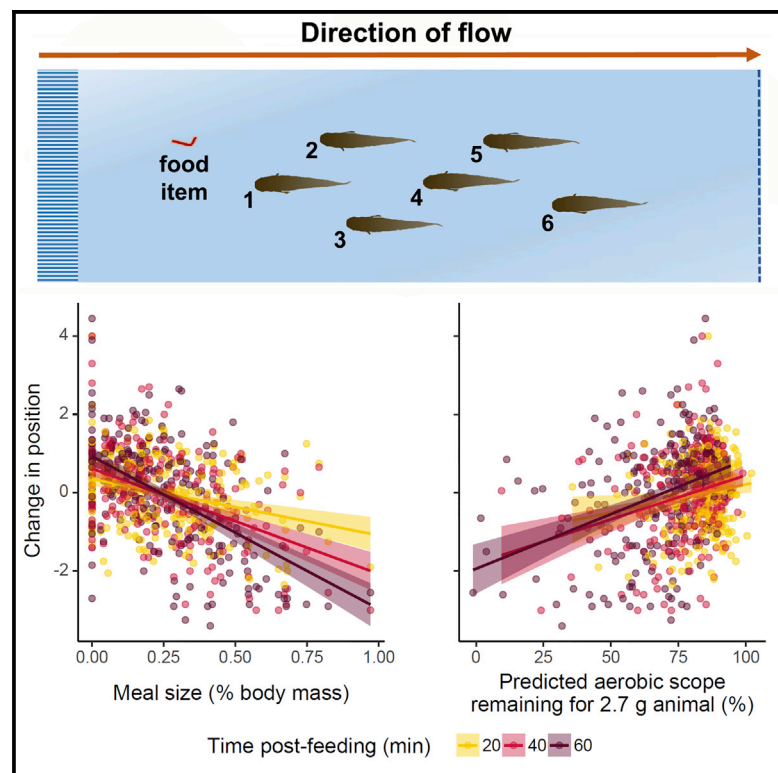


Current Biology

Metabolic Costs of Feeding Predictively Alter the Spatial Distribution of Individuals in Fish Schools

Graphical Abstract



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In Brief

The mechanisms of how individuals spatially arrange themselves within social groups remain unclear. Using observations of fish feeding in groups and estimates of metabolic rate, McLean et al. show that shifts in position within groups after feeding are related to aerobic metabolic scope remaining after accounting for energetic costs of digestion.

Highlights

- We examined individual positioning in groups of swimming fish after feeding
- Fish that ate most subsequently shifted to more posterior positions within groups
- Shifts in position were related to the remaining aerobic scope after feeding
- Feeding-related constraints could affect leadership and group functioning

Metabolic Costs of Feeding Predictively Alter the Spatial Distribution of Individuals in Fish Schools

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SUMMARY

Group living is ubiquitous among animals [1, 2], but the exact benefits of group living experienced by individual groupmates is related to their spatial location within the overall group [3–5]. Individual variation in behavioral traits and nutritional state is known to affect interactions between individuals and their social group [6, 7], but physiological mechanisms underpinning collective animal behavior remain largely unexplored [8]. Here, we show that while fish at the front of moving groups are most successful at capturing food items, these individuals then show a systematic, post-feeding movement toward the rear of groups. Using observations of fish feeding in groups coupled with estimates of metabolic rate in fish consuming different meal sizes, we demonstrate that the magnitude of this shift in spatial position is directly related to the aerobic metabolic scope remaining after accounting for energetic costs of digestion. While previous work has shown that hungry individuals occupy anterior positions in moving groups [9, 10], our results show that the metabolic demand of food processing reduces the aerobic capacity available for locomotion in individuals that eat most, thus preventing them from maintaining leading positions. This basic trade-off between feeding and locomotor capacity could fundamentally dictate the spatial position of individuals within groups, perhaps obviating the role of individual traits in determining spatial preferences over shorter timescales (e.g., hours to days). This may be a general constraint for individuals within animal collectives, representing a key, yet overlooked, mediator of group functioning that could affect leadership, social information transfer, and group decision making.

RESULTS

The spatial positioning of individuals within social groups affects the resources they obtain, the predation risk they experi-

ence, and their influence on group decision making [11, 12]. Individuals with relatively high boldness [11], locomotor capacity [6, 13], or metabolic demand may be found at the front of moving groups more often, where they can maximize food intake [9, 10, 14]. Receiving less attention in the context of group behavior is the fact that, at acute timescales, feeding is associated with an increase in metabolic rate due to the energetic costs of the mechanical and biochemical digestion of food and uptake of nutrients [15, 16]—the so-called specific dynamic action (SDA) response. These SDA costs can be substantial and, at their peak, approach an animal's maximum aerobic metabolic rate, thus occupying a significant portion of the aerobic scope (AS) available for other physiological functions, including locomotion [17, 18]. It is therefore possible that, following feeding, individuals that prefer frontal positions within moving groups may be physiologically constrained from occupying these positions.

We examined whether the remaining AS during SDA was related to shifts in spatial positioning by individual common minnows (*Phoxinus phoxinus*) in moving groups following feeding. We observed individual variation in food intake while drift feeding on dead chironomid larvae (“bloodworms”) within a swimming flume (Figure 1A and Movie S1). For all fish, we also estimated metabolic rates via rates of oxygen uptake (\dot{M}_{O_2}) using intermittent flow respirometry [19]. Separate fish were measured for changes in oxygen uptake rate after consuming different amounts of food. The metabolic responses of these fish to feeding were used to predict the AS remaining, after accounting for the costs of SDA, for fish in the swimming experiments in relation to food intake and time since feeding.

Each individual was tested for group behavior twice, across two trials with different groups of conspecifics. The number of food items captured showed strong repeatability across the two trials (Figure 1B; $R = 0.667$, 95% CI = 0.593–0.722, $p < 0.0001$). Within trials, the mean position was also repeatable across time periods ($R = 0.196$, 95% CI = 0.140–0.253, $p < 0.0001$). Within each time period but between the two trials (Figure 1C), repeatability was maintained but lowered during and 20 min after feeding (Figure 1D). Absolute SMR (SMR; the minimum energy needed to sustain life in an ectotherm), maximum metabolic rate (MMR), and AS were all positively correlated with body mass (Figure S1). Mass-standardized SMR and AS were not related to the amount of food captured by individuals nor their position within groups before, during,

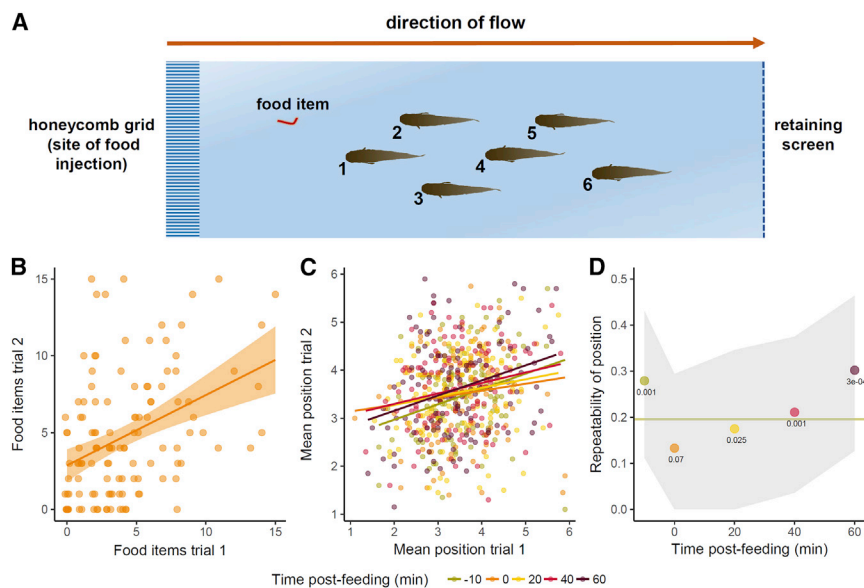


Figure 1. Repeatability of Behavior during Group Feeding and Swimming Trials

(A) Top view of swim flume during tests of schooling behavior in common minnows (*P. phoxinus*). Fish were ranked according to the position of the tip of the snout in relation to the front of the school. Positions in this theoretical example are indicated by numbers to the bottom left of fish. (B) Repeatability of food items captured. (C) Repeatability of mean spatial position occupied within a school of six *P. phoxinus* across two group trials. Each data point represents one individual fish. For illustrative purposes, solid lines in (B) and (C) represent linear regressions. To make overlapping points visible, points in (B) are slightly offset (horizontally) from their true value. Shaded area in (B) represents the 95% CI (not shown on C for visual clarity).

(D) Changes in repeatability within each time interval before, during, and after feeding, using data collected during the two trials. The gray shaded area in (D) represents the 95% CI on repeatability estimates. The green horizontal line represents repeatability of mean position across time periods (see Results for details). Numbers beneath data points equal p values for repeatability estimates. Sample size is $n = 123$ individuals tested in two replicate trials. See also Movie S1.

or after feeding (Table S1). Fish at the front of groups and those that were larger at a given position captured the most food items (Figure 2; $t = 5.475$, $p < 0.0001$). Fish that consumed the most food had moved to more posterior positions within groups by 40 and 60 min post feeding (Figure 2; Table S1).

Next, to quantify the increase in metabolic rate during the SDA response, fish separate to those used during the swimming trials were fed known quantities of food then measured for \dot{M}_{O_2} . The SDA response of each individual was modeled by applying a polynomial function to the \dot{M}_{O_2} data for 40 hr after feeding (Figure 3A). Fish that ate larger meals during feeding trials showed

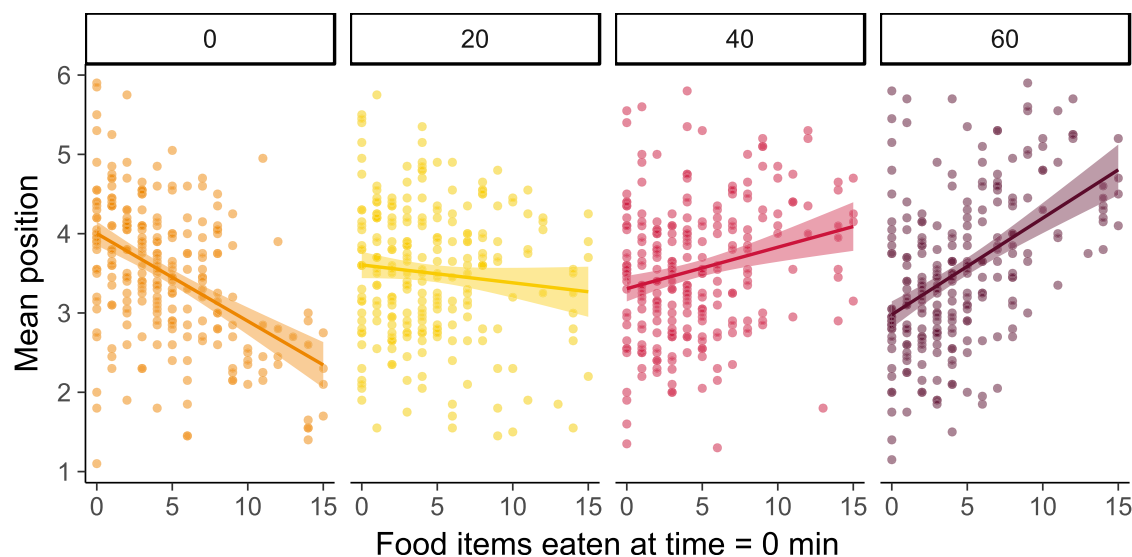


Figure 2. The Relationship between Mean Spatial Position of Individual Minnows within a School of Six Fish and the Amount of Food Items Eaten during a Period of Feeding

The feeding period is designated as time 0. For positions, 1 = at the front of the school and 6 = at the back of the school. There are two points per individual (each fish was tested twice). For illustrative purposes, solid lines represent linear regressions. Shaded areas around lines represent the 95% CI. From left to right, each plot shows data during feeding (0) and at 20, 40, and 60 min post feeding. Sample size is $n = 123$ individuals tested in two replicate trials. See also Table S1.

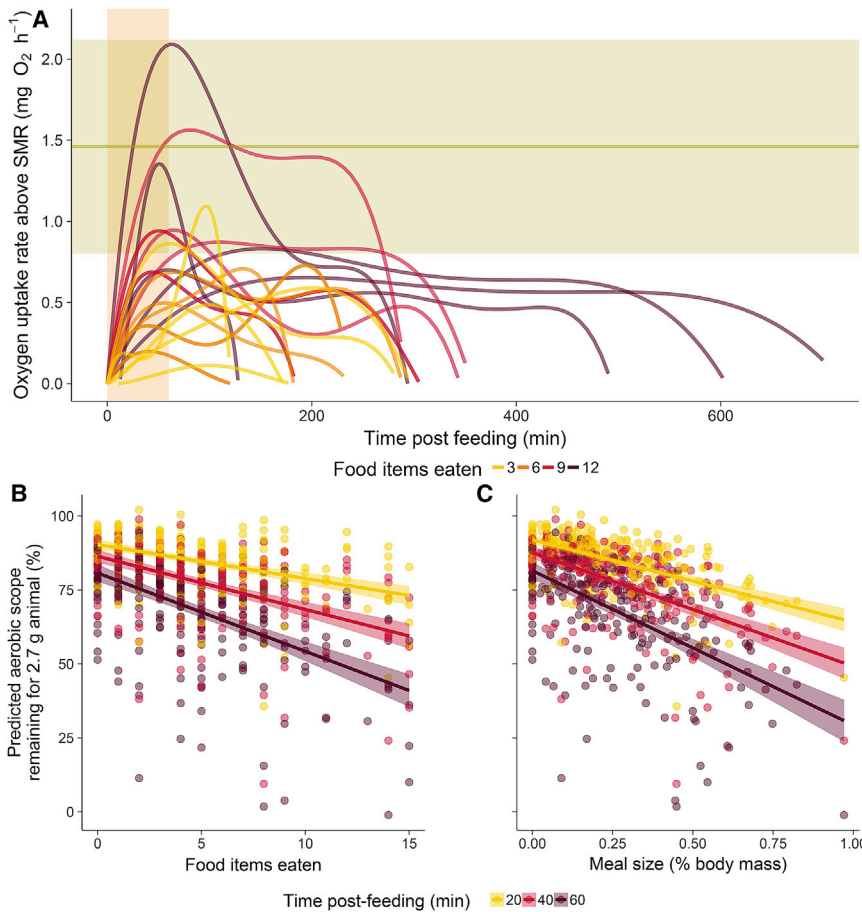


Figure 3. The Effects of Feeding on Oxygen Uptake Rate and Remaining Aerobic Scope

(A) Changes in \dot{M}_{O_2} with time in individual common minnows after consuming various amounts of food. Each curve represents data for one individual and is a polynomial function (detailed in the main text). Green horizontal line represents the mean maximum metabolic rate of fish used in the schooling trials; the green shaded area represents the upper and lower standard deviations. The vertical orange shaded area is the time period corresponding to feeding and the subsequent 60 min in the group swimming trials.

(B and C) Predicted percentage of aerobic scope remaining for individual fish in group swimming trials after feeding based on the amount of food consumed by each individual (B: food items eaten; C: meal size in terms of percent of body mass) and time since feeding. Each data point represents data for one individual; there are two points per individual (each fish was tested twice). For illustrative purposes, solid lines represent linear regressions. Shaded areas around lines represent the 95% CI. Sample size is $n = 123$ individuals tested in two replicate trials. See also [Tables S2 and S3](#).

higher peak levels of \dot{M}_{O_2} after feeding, associated with the digestion and assimilation of the food, and larger fish had a higher peak \dot{M}_{O_2} ([Table S2](#)). Fish that ate more also took longer for \dot{M}_{O_2} to return to baseline ([Table S2](#)). Neither meal size nor body mass affected the time post feeding at which peak \dot{M}_{O_2} occurred, with peak \dot{M}_{O_2} occurring on average 93.90 ± 11.39 min post feeding. There was individual variation in this response, however, and when time was broken into the 12-min intervals during which \dot{M}_{O_2} was recorded, the median time at peak \dot{M}_{O_2} post feeding was 60 min ([Figure 3A](#)).

Based on measurements of \dot{M}_{O_2} during these feeding trials, a multiple regression was constructed to estimate the \dot{M}_{O_2} of fish during the swimming trials according to the following equation ($r^2 = 0.385$, $p < 0.0001$):

$$\text{Log}_{10} \dot{M}_{O_2, \text{increase}} = -1.859 + (0.00727t) + (0.814B) + (1.643 \log_{10} \text{mass}), \quad \text{eq. 1}$$

where $\dot{M}_{O_2, \text{increase}}$ = the increase in \dot{M}_{O_2} above SMR, t = time post feeding (min), B = meal size (percent of body mass), and mass = fish body mass (g).

Applying this equation to fish observed in swimming trials, the predicted percentage of AS remaining, after accounting for the predicted rise in \dot{M}_{O_2} post feeding, decreased with meal size and time ([Figures 3B and 3C](#) and [Table S3](#)). Fish that ate larger

meals showed greater posterior movement within groups, and this effect strengthened over time ([Figures 4A and 4B](#) and [Table S4](#)). Individuals with a lower AS remaining after feeding (standardized to a common body mass) moved toward the back of groups ([Figure 4C](#) and [Table S4](#)), an effect that did not interact with time post feeding. This backward movement was not due to those fish spending more time at the front of the group and becoming fatigued or having nowhere to move besides back: during control trials in which fish were not fed, repeatability of positioning across observation times was high ($R = 0.595$, 95% CI = 0.459–0.707, $p = 0.001$; [Figure S2](#) and [Table S5](#)).

DISCUSSION

The magnitude of the shift in spatial position by individuals within schools after feeding was systematic and related to meal size during feeding and the AS remaining after accounting for SDA. Previous work has shown that individual boldness [[11](#), [12](#)], movement speed [[6](#)], and AS [[13](#), [20](#)] can all influence individual position within groups. However, our results suggest that, at least over short time frames on the scale of hours, the physiological costs of feeding could negate effects of intrinsic traits on spatial positioning and leadership within groups, with individuals moving toward the rear of groups after they feed. In the wild, it is possible that individuals at the front of groups may eat the most or highest quality food items [[21](#)] but then be forced to move back within the group, with others that have fed less taking over anterior positions. After feeding, individuals that might otherwise act as leaders may be physiologically unable to occupy the spatial positions that would allow them to influence the group.

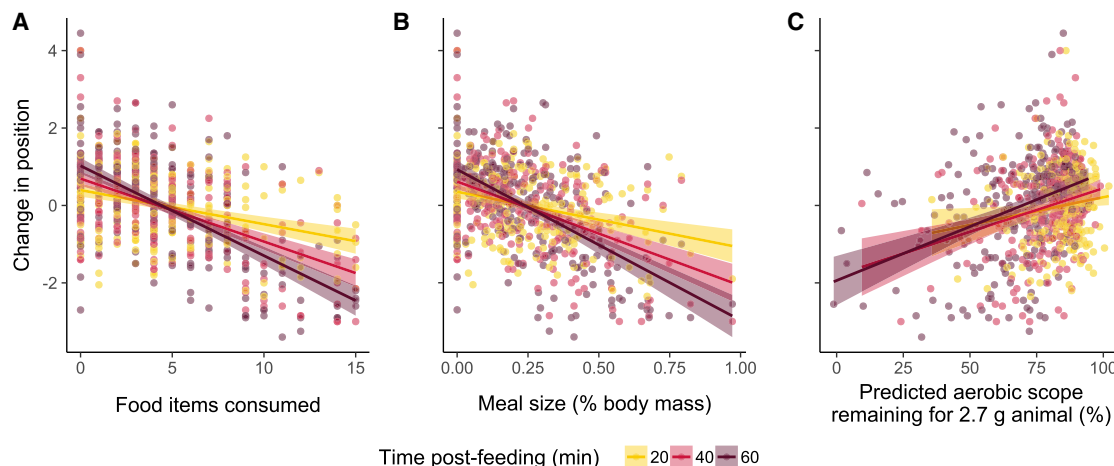


Figure 4. The Effect of Food Intake and Remaining Aerobic Scope on Changes in Position at Various Times since Feeding

(A) Effect of food items consumed.

(B) Effect of relative meal size (percent of body mass).

(C) Effect of the percent of aerobic scope remaining.

Positive values indicate the number of positions moved toward the front of the school, while negative values indicate the number of positions moved toward the back of the school. Each data point represents data for one individual; there are two points per individual (each fish was tested twice). For illustrative purposes, solid lines represent linear regressions. Shaded areas around lines represent the 95% CI. Sample size is $n = 123$ individuals tested in two replicate trials. See also [Figure S2](#) and [Tables S4](#) and [S5](#).

The position occupied by individuals within groups after feeding was related to (1) the amount of food consumed during feeding, (2) the time since feeding, and (3) the proportion of AS remaining during meal processing. Feeding motivation may have influenced spatial positioning, as individuals consuming little or no food during feeding consistently obtained positions at or near the front of schools within the hour after feeding. This agrees with previous observations in which food-deprived individuals occupied frontal positions more frequently than satiated conspecifics [10, 22]. Although positions at the front of fish schools are associated with the highest degrees of food intake, the available evidence suggests that, in many systems, they also have the highest risk of predation [23, 24, but see 25]. It therefore stands to reason that, once an individual has fed to satiation or exhausted the food resource, it should move to a less risky position within the group. Indeed, in the current study, individuals consuming larger meals had moved toward the back of the school by 60 min after feeding. However, the persistent effect of remaining AS on the positioning of fish after feeding within groups strongly suggests that metabolic constraints imposed by meal processing had a direct role in dictating positions occupied by individuals. Notably, the effect of meal size increased with time since feeding, while the effect of available AS was consistent across observation periods. This suggests that fish moved back within groups as they reached thresholds for available AS during meal digestion and assimilation regardless of the time since feeding. If motivation was the primary factor causing individuals to change their position, then fish that ate the most should have moved back within the group almost immediately after feeding.

The increase in \dot{M}_{O_2} following feeding can be considerable, and depending on meal size, it can occupy a large proportion of an individual's total AS at its peak [17, 18, 26, 27]. Several

fish in the current study were estimated to have substantial decreases in AS after feeding, which would be expected to constrain locomotor ability [27, 28]. Individuals consuming the largest meals, and thus exhibiting the highest peak rise in metabolic costs during digestion, adjusted spatial positions to the back of groups where the costs of locomotion are generally reduced due to vortices produced by anterior groupmates [29, 30]. There may also be interactions among intrinsic physiological traits, feeding motivation or ability, and the magnitude of the SDA response. Fish with a larger AS feed more when given the opportunity [31], and SDA is positively linked to meal size [26]. It is therefore plausible that, in scenarios where food is abundant, individuals with a higher AS may eat more and, paradoxically, be more constrained than fish with a lower AS but that eat less. Furthermore, fish that ate more in the current study were found to have \dot{M}_{O_2} elevated for longer periods (up to 11 hr post feeding), suggesting that fish that eat more will also be constrained by SDA for a longer duration. Notably, there was variation in the digestive “strategy” employed by individuals. Some fish had a high peak \dot{M}_{O_2} but returned to baseline more quickly, while others took longer to digest but with a lower peak \dot{M}_{O_2} . The cause of this variation is unknown but undoubtedly contributed to uncertainty in predictions of remaining aerobic scope after feeding. Our estimates of the energetic costs of foraging may have also been underestimated for some individuals because we could not account for the physical acceleration and turning to capture food ([Movie S1](#)).

Despite changes in spatial positioning among individuals after feeding, there remained a degree of repeatability in positions occupied by individuals. Repeatability of positioning between trials before feeding suggests that individuals prefer specific positions within groups in the absence of food. This is corroborated by trials in which fish were not fed, where repeatability

of positioning was maintained throughout trials (Figure S2). Repeatability decreased during feeding, probably because fish were shuffling positions during competition for food items, but then restabilized as time after feeding increased. Future work is needed to understand the consequences of these changes in repeatability caused by feeding constraints for the costs and benefits of group membership experienced by individuals and for the potential selection on phenotypic traits that are normally assumed to correlate with spatial positioning within groups (e.g., boldness) [32, 33]. For example, reshuffling of individuals within moving groups, or changes in repeatability due to SDA, could disrupt social niche formation [34, 35] or influence group cohesion.

In conclusion, data from the present study demonstrate a series of complex interactions between feeding and intrinsic physiological mechanisms that determine the spatial positions that individuals will occupy within moving animal groups. Individuals that obtained more food during foraging showed a predictable pattern of movement to more posterior positions within the group that was tied directly to available AS during meal digestion. Changes in position will alter the costs and benefits experienced by individuals in different locations within the group. This information is critical for understanding how our current knowledge of collective behavior may extend to ecologically relevant scenarios [36, 37]. Additional work is required to understand how these processes interact with factors such as boldness on determining spatial positioning within groups. Over prolonged timescales, intrinsic behavioral or physiological traits may influence positional preference, but these effects may be overridden over shorter timescales due to locomotor constraints after feeding. More research is also needed to understand the consequences of locomotor constraints for group leadership, group learning, and group decision making, particularly if group leaders or demonstrators are physiologically incapable of occupying specific positions within groups.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- [KEY RESOURCES TABLE](#)
- [CONTACT FOR REAGENT AND RESOURCE SHARING](#)
- [EXPERIMENTAL MODEL AND SUBJECT DETAILS](#)
 - Animal model
- [METHOD DETAILS](#)
 - Protocol Overview
 - Estimation of metabolic rates
 - Metabolic cost of feeding (SDA)
 - Spatial positioning and feeding of schooling fish
- [QUANTIFICATION AND STATISTICAL ANALYSIS](#)
- [DATA AND SOFTWARE AVAILABILITY](#)

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures, five tables, and one movie and can be found with this article online at <https://doi.org/10.1016/j.cub.2018.02.043>.

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AUTHOR CONTRIBUTIONS

S.S.K. and S.M. conceived the study; S.M., A.P., and T.N. collected the data; S.M., S.S.K., A.P., and T.N. analyzed the data; S.M. and S.S.K. drafted the manuscript; and all authors contributed to further manuscript development and gave final approval for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Raw data	This paper	https://data.mendeley.com/datasets/8g955t3r9g/1
Experimental Models: Organisms/Strains		
Common minnow	Wild caught (River Kelvin, UK)	<i>Phoxinus phoxinus</i>
Software and Algorithms		
R version 3.4.0	R Core Team	https://www.r-project.org/

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Shaun S. Killen (shaun.killen@glasgow.ac.uk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal model

Approximately 180 common minnows were collected from the River Kelvin, Glasgow, United Kingdom (55° 51' 59.99" N –4° 18' 60.00" W) using large dip-nets. Animals were in a non-reproductive state and thus sex was unable to be identified. Sex was therefore not included as a factor in analyses but was unlikely to affect results given that individuals were non-reproductive at the time of experiments. In addition, individuals were randomly assigned to test groups (as described below) and so males and females should have been equally distributed among groups and time points in relation to feeding. To reduce capture bias at the time of collection from the wild, shallow-side channels along the river were used to initially herd fish before capture. Fish were maintained in the laboratory in four stock tanks (640 mm L x 620 mm W x 330 mm H) in recirculating, aerated freshwater at 13°C. All tanks had pebble substrate, artificial plants, and plastic tubes for environmental enrichment and were maintained on a 12 L:12 D photoperiod. All individuals were fed daily using commercial feed (flakes) and bloodworms. Fish were held in these conditions for approximately 4 months before starting experiments (at this point, fish mass = 2.7 ± 0.09 (SEM) g; total length = 6.74 ± 0.07 cm). Before experiments, however, individuals were deprived of food for a minimum of 36 h. The maintenance, handling, and non-lethal experiments conducted on fish during this study were carried out in strict accordance with the Home Office legislation (Project License Number: 60/4461) in the United Kingdom.

METHOD DETAILS

Protocol Overview

The overall protocol consisted of three main parts, details of which are given below. First, the standard and maximum metabolic rates (SMR and MMR, respectively) of individual minnows ($n = 130$) were estimated from rates of oxygen uptake (\dot{M}_{O_2}) using intermittent-closed respirometry. Second, and approximately 3 weeks later, these same fish were used in group behavioral trials within a swimming flume (6 fish per group). These trials were video recorded and later analyzed to estimate the spatial position of individual fish. Positions were estimated for each fish before, during, and after a period of feeding in which drifting food items (bloodworms) were injected into the flume. The total number of food items eaten by each fish was also quantified. In these behavior trials, each individual fish was tested twice (about 2 weeks between trials), with each trial being performed with a different set of conspecifics. Behavior was recorded for 45 groups in total. Due to a small number of mortalities, some individuals were not used in both trials. Only individuals that were exposed to all trials and metabolic trait measurements were used in statistical analyses ($n = 123$ fish). Third, a different set of minnows were measured for \dot{M}_{O_2} in the 40 h following feeding on different amounts of food items. The data from these trials were used to construct a predictive equation (see Results) of the rise in \dot{M}_{O_2} induced by feeding (i.e., the 'specific dynamic action'; SDA) in relation to the amount of food eaten and the time since feeding. This equation was then applied to fish used in the behavioral trials within the swimming flume, to estimate the percentage of remaining aerobic scope (AS) each fish would have possessed according to food it had consumed and the timing of the observations.

Estimation of metabolic rates

Fasted fish were carefully removed from their holding tanks using a dip-net. Maximum metabolic rate was measured after exhaustive exercise for a 2 min duration by manually chasing individual fish in a circular tank (50 cm diameter) with a water depth of 10 cm. All fish

were exhausted after this time period and would not swim with further stimulation. This method assumes that maximum \dot{M}_{O_2} is achieved during the recovery from the bout of exhaustive and partially anaerobic exercise, which is generally well supported [38, 39]. After complete exhaustion, fish were immediately transferred into individual cylindrical 96 mL glass respirometry chambers connected to an intermittent-closed respirometry system; the time until transfer was always less than 10 s [40, 41]. Water oxygen content in the respirometry chambers was quantified once every 2 s using a FireStingO₂ 4-channel optical oxygen meter and associated sensors and software (Pyro Science GmbH, Aachen, Germany). The respirometry chambers were kept in a 50 L rectangular experimental tank which was aerated and temperature regulated to 13°C. The setup was shielded from disturbance and direct lighting by an opaque plastic blind. Water mixing within each respirometry chamber was achieved with a peristaltic pump that moved water through the chamber and around an external circuit of gas-tight tubing. An automated flush pump allowed the chambers to switch between being flushed with fresh and fully aerated water for 3 min, and then switched off for 8 min, during which time the chambers were sealed to allow the decrease in oxygen content to be analyzed to indicate the rate of oxygen uptake (\dot{M}_{O_2}). To estimate MMR, we calculated \dot{M}_{O_2} for each 2 min time interval throughout the first 20 min of recovery immediately following the exhaustive exercise, and MMR (mg O₂ h⁻¹) was taken as the highest \dot{M}_{O_2} during this period.

After measurement of MMR, fish remained in the same respirometry chambers overnight to allow the estimation of SMR. Individuals were then removed from the respirometry chambers at around 09:00 the following day, having remained in the respirometry chambers for approximately 20 h in total. The fish were then lightly anaesthetised using benzocaine, given a unique combination of colored visible implant elastomer tags (Northwest Marine Technology, Shaw Island, USA) to allow individual identification when in groups, measured for wet mass and total length, and returned to their holding tanks for 4 days before continuing with the remainder of the study. Whole-animal SMR (mg O₂ h⁻¹) was estimated as the lowest 10th percentile of measurements taken throughout the measurement period [42]. The first 5 h of confinement in the chambers was excluded from analyses of SMR because the \dot{M}_{O_2} of the fish was often elevated at this time. AS was calculated as the difference between MMR and SMR. Before and after fish \dot{M}_{O_2} measurements, three full respirometry cycles were run with empty chambers to measure any background \dot{M}_{O_2} by bacteria. The increase in this background respiration from start to end of a respirometry trial was assumed to be linear and was subtracted from each measure of \dot{M}_{O_2} for individual fish.

Metabolic cost of feeding (SDA)

The increase in \dot{M}_{O_2} following feeding (i.e., the SDA response) was measured using the intermittent-closed respirometry setup described above. In this case, however, fish were fed a set amount of bloodworms immediately before being placed within the respirometry chambers. Pilot studies revealed that minnows would not eat food that was directly injected into the respirometry chambers, thus fish were fed before placement into the chambers. Individuals (separate fish from those used in previous swimming trials and measurements of metabolic attributes) were fasted for 4 days to elevate hunger levels and ensure that each fish would eat all of their assigned food during trials. From one of the 4 holding tanks, individuals were randomly selected and assigned to receive either 0, 3, 6, 9, or 12 bloodworms (covering the range of food items consumed by fish in the schooling trials; detailed below). The fish receiving 0 bloodworms acted as a control for handling involved in transfer to the respirometry chambers. Fish were fed their prescribed amount of food in a temperature-regulated tank kept at 13°C. Once each fish had eaten the desired number of food items, they were immediately placed into the respirometry chamber and \dot{M}_{O_2} was measured as described above for the next 40 h. In total, 32 fish were measured for \dot{M}_{O_2} in these feeding trials ($n = 6$ individuals per feeding level; $n = 8$ control individuals). For the calculation of SMR in these individuals, the lowest 10th percentile of data was used after oxygen consumption had reached a clear baseline; data collected while \dot{M}_{O_2} was still elevated due to feeding was not used for the calculation of SMR (all fish had reached a plateau in their \dot{M}_{O_2} within 11 h). To account for any effects of handling during feeding or transfer to the respirometry chambers during the period over which SDA was measured, the mean \dot{M}_{O_2} for the control fish was subtracted from all fed individuals.

For subsequent analyses, meal sizes were also quantified in terms of the percentage body mass each fish consumed. This was achieved by weighing samples of 20 bloodworms (blotted dry to remove excess water) and using the mean mass of these, divided by the total number of bloodworms per sample, as an estimate of the mass of one bloodworm.

Spatial positioning and feeding of schooling fish

Measurements of fish behavior in swimming schools (Movie S1) were performed in a 30 L Steffensen-type swimming flume (Loligo Systems, Viborg, Denmark), designed to cause fish to swim at controlled speeds in non-turbulent water with a uniform velocity profile. Water in the flume was regulated to 13°C and the tunnel had a working (swimming) section that was 45 cm long, 14 cm wide, and 14 cm high. During group trials, the speed of the tunnel was set to 13 cm s⁻¹ (~2 body lengths s⁻¹) to ensure individuals swam aerobically with a steady pace.

To begin a trial, six individuals that had been fasted for 36 h were carefully removed from their holding tanks with dip-nets. This group size was chosen because it allowed the intake of food per individual to be easily quantified and it prevented crowding within the flume. To reduce capture bias, individuals were selected by eye then pursued until capture. Fish were then introduced as a group to the working section of the swim tunnel and water velocity was gradually increased from 0 to 13 cm s⁻¹. Each school was then allowed to settle in the swim flume for 30 min prior to conducting any behavioral observations. Following this period, the behavior of the schools was recorded using a camera (GoPro Hero4, GoPro, California, USA) positioned directly above the flume. Specifically, trials were recorded during 10 min intervals starting at: (1) 10 min before the introduction of food; (2) during a feeding period; (3) 20 min following the conclusion of feeding; (4) 40 min following feeding; and (5) 60 min following feeding. During the feeding

period, individual bloodworms were injected (using a 10 mL syringe) into the flow of the swim tunnel in a randomized location through one of five pieces of aquarium tubing. The tube used for each injection was determined using a random number generator. The aquarium tubing was inserted halfway into the honeycomb flow straightener at the front of the swimming section and was not visible to the fish (Figure S1). During the injection of food, the fish were shielded from the experimenter in order to minimize stress or any cues that would signal the onset of incoming food. During the feeding period (time 0 min), a total of 30 bloodworms were introduced into the flow of the flume with approximately 8 s between injections. This number of food items ensured that there was enough food for some, but not all, individuals within the school to become satiated. Additional control trials (8 groups with 6 fish each) were conducted in which fish were not fed at time 0, but were still video recorded at –10, 0, 20, 40 and 60 min. The fish used for these trials were different fish than those used in the treatment trials, and each control fish was tested in one trial.

All videos were later analyzed to quantify: (1) the number of food items consumed by each individual, and (2) the spatial positioning behavior of individuals within schools during each time period. To determine positioning, still frames of each recording were analyzed at 30 s intervals. The fish in each frame (identified by their unique elastomer tag) were ranked according to their proximity to the front of the school according to the position of their snout (Figure S1). This produced 20 observations per individual per trial for each of the five time intervals described above (i.e., 100 observations per individual per behavioral trial).

QUANTIFICATION AND STATISTICAL ANALYSIS

All models were produced using R v. 3.4.0 (R Development Core Team 2016) using the function `lmer` in package `lme4` [43]. Outputs of statistical analysis are mentioned where appropriate in the Results. Additionally, outputs for all models are presented in Tables S1, S2, S3, S4, and S5. Before inclusion in statistical models, all metabolic attributes (SMR, MMR, and AS) were standardized to a common body mass (2.7 g, the mean mass of all fish in the study at the time of metabolic rate measurements) using the residuals of the relationships between the log-transformed value of each variable and log body mass (see Figure S1). For all models in which AS is described as being used as an explanatory variable, separate models were also constructed which instead used MMR as an index of aerobic capacity. All model outputs are given in Tables S1, S2, S3, S4, and S5, however in the Results section specific values refer to models that used AS. Importantly, the use of either MMR or AS in models did not affect conclusions. Similarly, in cases where meal size (as a percentage of body mass) was used as an explanatory variable, alternative models were run using the absolute number of food items as an index of food intake. Again, all models are given in Tables S1, S2, S3, S4, and S5, but Results refer to models using meal size as a percentage of body mass, and conclusions were not affected by the use of either meal size or number of food items eaten in models.

The factors affecting the position of fish within groups were assessed using a linear mixed effects model (LME) with mean position (within a given time period and trial) as the dependent variable, body mass, SMR, AS (or MMR), and time post-feeding (categorical with five levels: –10, 0, 20, 40, and 60) as fixed factors, and fish ID nested within group and trial number (either trial one or two for an individual fish) as random factors. Repeatability (R) of individual positioning within schools across and within time periods (before, during, and after feeding) was calculated as adjusted (consistency) repeatability using the variance components from this first LME model [44]. The amount of food eaten by each individual while swimming in groups was analyzed using a second LME model with the number of food items obtained as the dependent variable, mass, SMR, AS (or MMR), and position as fixed effects, and fish ID, group number, and trial number as random effects. Within-context repeatability for individual positioning within each time period, as well as repeatability of food captured during feeding, across the two trials was calculated using the variance components from this second LME model.

To examine the effects of feeding on the amount of AS available for swimming, a predictive multiple regression was constructed from the data for fish measured for \dot{M}_{O_2} following feeding. This model used \dot{M}_{O_2} as the response variable, and meal size (in terms of percentage body mass), time post-feeding, and body mass as explanatory variables (see Results). Before inclusion in statistical models, the predicted percentage of AS remaining was standardized to a common body mass (2.7 g, the mean mass of all fish in the study at the time of metabolic rate measurements) using the residuals of the relationships between percent remaining AS and body mass. After applying this model to fish in the swimming trials, the predicted percentage of AS remaining after feeding was analyzed using an LME with standardized percentage of remaining AS as the dependent variable, mass, meal size (or number of food items), SMR, AS (or MMR), and time as fixed effects, and fish ID nested within group and trial number as random effects. Finally, the change in position between the feeding period (time 0 min) and 60 min post-feeding for each individual was analyzed using an LME with the change in position as the dependent variable, mass, meal size (percentage body mass or food items eaten), standardized percentage AS remaining, and time as fixed effects, and fish ID nested within group and trial number as random effects. For all models, model selection proceeded by using maximum likelihood estimation, dropping variables one by one, starting with the variables with smallest t values. Variables were kept in the model if their removal resulted in significantly larger Akaike information criterion value as indicated by likelihood ratio tests. The assumptions of homoscedasticity and normality of residuals were confirmed by visual inspection of residual-fit plots and Q-Q plots. To conform to model assumptions, mass, SMR, MMR, and AS were \log_{10} -transformed.

Significance testing was employed to provide some indication of the strength of evidence for observed patterns, along with model r^2 values using the `MuMIn` 1.9.13 package for R [45]. This included marginal r^2 (r^2_m) and conditional r^2 (r^2_c), which indicate the variance explained by fixed factors and by both fixed and random factors, respectively [46]. P values are generally imprecise

in model outputs and are arbitrary when used as thresholds for declaring statistical significance and problematic and limiting in several ways [47, 48]. Thus, for all models we treat p values as a continuous measure providing an approximate level of evidence against the null hypothesis [49].

DATA AND SOFTWARE AVAILABILITY

All data are available in the Mendeley Data Repository (<https://data.mendeley.com/datasets/8g955t3r9g/1>).

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Supplemental Information

Metabolic Costs of Feeding Predictively

Alter the Spatial Distribution

of Individuals in Fish Schools

Stephanie McLean, Anna Persson, Tommy Norin, and Shaun S. Killen

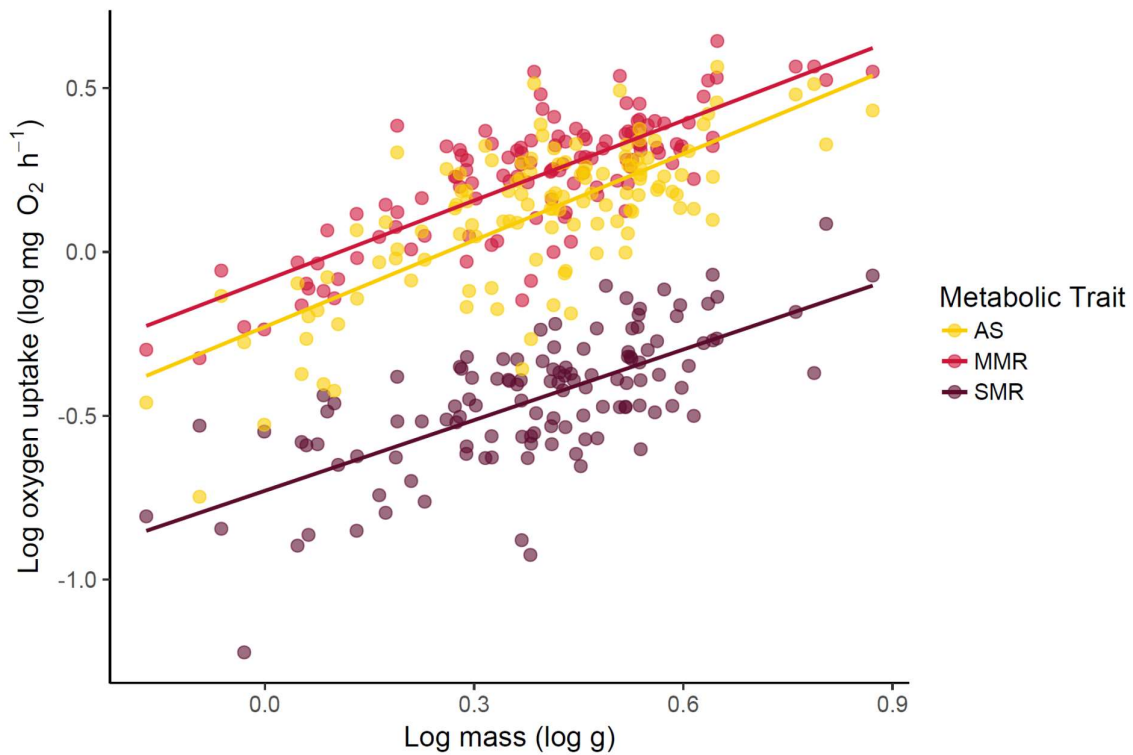


Figure S1. Relationship between metabolic traits measured in common minnows and body mass. Related to mass-standardisation of variables described in STAR Methods. SMR = standard metabolic rate; MMR = maximum metabolic rate; AS = aerobic scope. Regression equations are as follows: $\log \text{SMR} = -0.732 + 0.721(\log \text{mass})$, $r^2 = 0.470$, $p < 0.0001$; $\log \text{MMR} = -0.087 + 0.813(\log \text{mass})$, $r^2 = 0.638$, $p < 0.0001$; $\log \text{AS} = -0.227 + 0.878(\log \text{mass})$, $r^2 = 0.532$, $p < 0.0001$.

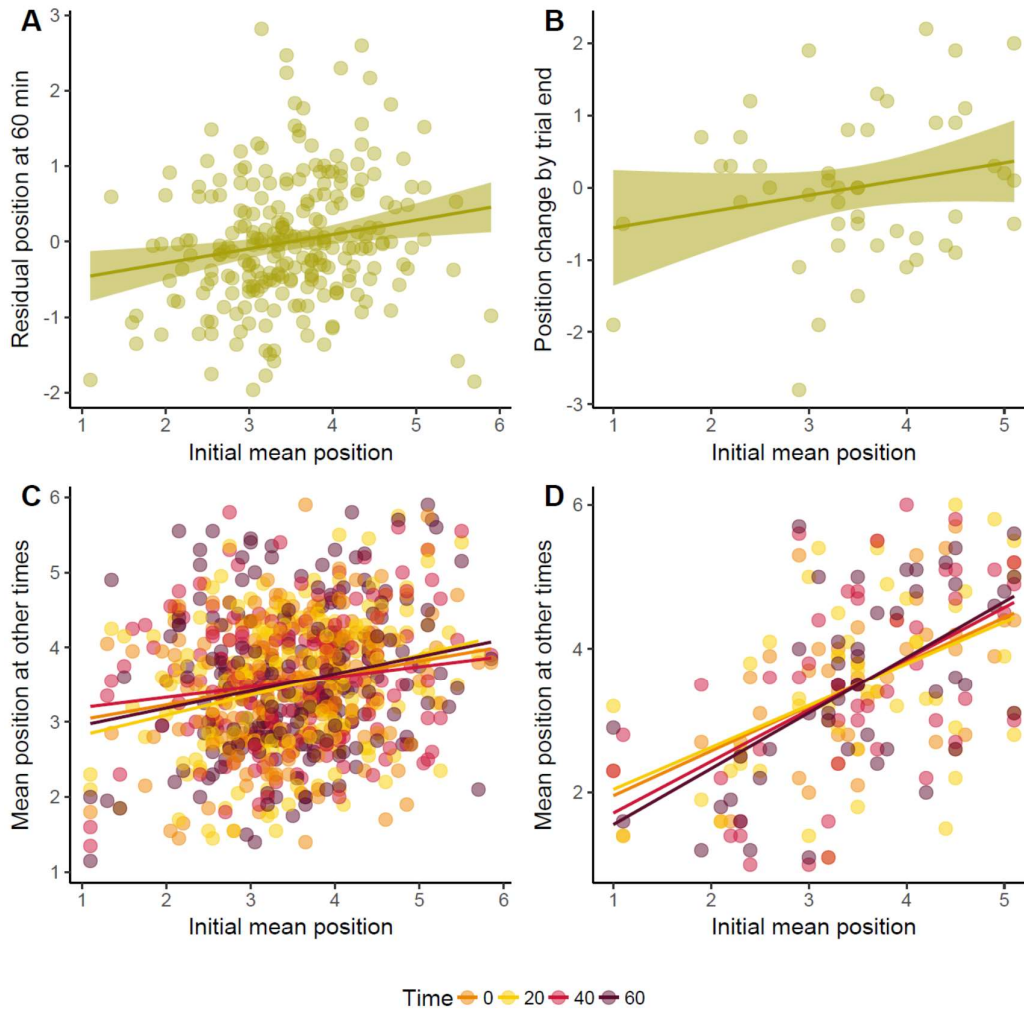


FIGURE S2. Changes in spatial position during trials were not due to fish at the front early in the trial becoming fatigued (independent of food intake) or the result of only being able to move toward the back. Related to Figure 4. (A) For treatment trials, the relationship between residual position at 60 min (after controlling for the total number of food items consumed) and initial spatial position at the beginning of the trial. (B) For control trials in which fish were not fed (8 groups of 6 fish each, with each fish tested one time in one group), the relationship between total change in mean position during the course of the trial and the initial position of each individual (10 minutes before time 0, the point at which fish would have been fed in treatment trials). (C) For treatment trials, spatial positioning of individuals at various times post-feeding, in relation to their initial position 10 minutes before feeding; (D) Spatial positioning during control trials in which fish were not fed (8 groups of 6 fish each, with each fish tested one time in one group) at various times in relation to the position of fish when the trial first began (10 minutes before time 0). Points are coloured according to the variable “Time”, which represents the time in relation to when fish would have been fed in the treatment trials (i.e. time 0, and at 20, 40, and 60 min after this time). In all panels, each data point = 1 individual fish. For visual reference, lines in each panel represent linear regression through points; shaded area is 95% CI for regression. Model outputs are given in Table S5.

	estimate	s.e.	df	t	p	r ² _m	r ² _c
<i>model with AS as a fixed factor</i>							
intercept	3.239	0.169	190.70	19.165	< 0.0001	0.129	0.427
log mass (log g)	0.695	0.252	239.70	2.757	0.006		
food items	-0.041	0.014	751.00	-2.811	0.005		
log SMR (log mg O ₂ h ⁻¹)	-0.255	0.249	240.00	-1.026	0.306		
log AS (log mg O ₂ h ⁻¹)	-0.016	0.237	240.00	-0.067	0.947		
time (min)							
0	0.364	0.094	976.00	3.890	0.0001		
20	-0.032	0.094	976.00	-0.338	0.735		
40	-0.331	0.094	976.00	-3.530	0.0004		
60	-0.659	0.094	976.00	-7.027	< 0.0001		
food items x time (min)							
0	-0.080	0.016	976.00	-5.026	< 0.0001		
20	0.0076	0.016	976.00	0.480	0.631		
40	0.082	0.016	976.00	5.162	< 0.0001		
60	0.152	0.016	976.00	9.517	< 0.0001		
<i>model with MMR as a fixed factor</i>							
intercept	3.238	0.194	219.30	16.710	< 0.0001	0.129	0.427
log mass (log g)	0.694	0.253	249.70	2.756	0.006		
food items	-0.041	0.014	751.30	-2.816	0.005		
log SMR (log mg O ₂ h ⁻¹)	-0.252	0.246	240.00	-1.024	0.306		
log MMR (log mg O ₂ h ⁻¹)	-0.003	0.312	240.10	0.000	0.999		
time (min)							
0	0.364	0.094	976.00	3.890	0.0001		
20	-0.032	0.094	976.00	-0.338	0.735		
40	-0.331	0.094	976.00	-3.530	0.0004		
60	-0.659	0.094	976.00	-7.027	< 0.0001		
food items x time (min)							
0	-0.080	0.016	976.00	-5.026	< 0.0001		
20	0.008	0.016	976.00	0.480	0.631		
40	0.082	0.016	976.00	5.162	< 0.0001		
60	0.152	0.016	976.00	9.517	< 0.0001		

TABLE S1. Results of linear mixed effects models examining factors affecting the mean position of individual fish within swimming schools. Related to Figure 2. SMR = standard metabolic rate; MMR = maximum aerobic scope; AS = aerobic scope. Note that each of SMR, MMR, and AS were standardised to a common mass of 2.7 g (the mean mass of all fish in the study at the time of oxygen uptake measurements) before use in models. The term ‘mass’ refers to body mass at the time of the group swimming trials in the flume. Separate models were constructed to use either MMR or AS as indices of aerobic capacity. For each model, fish ID nested within group and trial number (each fish was tested twice in two different groups) were used as random effects.

	estimate	s.e.	t	p	r ²
<i>response: peak oxygen uptake</i>					
intercept	-1.325	0.645	-2.056	0.055	0.599
log mass (log g)	3.067	0.775	0.959	0.001	
log SMR (log mg O ₂ h ⁻¹)	-0.373	0.392	-0.952	0.355	
meal size (%)	1.019	0.324	3.146	0.006	
intercept	-0.589	0.518	-1.139	0.271	0.557
log mass (log g)	1.614	0.530	3.044	0.007	
log SMR (log mg O ₂ h ⁻¹)	-0.311	0.414	-0.750	0.463	
food items	0.063	0.024	2.678	0.016	
<i>response: time at peak (min)</i>					
intercept	67.659	123.729	0.547	0.592	0.056
log mass (log g)	4.153	148.685	0.028	0.978	
log SMR (log mg O ₂ h ⁻¹)	-41.370	75.282	-0.550	0.590	
meal size (%)	16.094	62.202	0.259	0.799	
intercept	110.034	94.222	1.168	0.259	0.056
log mass(log g)	-34.078	96.498	-0.353	0.728	
log SMR (log mg O ₂ h ⁻¹)	-21.589	75.436	-0.286	0.778	
food items	-1.037	4.302	-0.241	0.812	
<i>response: time until return to SMR (min)</i>					
intercept	-190.4	253.700	-0.751	0.463	0.509
log mass (log g)	455.0	304.900	1.492	0.154	
log SMR (log mg O ₂ h ⁻¹)	-105.5	154.400	-0.683	0.504	
meal size (%)	484.9	127.500	3.802	0.001	
intercept	115.134	195.502	0.589	0.564	0.498
log mass (log g)	-214.013	200.226	-1.069	0.300	
log SMR (log mg O ₂ h ⁻¹)	-102.991	156.523	-0.658	0.519	
food items	33.041	8.926	3.702	0.002	

TABLE S2. Results of general linear models examining factors affecting oxygen uptake following feeding in minnows. Related to Figure 3A. SMR = standard metabolic rate. For each response variable, separate models were constructed to use either meal size (% body mass) or number of food items consumed as indices of food intake.

	estimate	s.e.	df	t	p	r ² _m	r ² _c
<i>model with meal size as a fixed factor</i>							
intercept	80.178	2.311	68.30	34.700	< 0.0001	0.799	0.937
log mass (log g)	-32.091	2.772	240.50	-11.578	< 0.0001		
meal size (%)	-32.581	2.452	369.80	-13.287	< 0.0001		
log SMR (log mg O ₂ h ⁻¹)	-11.716	2.884	240.00	-4.053	< 0.0001		
log MMR (log mg O ₂ h ⁻¹)	80.068	3.645	240.00	21.974	< 0.0001		
time (min)							
40	-4.299	0.601	488.00	-7.149	< 0.0001		
60	-10.307	0.601	488.00	-17.139	< 0.0001		
meal size (%) x time (min)							
40	-10.661	1.923	488.00	-5.533	< 0.0001		
60	-25.558	1.923	488.00	-13.265	< 0.0001		
<i>model with food items as a fixed factor</i>							
intercept	69.077	2.320	123.20	29.780	< 0.0001	0.791	0.946
log mass (log g)	-10.623	3.101	241.00	-3.427	0.0007		
food items	-1.661	0.137	330.30	-12.104	< 0.0001		
log SMR (log mg O ₂ h ⁻¹)	-12.938	3.021	240.00	-4.286	< 0.0001		
log MMR (log mg O ₂ h ⁻¹)	78.748	3.810	240.00	20.671	< 0.0001		
time (min)							
40	-3.966	0.545	488.00	-7.272	< 0.0001		
60	-9.509	0.545	488.00	-17.433	< 0.0001		
food items x time (min)							
40	-0.650	0.093	488.00	-6.993	< 0.0001		
60	-1.560	0.093	488.00	-16.766	< 0.0001		

TABLE S3. Results of linear mixed effects models examining the estimated percentage of aerobic scope remaining after feeding, after accounting for the approximate costs of digestion and meal assimilation. Related to Figure 3B and 3C. SMR = standard metabolic rate; AS = aerobic scope. Note that each of SMR and AS were standardised to a common mass of 2.7 g (the mean mass of all fish in the study at the time of oxygen uptake measurements) before use in models. The term ‘mass’ refers to body mass at the time of the group swimming trials in the flume. Separate models were constructed to use either meal size (% body mass) or number of food items consumed as indices of food intake. For each model, fish ID nested within group and trial number (each fish was tested twice in two different groups) were used as random effects.

	estimate	s.e.	df	t	p	r ² _m	r ² _c
<i>model with food items as a fixed factor</i>							
intercept	0.494	0.183	282.40	2.697	0.0074	0.310	0.722
log mass (log g)	-0.230	0.367	243.10	-0.627	0.531		
food items	-0.085	0.017	404.00	-4.935	< 0.0001		
time (min)							
40	0.297	0.086	487.30	3.446	0.0006		
60	0.627	0.086	487.20	7.279	< 0.0001		
food items x time (min)							
40	-0.075	0.015	487.20	-5.089	< 0.0001		
60	-0.144	0.015	487.20	-9.820	< 0.0001		
<i>model with meal size as a fixed factor</i>							
intercept	1.163	0.200	278.70	5.803	< 0.0001	0.279	0.710
log mass (log g)	-1.648	0.352	243.10	-4.681	< 0.0001		
meal size (%)	-1.468	0.322	425.00	-4.559	< 0.0001		
time (min)							
40	0.264	0.090	487.30	2.924	0.0036		
60	0.575	0.090	487.10	6.385	< 0.0001		
meal size (%) x time (min)							
40	-1.246	0.289	487.20	-4.315	< 0.0001		
60	-2.455	0.289	487.10	-8.504	< 0.0001		
<i>model with remaining AS as a fixed factor</i>							
intercept	-1.581	0.376	253.60	-4.204	< 0.0001	0.150	0.700
log mass (log g)	-1.605	0.395	241.80	-4.060	< 0.0001		
remaining AS (%)	0.028	0.004	485.60	7.564	< 0.0001		
time (min)							
40	0.151	0.064	593.50	2.373	0.0179		
60	0.432	0.084	730.70	5.156	< 0.0001		

TABLE S4. Results of linear mixed effects models examining factors affecting the change in mean position of individual fish after feeding in swimming schools. Related to Figure 4. SMR = standard metabolic rate; AS = aerobic scope. Note that each of SMR and AS were standardised to a common mass of 2.7 g (the mean mass of all fish in the study at the time of oxygen uptake measurements) before use in models. The term 'mass' refers to body mass at the time of the group swimming trials in the flume. Separate models were constructed to use either meal size (% body mass) or number of food items consumed as indices of food intake. The percentage of AS remaining was not included in models with food intake because of the strong correlations between variables. For each model, fish ID nested within group and trial number (each fish was tested twice in two different groups) were used as random effects.

	estimate	s.e.	df	t	p	r ² _m	r ² _c
<i>residual position at 60 min</i>							
intercept	-0.917	0.302	152.10	-3.034	0.003	0.042	0.295
log mass (log g)	0.649	0.383	153.33	1.695	0.092		
initial position	0.171	0.066	240.54	2.592	0.010		
<i>mean position during control trials</i>							
intercept	0.004	0.511	143.59	0.008	0.993	0.375	0.610
log mass (log g)	-0.052	0.416	45.00	-0.125	0.901		
initial position	1.002	0.142	141.60	7.067	< 0.0001		
time (min)							
0	1.335	0.569	184.00	2.344	0.02		
20	1.459	0.569	184.00	2.563	0.011		
40	1.000	0.569	184.00	1.757	0.081		
60	0.780	0.569	184.00	1.371	0.172		
initial position x time (min)							
0	-0.389	0.157	184.00	-2.416	0.017		
20	-0.417	0.157	184.00	-2.651	0.009		
40	-0.285	0.157	184.00	-1.813	0.071		
60	-0.225	0.157	184.00	-1.431	0.154		
<i>change in position during control trials</i>							
intercept	-0.796	0.561		-1.419	0.163	0.045	
log mass (log g)	0.197	0.645		0.306	0.761		
initial position	0.217	0.156		1.382	0.174		

TABLE S5. Summary of analyses demonstrating that changes in position are not due to individuals spending more time at the front of the group or having nowhere to move besides moving backward. Related to Figure 4 and Figure S2. The first linear mixed effects model examines the relationship between residual position at 60 min post-feeding (after correcting for total food items consumed) and initial position occupied by fish at the beginning of trials. Model also included fish ID, group, and trial number as random factors. The second linear mixed effects model examines factors affecting the mean position occupied by fish during control trials in which individuals were not fed over a 70 min time period. Model also included fish ID nested within group as a random factor. The final model is a general linear model examining factors affecting change in positioning in fish during control trials in which individual were not fed, over a 70 min time period.