subset of individuals may have been sufficient to alter the influence of SMR on sociability among FD fish. These findings support the generally emerging view that links between physiological and behavioural traits are extremely complex and context-dependent (Killen *et al.* 2013; Mathot *et al.* 2015).

While control fish moved further from stimulus shoals over the course of the trial, FD fish tended to remain relatively close to the stimulus shoal. This contrasts with previous studies which have shown that hungry individuals become less sociable, decreasing associations with groups of conspecifics (Krause 1993a; Krause, Hartmann & Pritchard 1999). These prior studies have examined shorter-term fasting, however, whereas the current study examined more prolonged periods of reduced nutrition. Over the short-term, hunger may promote aggression and competition for food within groups (Webster & Hart 2006). Individuals near the periphery of groups also tend to be the first to find food items and consume the highest quality food items (Krause 1993b; DeBlois & Rose 1996). However, group membership can carry numerous foraging benefits, and the importance of these benefits may increase as animals experience FD for longer durations. Animals foraging in groups, for example, find food more consistently with less variable time frames between patch discoveries (Ekman & Hake 1988; Krause & Ruxton 2002). Furthermore, decreased individual vigilance, due to increased food-searching behaviour, may increase the degree to which individual fish need to rely on social information to alert them of predatory threats. Long-term FD can also decrease swimming performance in fish, including for *S. simensis* (Zhao *et al.* 2012), particularly the capacity for burst-type swimming that is employed during escapes from predators (Killen, Marras & McKenzie 2014). Therefore, FD may alter the ability of individual fish to detect and avoid predators, thereby increasing

shoaling tendency to at least partially mitigate increased risk of predation.

Control fish were less sociable when they were exposed to shoals of FD fish. This finding agrees with previous work showing that individuals show less sociability towards hungry or food-deprived conspecifics (Krause, Hartmann & Pritchard 1999). It is likely that well-fed individuals avoid these groups because hungry individuals may be more intense competitors for discovered food items. It is therefore interesting that, in the current study, FD focal fish showed no tendency to avoid shoals comprised of FD fish. Again, this suggests that longer-term FD may exert effects on sociability that differ from that caused by shorter periods of FD. It also has been shown that fish within species sort into shoals according to body size (Croft *et al.* 2005), and so it is possible that control fish were less attracted to food-deprived shoals due to differences in size. While fish in both treatments showed no differences in body length (GLM, effect of treatment on standard length,  $F = 0.144$ ,  $P = 0.706$ ), control fish had a greater body mass than the FD individuals (GLM, effect of treatment on body mass,  $F = 4.13$ ,  $P = 0.046$ ). However, FD fish showed no tendency to move away from shoals comprised of control fish and so it is unlikely that body size preferences played an appreciable role in measures of sociability in the current study. An additional possibility is that reduced mass for a given body length is a cue of overall poor condition within a group. This could in turn signal poor foraging success or even disease, therefore making them undesirable shoal-mates unless the benefits of decreased predation risk obtained from group membership are especially valued in a given context (though see Metcalfe & Thomson 1995; who show that fish can prefer to shoal with weaker competitors).

Fish in both treatments showed wide variation in sociability, but despite the changes in sociability generated by exposure to control vs. FD shoals, there was consistency in sociability among individuals between trials. This agrees with previous work demonstrating consistent interindividual variation in sociability (Réale *et al.* 2007; Careau & Garland 2012). Interestingly, sociability was correlated with SMR among control fish but not among FD fish. This observation contrasts with expectations that FD would strengthen any link between metabolic rate and behaviour (Killen *et al.* 2013). It is worth noting, however, that there was no correlation between SMR and mass loss in FD fish in this study, and so prior differences in metabolic demand did not necessarily amplify differences in foraging requirements as FD progressed. Links between metabolic demand and mass loss may be obscured by differences in fuel use (e.g. protein vs. lipid) among individuals during a period of fasting (McKenzie *et al.* 2014).

In the light of the current results, an interesting avenue for future research would be to examine how SMR and FD interact with choice of shoal size. Hungrier fish, for example, show a preference for smaller shoals, presumably because there is less competition for food (Krause 1993a).

Similarly, the optimal group size could vary among individuals in relation to metabolic demand: lower metabolic rate individuals may tend towards larger groups that can find food patches more consistently and provide increased protection from predators, even though competition for food may be higher and so meals may be smaller in larger groups. Another area requiring further investigation in this context is the role of non-visual cues. In the current study, we focussed on the role of visual cues in sociability. In the wild, qingbo prefer relatively clear and flowing sections of rivers and streams (S.J. Fu, personal observation), and so are likely to be capable of relying on visual cues. It should be noted, however, that fish are capable of using olfactory information when making social decisions (Ward & Currie 2013; Kleinhappel *et al.* 2014). It is therefore possible that links between metabolic rate and sociability, or the effects of FD, may be greater than that observed in the present study in situations where individuals can take advantage of olfactory cues. Non-visual cues may also be of increased importance for other species that inhabit more turbid waters.

In summary, this study shows that a period of FD can increase sociability in shoaling fish. Increased requirements for nutrition may heighten the need for group-assisted foraging, or decreased locomotor performance may increase the need for risk mitigation. Results also provide evidence that links between sociability and metabolic rate are labile and may vary according to factors such as food availability. If sociability or spatial positioning in relation to groups is associated with fitness (Krause & Ruxton 2002; Silk 2007), then selection on sociability could generate correlated selection on additional traits (e.g. SMR) depending on context.

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## Data accessibility

Data are available in Table S1.

## References

- Auer, S.K., Salin, K., Rudolf, A.M., Anderson, G.J. & Metcalfe, N.B. (2015) Greater flexibility in metabolic rate confers a growth advantage under changing food availability. *Journal of Animal Ecology*, **84**, 1405–1411.
- Biro, P.A. & Stamps, J.A. (2010) Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution*, **25**, 653–659.
- Burton, T., Killen, S.S., Armstrong, J.D. & Metcalfe, N.B. (2011) What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B: Biological Sciences*, **278**, 3465–3473.
- Burns, A.L., Herbert-Read, J.E., Morrell, L.J. & Ward, A.J. (2012) Consistency of leadership in shoals of mosquitofish (*Gambusia holbrooki*) in novel and in familiar environments. *PLoS One*, **7**, e36567–e36567.
- Careau, V. & Garland, T. Jr (2012) Performance, personality, and energetics: correlation, causation, and mechanism. *Physiological and Biochemical Zoology*, **85**, 543–571.
- Cote, J., Fogarty, S. & Sih, A. (2012) Individual sociability and choosiness between shoal types. *Animal Behaviour*, **83**, 1469–1476.
- Croft, D.P., James, R., Ward, A.J.W., Botham, M.S., Mawdsley, D. & Krause, J. (2005) Assortative interactions and social networks in fish. *Oecologia*, **143**, 211–219.
- DeBlois, E.M. & Rose, G.A. (1996) Cross-shoal variability in the feeding habits of migrating Atlantic cod (*Gadus morhua*). *Oecologia*, **108**, 192–196.
- Ding, R.H. (1994) *The Fishes of Sichuan*. Publishing House of Science and Technology, Chengdou, China.
- Ekmann, J. & Hake, M. (1988) Avian flocking reduces starvation risk: an experimental demonstration. *Behavioral Ecology and Sociobiology*, **22**, 91–94.
- Frommen, J.G., Luz, C. & Bakker, T.C.M. (2007) Nutritional state influences shoaling preference for familiars. *Zoology*, **110**, 369–376.
- Fu, S.-J., Pang, X., Cao, Z.-D., Peng, J.-L. & Yan, G. (2011) The effects of fasting on the metabolic interaction between digestion and locomotion in juvenile southern catfish (*Silurus meridionalis*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **158**, 498–505.
- Gingerich, A., Philipp, D. & Suski, C. (2010) Effects of nutritional status on metabolic rate, exercise and recovery in a freshwater fish. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, **180**, 371–384.
- Hopkins, K.D. (1992) Reporting fish growth: a review of the basics. *Journal of the World Aquaculture Society*, **23**, 173–179.
- Jolles, J.W., Fleetwood-Wilson, A., Nakayama, S., Stumpe, M.C., Johnstone, R.A. & Manica, A. (2015) The role of social attraction and its link with boldness in the collective movements of three-spined sticklebacks. *Animal Behaviour*, **99**, 147–153.
- Jones, K.A., Croft, D.P., Ramnarine, I.W. & Godin, J.-G.J. (2010) Size-assortative shoaling in the Guppy (*Poecilia reticulata*): the role of active choice. *Ethology*, **116**, 147–154.
- Killen, S.S. (2014) Growth trajectory influences temperature preference in fish through an effect on metabolic rate. *Journal of Animal Ecology*, **83**, 1513–1522.
- Killen, S.S., Marras, S. & McKenzie, D.J. (2014) Fast growers sprint slower: effects of food deprivation and re-feeding on sprint swimming performance in individual juvenile European sea bass. *The Journal of Experimental Biology*, **217**, 859–865.
- Killen, S.S., Marras, S., Metcalfe, N.B., McKenzie, D.J. & Domenici, P. (2013) Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology & Evolution*, **28**, 651–658.
- Killen, S.S., Mitchell, M.D., Rummer, J.L., Chivers, D.P., Ferrari, M.C.O., Meekan, M.G. *et al.* (2014) Aerobic scope predicts dominance during early life in a tropical damselfish. *Functional Ecology*, **28**, 1367–1376.
- Kleinhappel, T.K., Burman, O.H.P., John, E.A., Wilkinson, A. & Pike, T.W. (2014) Diet-mediated social networks in shoaling fish. *Behavioral Ecology*, **25**, 374–377.
- Krause, J. (1993a) The influence of hunger on shoal size choice by three-spined sticklebacks, *Gasterosteus aculeatus*. *Journal of Fish Biology*, **43**, 775–780.
- Krause, J. (1993b) The relationship between foraging and shoal position in a mixed shoal of roach (*Rutilus rutilus*) and chub (*Leuciscus cephalus*): a field study. *Oecologia*, **93**, 356–359.
- Krause, J., Hartmann, N. & Pritchard, V.L. (1999) The influence of nutritional state on shoal choice in zebrafish. *Danio rerio*. *Animal Behaviour*, **57**, 771–775.
- Krause, J. & Ruxton, G.D. (2002) *Living in Groups*. Oxford University Press, New York, NY, USA.
- Lessells, C.M. & Boag, P.T. (1987) Unrepeatable repeatabilities: a common mistake. *The Auk*, **104**, 116–121.
- Marchini, A., Gauzer, K. & Occhipinti-Ambrogi, A. (2004) Spatial and temporal variability of hard-bottom macrofauna in a disturbed coastal lagoon (Sacca di Goro, Po River Delta, Northwestern Adriatic Sea). *Marine Pollution Bulletin*, **48**, 1084–1095.
- Martinez, M., Guderley, H., Nelson, J.A., Webber, D. & Dutil, J.D. (2002) Once a fast cod, always a fast cod: maintenance of performance hierar-

- chies despite changing food availability in cod (*Gadus morhua*). *Physiological and Biochemical Zoology*, **75**, 90–100.
- Martinez, M., Bedard, M., Dutil, J.-D. & Guderley, H. (2004) Does condition of Atlantic cod (*Gadus morhua*) have a greater impact upon swimming performance at Ucrit or sprint speeds? *Journal of Experimental Biology*, **207**, 2979–2990.
- Mathot, K.J., Nicolaus, M., Araya-Ajoy, Y.G., Dingemanse, N.J. & Kempenaers, B. (2015) Does metabolic rate predict risk-taking behaviour? A field experiment in a wild passerine bird. *Functional Ecology*, **29**, 239–249.
- McKenzie, D.J., Vergnet, A., Chatain, B., Vandeputte, M., Desmarais, E., Steffensen, J.F. *et al.* (2014) Physiological mechanisms underlying individual variation in tolerance of food deprivation in juvenile European sea bass, *Dicentrarchus labrax*. *The Journal of Experimental Biology*, **217**, 3283–3292.
- Metcalf, N.B. & Thomson, B.C. (1995) Fish recognize and prefer to shoal with poor competitors. *Proceedings of the Royal Society of London B: Biological Sciences*, **259**, 207–210.
- Mills, A.D. & Faure, J.M. (2000) Ease of capture in lines of Japanese quail (*Coturnix japonica*) subjected to contrasting selection for fear or sociability. *Applied Animal Behaviour Science*, **69**, 125–134.
- Morgan, M. (1988) The effect of hunger, shoal size and the presence of a predator on shoal cohesiveness in bluntnose minnows, *Pimephales notatus* Rafinesque. *Journal of Fish Biology*, **32**, 963–971.
- Nakayama, S., Harcourt, J.L., Johnstone, R.A. & Manica, A. (2012) Initiative, personality and leadership in pairs of foraging fish. *PLoS One*, **7**, e36606.
- Nicieza, A. & Álvarez, D. (2009) Statistical analysis of structural compensatory growth: how can we reduce the rate of false detection? *Oecologia*, **159**, 27–39.
- Pang, X., Yuan, X.-Z., Cao, Z.-D. & Fu, S.-J. (2014) The effects of fasting on swimming performance in juvenile qingbo (*Spinibarbus sinensis*) at two temperatures. *Journal of Thermal Biology*, **42**, 25–32.
- Pérez-Escudero, A., Vicente-Page, J., Hinz, R.C., Arganda, S. & de Polavieja, G.G. (2014) idTracker: tracking individuals in a group by automatic identification of unmarked animals. *Nature Methods*, **11**, 743–748.
- Réale, D., Reader, S.M., Sol, D., McDougall, P.T. & Dingemanse, N.J. (2007) Integrating animal temperament within ecology and evolution. *Biological Reviews*, **82**, 291–318.
- Reeb, S.G. & Saulnier, N. (1997) The effect of hunger on shoal choice in golden shiners (Pisces: Cyprinidae, *Notemigonus crysoleucas*). *Ethology*, **103**, 642–652.
- Rosenfeld, J., Van Leeuwen, T., Richards, J. & Allen, D. (2015) Relationship between growth and standard metabolic rate: measurement artefacts and implications for habitat use and life-history adaptation in salmonids. *Journal of Animal Ecology*, **84**, 4–20.
- Ruxton, G., Hall, S. & Gurney, W. (1995) Attraction toward feeding conspecifics when food patches are exhaustible. *American Naturalist*, **145**, 653–660.
- Silk, J.B. (2007) The adaptive value of sociality in mammalian groups. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **362**, 539–559.
- Van Leeuwen, T.E., Rosenfeld, J.S. & Richards, J.G. (2012) Effects of food ration on SMR: influence of food consumption on individual variation in metabolic rate in juvenile coho salmon (*Onchorhynchus kisutch*). *Journal of Animal Ecology*, **81**, 395–402.
- Wang, T., Hung, C.C.Y. & Randall, D.J. (2006) The comparative physiology of food deprivation: from feast to famine. *Annual Review of Physiology*, **68**, 223–251.
- Ward, A.W. & Currie, S. (2013) Shoaling fish can size-assort by chemical cues alone. *Behavioral Ecology and Sociobiology*, **67**, 667–673.
- Webster, M.M. & Hart, P.J. (2006) Kleptoparasitic prey competition in shoaling fish: effects of familiarity and prey distribution. *Behavioral Ecology*, **17**, 959–964.
- Webster, M. & Laland, K. (2015) Space-use and sociability are not related to public-information use in ninespine sticklebacks. *Behavioral Ecology and Sociobiology*, **69**, 895–907.
- Zhao, W.-W., Pang, X., Peng, J.-L., Cao, Z.-D. & Fu, S.-J. (2012) The effects of hypoxia acclimation, exercise training and fasting on swimming performance in juvenile qingbo (*Spinibarbus sinensis*). *Fish Physiology and Biochemistry*, **38**, 1367–1377.

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## Supporting Information

Additional Supporting information may be found in the online version of this article:

**Table S1.** Data collected and analysed in the current study.

**Video S1.** Example footage of individual *Spinibarbus sinensis* with relatively high and low sociability scores, showing their respective tendencies to associate with the stimulus shoal.