Solving post-prandial reduction in performance by adaptive regurgitation in a freshwater fish

Jian Zhao[1,#], Yanci Wen[1,#], Songming Zhu[1,2], Jinyun Ye[3], Junjie Zhu[3], Zhangying Ye[1,2,*], Alex Jordan[4,5*]

1. College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, 310000, China

2. Ocean Academy, Zhejiang University, Zhoushan, 316000, China

3. School of Life Sciences, Huzhou Normal University, Huzhou, 313000, China

4. Department of Collective Behaviour, Max Planck Institute of Animal Behaviour, Konstanz, 78467, Germany

5. Centre for the Advanced Study of Collective Behaviour, University of Konstanz, Konstanz, 78467, Germany

Correspondence and requests for materials should be addressed to Z. Y. (email: yzyzju@zju.edu.cn) and A. L. J. (email: ajordan@ab.mpg.de)

<u>Running Head</u>

Adaptive regurgitation in the face of threat

<u>Abstract</u>

Foraging animals must balance benefits of food acquisition with costs induced by post-prandial reduction in performance. Eating to satiation can lead to a reduction in locomotor and escape performance, which increases risk should a threat subsequently arises, but limiting feeding behaviour may be maladaptive if food intake is unnecessarily reduced in prediction of threats that do not arise. The efficacy of the trade-off between continued and interrupted feeding therefore relies on information about the future risk, which is imperfect. Here we find that black carp (*Mylopharyngodon piceus*) can balance this trade-off using an *a posteriori* strategy; by eating to satiation but regurgitating already ingested food when a threat arises. While degrees of satiation (DS) equal to or greater than 60% reduce elements of escape performance (turning angle, angular velocity, distance moved, linear velocity), at 40% DS or lower performance in these tasks approaches levels comparable to that at 0% satiation. After experiencing a chasing event, we find that fish are able to regurgitate already ingested food, thereby changing the amount of food in their gastrointestinal tract to consistent levels that maintain high escape performance. Remarkably, regurgitation results in degrees of satiation between 40%-60% DS, regardless of whether they had previously fed to 40%, 60%, or 100% DS. Using this response, fish are able to maximize food intake, but regurgitate extra food to maintain escape performance when they encounter a threat. This novel strategy may be effective for continual grazers and species with imperfect information about the level of threat in their environment.

<u>Keywords</u>: foraging; escape; trade-off; performance; regurgitation

Introduction

Maximizing food intake has clear benefits for energy-dependent biological processes, including growth, movement, and reproduction. Indeed, understanding how animals manage energetic and temporal trade-offs in foraging contexts has seen the development of entire fields including optimal foraging theory [1]. For animals with indeterminate growth such as fish, higher food intake leads to faster growth, which is advantageous in contexts of predator avoidance [2], territory defence [3], and mate acquisition [4]. However, the increase in mass after feeding, as well as internal metabolic processes associated with digestion, may reduce performance in other essential traits such as locomotion and vigilance. In fishes, for example, feeding changes postprandial metabolism, body morphology, and mass [5,6], resulting in reduced swimming performance. Moreover, overfeeding reduces the capacity for fish to perform fast-start escape responses [6,7], which can be hazardous under natural contexts as the escape response is essential to fish survival when encountering predation threat [7,8]. In animals including humans [10,11] and fruit-flies [12] eating can induce post-prandial sleep, which may solve any potential deficits in performance, but is only possible when threats are absent. Yet for many animals post-prandial rest is not possible as it impedes vigilance and potential escape from threats including predators.

 A trade-off may therefore arise, in which the benefits of feeding are opposed by the costs of reduced performance and escape efficiency. To date, work in this area, focussed on fish, has suggested individuals may satisfy this trade-off by limiting food intake in threatening contexts to maintain swimming performance [2,13,14]. These studies primarily focus on decisions before feeding, assuming that an assessment about the future state of threat is made and current feeding decisions are based on that assessment. However, in unpredictable environments, for example those in which predation is a rare but highly consequential event, limiting current intake may be maladaptive as it results in type I, or false positive, error. Moreover, because of the advantages of faster growth, for many animals, and in particular grazing or continually feeding animals, feeding may take priority even in potentially dangerous situations, and this phenomenon may be more pronounced when food is scarce [15].

A potential solution to the performance problems associated with overfeeding is regurgitation. In fishes, regurgitation under threat has been observed in the context of fishing and capture stress [16,17]. However, the consequences of regurgitation for performance have not been explored, and it is not known whether this regurgitation behaviour is part of an *a posteriori* solution to the trade-off between feeding and escape. In this study, we investigated this link in juvenile black carp (*Mylopharyngodon piceus*, a common freshwater fish in Yangtze river basin, China, which is stomachless but with an enlarged esophagus to store food). We examined the relationship between escape performance and the amount of food ingested in juvenile black carp, as well as the ability of the fish to regurgitate already ingested food when faced with threat. Results indicate that *a posteriori* regurgitation allowed fish to solve the trade-off between maximising energy intake when foraging opportunities are present, and maintaining escape performance when threats arise.

Methods

(a)   Animal model

Black carp (*Mylopharyngodon piceus*; quantity: N = 108, mass: 11.85 ± 1.38 g, total length: 12.46 ± 0.51 cm) used in this study were collected using purse seine nets from a semi-wild fishpond at Hangzhou Jianfeng Agriculture Development Co. Ltd (Zhejiang, China). Before experiments, fish were maintained in the laboratory (Zhejiang University) using a recirculating aquaculture system (radius: 80 cm, height: 45 cm; water temperature: 23-25 °C, pH: 7.0-8.0, dissolved oxygen: 6.0-7.2 mg/L, ammonia nitrogen: <0.6 mg/L, chemical oxygen demand: <1.5 mg/L) on a 12 L:12 D photoperiod for approximately 5 months. All individuals were fed daily using commercial feed (pellets). In this study, fish were in a juvenile (non-reproductive) state and thus sex could not be reliably 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, and so males and females should have been equally distributed among groups.

(b)   Details of experiments

Protocol overview

We performed three experiments in this study. For experiment I, 81 experimental fish were used to characterize food-expulsion behaviour, exploring whether the food amount expelled by fish when chased with a hand net tended toward any particular ratio. In experiment II, 27 experimental fish were selected for dissection to determine whether the amount of food expelled was related to the degree of already digested food, specifically that only undigested food was being expelled. In experiment III, 80 experimental fish were used to evaluate fast-start escape responses of fish and disentangle whether and how the expelled food affected fast-start escape response of fish.

Experiment I and III were conducted in a Brett-type open swimming flume (Figure S1A), designed to cause fish to swim at controlled speeds in non-turbulent water with a uniform velocity profile. Water in the flume was regulated between 23.8-25 °C and the tunnel had a working section that was 50 cm long, 20.5 cm wide, and 10.3 cm high. During experiments, the speed of the water flow in the tunnel was set to 3 cm/s (~0.24 BL/s). This flow speed was selected to allow fish to rapidly adapt to the chamber environment while minimizing interference with food intake. During experiments, food pellets (diameter: ~2.40 mm, mass: ~6.34 mg) were delivered manually through a paper tube mounted above the flume. Meanwhile, fish in the flume were shielded from the experimenter and surroundings by sky blue screens and black curtains to minimize stress (Figure S1). Fish behaviour (including feeding and escape) was monitored and recorded using a camera (BASLER acA2040-90um, resolution: 2048×2048, frame rate: 90 fps, Germany) positioned directly above the flume.

For the feeding strategy during experiments, two food pellets per fish were fed at intervals of 10-12 seconds. This feeding procedure was stopped when the corresponding degree of satiety (DS) was met.

Following this feeding strategy, fish in a group had equal access to food, and consumption distribution was fairly equal (see experiment SII in Supplementary Methods and Results). Maximum food intake (which was used to calculate 100% DS; detailed in experiment I) was defined at the point when one or no pellet were eaten per event, in three successive feeding events. Following the criterion above, the feeding durations spent to achieve 40%, 60% and 100% DS were ~121 s, ~187 s, ~319 s, respectively.

All experiments were carried out between 9:30 and 18:00 daily to conform with established circadian rhythms. Before each experiment fish were deprived of food for 36-48 h to ensure empty gastrointestinal tracts. Mass and the total length of fish were measured after each experiment to minimize the impact of stress on behaviour during experiments.

Experiment I: characterization of food-expulsion behaviour

Three batches, each of 27 experimental fish, were haphazardly selected for this experiment. These batches were quantified under satiety regimes of 100%, 60% and 40% DS, respectively. For each batch, fish were distributed into nine groups of three. This group size was chosen because it allowed fish to adapt to the new environment quickly, leading to natural feeding behaviour, and prevented crowding within the flume. Single fish are typically not responsive in feeding trials due to isolation stress. For each group, two overfeeding trials were conducted, separated by intervals of 36-48 h to empty gastrointestinal tract, following the feeding strategy described above. The 100% DS of each group was then determined by averaging the two maximum food intakes. It should be noted that, for all groups, no food was expelled during the 20-min continuous observation after the feeding in the first overfeeding trial.

Before the experiment, fasted fish in each group were carefully removed from their holding tanks to the test flume using a dip-net. Each group was then allowed to settle in the test flume for 90 min prior to experiments. Each fasted group was then fed to the corresponding experimental DS (100%, 60%, 40%) based on pre-determined 100% DS. Five minutes after this feeding event, the threat stimulus was introduced using a dip-net. During the execution of the threat stimulus, each of the three fish was chased in turn for five seconds. However, if food expulsion behaviour occurred during chasing, the chasing duration was prolonged by five seconds (as the maximum time interval between the two adjacent food-expulsion behaviours, as well as the start of the chasing and the first food-expulsion behaviour, during the long chasing did not exceed 5 seconds; see experiment S1 in Supplementary Methods and Results), such that food expulsion behaviour was not interrupted by the cessation of the stimulus. Subsequently, individual expelled food pellets were removed from the flume, and counted to characterize the food-expulsion behaviour. Because there was a five-minute pause between feeding and chasing, there was sufficient time for pellets to be swallowed by fish (so they were not being mechanically expelled from the buccal cavity (i.e. the "mouth"), but not to be digested.

Experiment II: quantification of the undigested and formed food in fish

The remaining 27 fish were distributed among nine further groups, with three groups each at 100%, 60% and 40% DS, respectively. After the determination of 100% DS following the same steps in experiment I, groups were transferred to nine glass tanks (48 cm L × 28 cm W × 12 cm H). To begin the experiment, fish were fed to the corresponding DS, following the feeding strategy above, after a 90-minute acclimation. Five minutes after feeding, lethal doses of MS-222 (~550 mg/L) were mixed into each tank, euthanizing fish within 90 seconds. Fish were then dissected and the undigested and formed food pellets in each tank were counted within 10 minutes. Due to the improper storage, data from two tanks corresponding to 100% and 60% DS were lost.

Experiment III: evaluation of the fast-start escape response

Three weeks after experiment I, 80 experimental fish that had been used in that experiment were used once again in experiment III. These individuals were randomly assigned to four groups which corresponded to 100%, 60%, 40% and 0% DS, respectively. As neither the mass nor total length of fish changed appreciably between experiment I and experiment III, experiment III was carried out following the average 100% DS quantified in experiment I. A single fasted fish from each treatment group was introduced to the test flume to evaluate individual fast-start escape responses. After a 90-minute acclimation period, each fish was fed to its corresponding DS following the feeding strategy above. Within a second five-minute period, a mechanical stimulus was triggered to startle the fish (Figure 1A). This second period was set to provide fish enough time to swim to the designated stimulus onset area (the area within the dotted box as depicted in Figure 1B). Only when fish swam to the designated area and maintained stationary swimming would the stimulus be triggered (the stimulus was triggered behind the fish, within the 20-25% blind spot reported for cyprinid fishes [18]). Fast-start escape responses were recorded by the monitoring system once the stimulus was triggered. In this experiment, the stimulus adopted was an aeration stone (diameter: ~36 mm) hung close to the retaining screen using a thread. Once the stimulus was triggered, the aeration stone would hit the centre of the screen. This experiment was necessarily run with individual fish, and in isolation not all fish fed to the required amount DS. Fish that did not consume required DS targets were removed from further analyses and returned to their housing tanks.

To rule out interference from stressed individuals, fish displaying signs of stress during the experiment were not considered in data analysis. The definition of stressed individuals are as follows: fish in 0% DS-based group were fed one food pellet before experiments; if the fish did not eat or exhibited abnormal behaviours (such as startling and hiding in the corner) after feeding, it was considered stressed. For fish in 40%, 60% and 100% DS-based groups, any fish that did not consume the entire provided amount, or that exhibited abnormal behaviours after feeding were also considered stressed. Following these criteria, the quantities of the valid samples in 0%, 40%, 60% and 100% DS-based groups were 12, 14, 11 and 10, respectively. To evaluate the fast-start escape response, a "Deeplab v3+" [19] based in-house code, written in Matlab 2019a, was used to track the fish and extract boundary points and midline kinematics from the recorded

videos. For the evaluation, the occurrence rate of the immediate response, durations of Stage 1 and 2, Stage 1 kinematics (comprising turning angle, average, and peak angular velocities), Stage 2 kinematics (comprising distance moved, average and peak linear velocities following Stage 1) during the entire fast-start escape response were calculated [9,20,21].
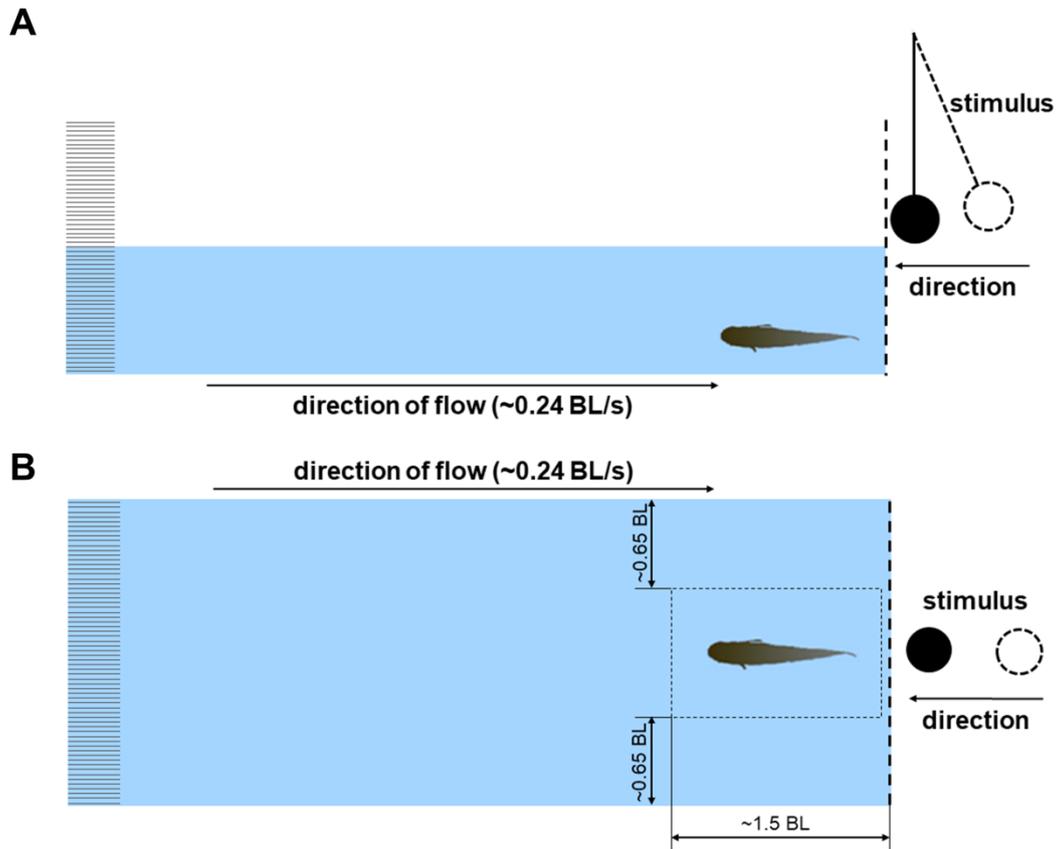

Figure 1. Views of swimming flume during the evaluation of the fast-start escape response
(A) Front view of swimming flume during the evaluation of the fast-start escape response. (B) Top view of swim flume during the evaluation of the fast-start escape response.

(c)   Statistical analysis

In this study, all statistical analysis was performed in SPSS Statistics 25. All data are presented as the means ± SD and analysed using one-way ANOVA with alpha set at 0.05 significance threshold, followed by LSD multiple-comparison tests (used for homoscedastic error) and Games-Howell multiple-comparison tests (used for heteroscedastic error). The normality and heteroscedasticity of all data were checked by One-sample Kolmogorov-Smirnov test and Levene's test, respectively.

Results

Experiment I: regurgitation leaves a consistent amount of food in the gastrointestinal tract, irrespective of initial amount

We allowed experimental fish to feed to satiation (figure. 2A), then exposed them to a threatening stimulus (chasing with hand net). We found that under this threat, fish were able to regurgitate and expel consumed food, while in the absence of threat no food was expelled. Because threats were introduced five minutes after feeding had ended, food had already been swallowed. Expelled food was therefore from the gastrointestinal tract rather than the buccal cavity.

In this study, we calculated food ingested as a proportion of maximum degree of satiation, where 100% DS corresponds to the total amount of food ingested until cessation of feeding. We then examined how much food was expelled compared to the maximal, or 100% degree of satiation (DS) by examining gastrointestinal contents after feeding and threat presentation. When fish were fed to 40% of satiation, the amount of expelled food was low – on average 1.00% was expelled and a mass of food corresponding to 39.0% DS of ingested food remained in the gastrointestinal tract. When fish were fed to 60% DS, on average 8.66% of food was expelled after threat, leaving a mass of food corresponding to 51.34% DS in the gastrointestinal tract. However, when fish were fully satiated the amount of expelled food was far higher, up to 49.16% of gastrointestinal contents were expelled following threat. Across the varying degrees of satiation, the residual amount of food that remained in the gastrointestinal tract tended to stabilize between 40% and 60% DS (figure. 2B), and no difference ($p > 0.05$, $F_{1,16} = 0.047$) was observed in residual amount of food remaining in gastrointestinal tract between 60% and 100% DS.

Experiment II: regurgitation here is not simply a process of expelling all undigested food

To explore the possibility that fish were expelling only all undigested food particles, we assessed the number of digested and undigested food pellets remaining in the gastrointestinal tract as a function of DS (figure. 2C). The total proportion of undigested food pellets remaining was high regardless of degree of satiation, demonstrating that it was not simply a process of all undigested food being expelled. Rather, following threat a consistent regurgitation of ingested food to between 40%-60% DS was observed.
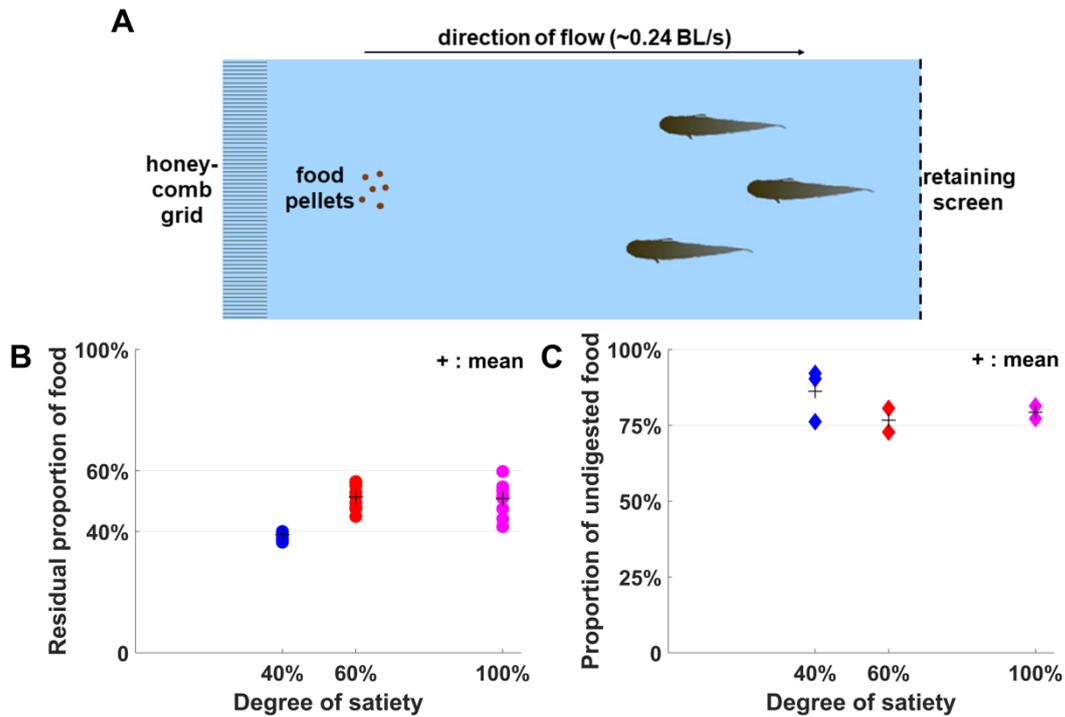
Figure 2. Characterization of food-expulsion behaviour

(A) Top view of swimming flume during characterization of food-expulsion behaviour. (B) Residual proportion of food (i.e., percentage of the residual amount of food to the relative feeding amount representing 100% DS) following fishing threat. (C) Proportion of undigested food (i.e., percentage of the amount of undigested food to the relative feeding amount) following the specific fishing threat.

Experiment III: increasing satiation lowers escape performance

We measured escape performance of individual fish, at different degrees of satiation, in an open swimming flume using a controlled threat stimulus (Figure 1A and 1B). During escape, the lower the escape latency, the higher the survival rate of fish is expected to be [9]. The most common response to the experimental threat stimulus was an immediate escape, across all levels of DS (figure. 3), however, the probabilities of the torpid response (i.e., fish escapes from original location but does so with a delay) and freezing response (i.e., fish is alert but unmoving) increase with increasing DS (see also mass-standardized DS in Figure S2), suggesting that higher degrees of satiation change escape strategy and may lower survival under natural conditions.
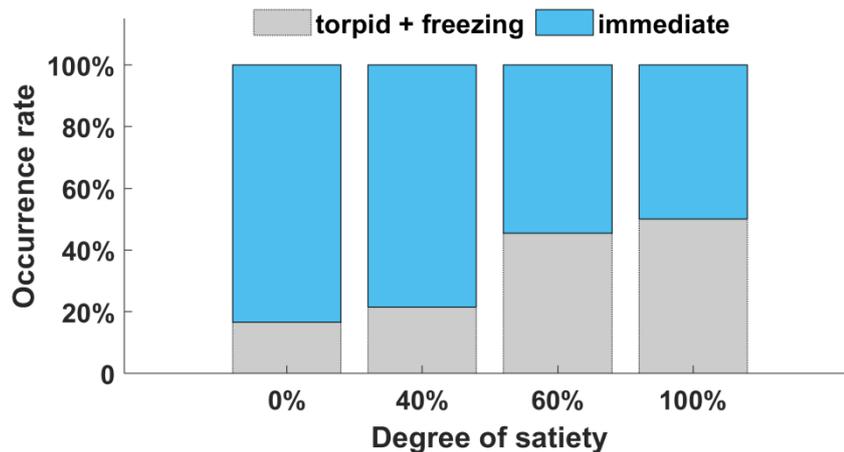
Figure 3. Evaluation of the fast-start escape response (occurrence rates of different escape responses)
The occurrence rates of different responses to the mechanical threat stimulus at varying degrees of satiation between
0 and 100%. "immediate" signifies immediate escape from the stimulus (blue shaded sections), whereas
torpid+freezing (grey shaded sections) indicate escape after a delay (torpid), or no escape at all (freezing).

We then measured the escape kinematics of the immediate responses, based on C-start (i.e., the most prevalent and best-known escape response [22,23]) and forward movement (i.e., fish escapes forward rather than towards the wall). For Stage 1 kinematics (the 'bending phase', which is defined as the onset of the movement of the head and ends when the head stops turning [24]); the turning angle (related to the propulsive force for stage 2 kinematics [25]); average and peak angular velocities (related to evasion from a threat [26]) were quantified. The highest turning angles were achieved at 40% DS (figure. 4A and table S1). Mean angular velocity was highest in 0% and 40% DS (although 40% and 60% DS mean values were not significantly different), and similarly peak angular velocity was highest at 0% and 40% DS (figure. 4B and table S2).

Following stage 1, fish rapidly swam away from the initial location (mainly reflected in Stage 2 kinematics, which is defined as the 'straightening phase' after a C-shaped turn [24]). The faster a fish flees location of the threat, the greater the chance of successful evasion of that threat. To assess this performance, we calculated the main behavioural components of the fast-start escape response: average and peak linear velocities, and the distance travelled in Stage 2. Both the linear velocities and distance travelled were lower with increasing DS (figure. 4C and 4D), and linear velocities were significantly different between DS ≤ 40% and DS ≥ 60% for most treatments (figure. 4D and tableS4).

We then analysed the distance travelled and the peak linear velocity during the entire fast-start escape response (figure. 5A). The temporal threshold we used to define the duration of the entire escape response – 44.4 ms – reflected the fastest duration of Stage 1 and 2 we observed in our experiments [20]). A negative trend with increasing DS was observed in these kinematic parameters. At 0% DS, total distance travelled

and peak linear velocity were significantly higher than at 60% and 100% DS. However, no significant differences for the total distance travelled nor peak linear velocity was detected between 0% and 40% DS (figure. 5B and table S5).
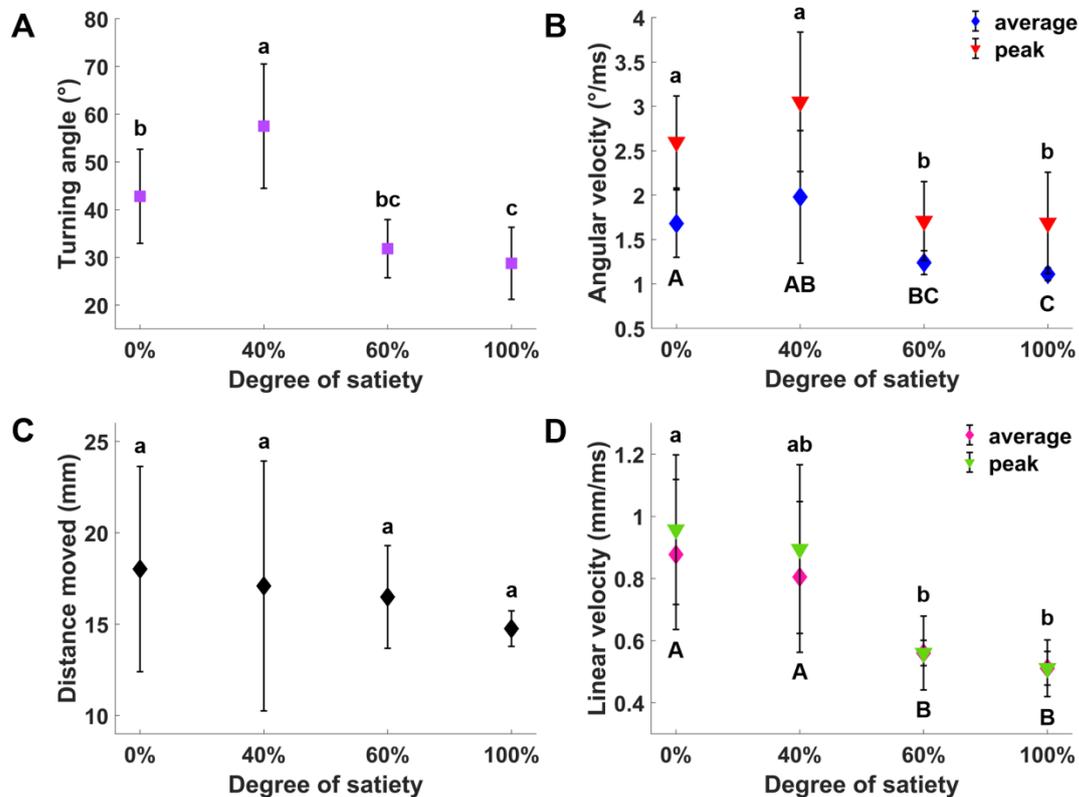

Figure 4. Evaluation of the fast-start escape response (Stage 1 and 2)

(A) Turning angle in stage 1 under different degrees of satiety. (B) Average and peak angular velocities in stage 1 under different degrees of satiety. Note that the duration of Stage 1 varies among individuals (figure S3). (C) Distance moved by fish (i.e., the centre of mass) in Stage 2 under the different degrees of satiety. (D) Average and peak linear velocities in Stage 2 under the different degrees of satiety. Note that the duration of Stage 2 varies from individual to individual (figure. S3). Upper case letters signify p < 0.05 for average angular velocity and average linear velocity; lower case letters signify p < 0.05 for turning angle, distance moved, peak angular velocity and peak linear velocity.
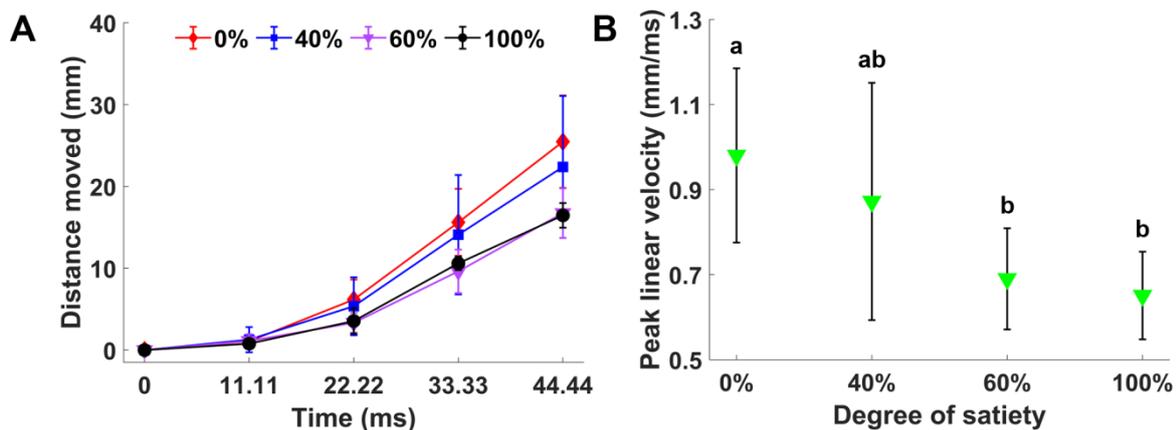
Figure 5. Evaluation of the whole fast-start escape response

(A) Distance moved by fish (based on centroid) during the fast-start escape response (within 44.44 ms) under the different degrees of satiety. (B) Peak linear velocity during the fast-start escape response (within 44.44 ms) under the different degrees of satiety. Lower case letter signify $p < 0.05$ for peak linear velocity.

Discussion

Here we demonstrate that a continually foraging fish, the black carp, is able to employ a novel strategy to solve the trade-off between foraging and escaping risk. Our experiments demonstrate that these fish will eat to satiation given the chance (which we here define as 100% degree of satiation DS), but at this DS performance in escape and locomotion is impaired. This post-prandial locomotor impairment is common for many animals, and can severely increase predation risk, and to avoid this risk, many animals interrupt feeding prior to satiation [27]. Based on our results, black carp appear able to solve this problem in another way – regurgitating and expelling already ingested food when threatened. This expulsion is not complete, but rather leaves the fish with approximately 40%-60% DS in the gastrointestinal tract (figure. 2B and 2C). This leaves an amount of residual food that approaches the 40% DS that brings many aspects of locomotor and escape performance to maximum levels (figure. 3, 4 and 5). As such, this strategy appears to approach the optimal trade-off between feeding to satiation and maintaining escape performance, but does so *a posteriori* only when a threat is encountered. Although this response is unlikely to provide an immediate benefit in avoiding a predator, if one predator encounter is indicative of increased probability of subsequent encounters, regurgitation and the associated improvements in performance would be adaptive in high risk environments, or when the perceived landscape of fear shifts [28]. In many ecological contexts, predation risk is clumped in space and time [29]. As such, animals may use recent history as a proxy for future contexts (for example in mating contexts in guppies [30]), thereby updating behaviour, or in this case amount of food in the gastrointestinal tract, in an adaptive manner. More generally, it is possible that feeding to satiation in the absence of immediate threat, and subsequently expelling food when threat arises, allows individuals to maximize food intake while maintaining escape performance in uncertain risk conditions.

The mechanism that generates these ratios of expelled food is unknown. In our experiments, the physical act of chasing led to regurgitation, but we cannot determine whether this response was based on

perception of threat, or the physical act of escape itself. At a physiological level, it is possible that expulsion behaviour functions to restore aerobic scope after the exercise associated with the threatening stimulus. To test for this possibility, experiments conducted under differing oxygen regimes would reveal how aerobic constraint and restoration affect expulsion behaviour. At the level of perception, further experiments testing varying sources and sensory modalities of threat would provide insight into this phenomenon. For example, using 'schreckstoff', a substance known to induce fear responses in fish, or purely visual cues of a predator threat, would reveal the interaction between perception of threat and the regurgitation response. If these stimuli did not elicit a regurgitation response, it would suggest that the perception of predation threat itself is not driving regurgitation, but rather the physical exertion of prolonged escape. In either scenario the outcome – reduction of DS to levels that improve performance – is the same, and has the same adaptive value whatever the mechanism producing it. Nevertheless, further research into the mechanisms underlying this behaviour would prove invaluable to understand both the evolution and generality of this strategy in this and other taxa.

Fast-start escape responses are anaerobic, and we therefore consider the main effect of feeding to be due to changes in mass and hydrodynamics rather than changes in energy distribution related to digestion [6,7,31]. This is supported by the rapid restoration of performance after expulsion, which would not be expected while digestion of retained food continued, but would be predicted if food expulsion altered body shape and improved hydrodynamic performance. The movements during Stage 1 of a fast-start response play a critically important role in successful escape [9], and in two kinematics parameters during this stage, fish at lower satiation levels performed better. The first, turning angle, has a strong influence on the linear kinematics in Stage 2, and subsequently affects the performance of the whole fast-start escape [26]. Fish at DS ≤ 40% show a significantly greater performance in Stage 1 turning than fish at 60% and 100% DS. Surprisingly, fish at 40% DS performed better than fish at 0% DS (see Supplementary Discussion). Similarly, Stage 1 angular velocity, or the speed of turning in response to a threat, also differed between satiation levels, with higher peak turning speeds observed at DS ≤ 40% (figure. 4A and 4B). As the duration of Stage 1 varies from individual to individual (figure. S3), the relationship between turning angle (an absolute measure) and average angular velocity (a temporally integrated measure) differ in their response to treatment.

For Stage 2, no significant differences were observed in distance travelled (figure. 4C), however fish at DS ≤ 40% have significantly higher average and peak linear velocities than fish at DS ≥ 60% (figure. 4D). Likewise, as the duration of Stage 2 varies among individuals, the distance travelled and the average linear velocity differ in their response to treatment. Over the entire fast-start response (here defined as the shortest observed duration for individuals – 44.44 ms), the total distance travelled and peak linear velocity were significantly greater at 0% DS than at DS ≥ 60%, but no significant differences were observed between 0% and 40% DS (figure. 5). Combined, these results demonstrate greater performance in the initial stages of escape from a threat at lower levels of satiation. Optimal performances were observed at 40% DS, although we did not test performance at 50% DS, which was closer to the DS ratios of fish which expelled food

under threat. It would be enlightening to examine the finer detail of the relationship between Stage 1 and Stage 2 performance and DS ratios between 30 and 60%, as well as expulsion amounts when fish have DS between 70% and 90%, but at this point we can only conclude that this *a posteriori* strategy brings individuals closer to the range of peak performance without actually achieving it. Additionally, we tested expulsion and performance in two separate experiments. Under natural conditions, expulsion would have to occur during exposure a potential threat, for example a chasing predator. The act of expulsion itself may impede locomotor performance during this phase, but would also provide potential other food sources or even occlusion for the chased fish. Clearly, further experiments examining the sequence and interactions of these behaviours should be conducted.

In summary we find that black carp can employ a novel solution to the ecological trade-off between resource acquisition and risk avoidance. The primary effect of reduced satiation comes in improved performance in the fast-start escape response (Stage 1), and improves performance in subsequent stages of escape, which appear to be related to changes in body shape and hydrodynamics. The mechanisms that produce the phenomenon require further attention, in particular to establish whether this expulsion is a voluntary behaviour or an involuntary physiological consequence of physical exertion during escape, and how fluid mechanics may influence escape performance [32]. Further inquiries with black carp into the mechanisms underlying this phenomenon would combine well with broader taxonomic studies to establish whether these *a posteriori* strategies are present and even possible in other species.

Contribution Statement

J.Z., Z.Y., S.Z. and A.J. conceived the study. Y.W., J.Z. and J.Z. (Junjie Zhu) collected the data. J.Z. and Y.W. analysed the data. J.Z., A.J. and Y.W. drafted the manuscript. A.J., Z.Y, and J.Y. contributed to further manuscript development. Z.Y., S.Z., J. Z. and A.J. provided financial support, and all authors gave final approval for publication.

Data Accessibility

All associated data are available at DRYAD (doi: to be uploaded upon publication).

Conflict of Interest

The authors declare no conflict of interest

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