Refuge use by fish as a function of body length-related metabolic expenditure and predation risks

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Theory predicts that staying in a refuge has benefits in terms of predator avoidance and costs in terms of lost feeding opportunities. In this study, we investigated how the relative importance of these costs and benefits changes with increasing body length. This is of particular interest in animals such as fish, which show continuous growth throughout their lives. Our results suggest that larger fish are subject to lower predation risks and are less affected by food deprivation than small fish, with fish decreasing their responses to food-deprivation treatments more strongly with increasing body length than to predation treatments. This may explain our observation that large fish emerged later from a refuge than small ones and spent shorter times outside the refuge. The key role of differential responses to food deprivation was further illustrated by the finding that the relative weight loss of individual fish was strongly correlated with a reduction in hiding time even in the absence of body length differences. The importance of inter-individual differences in metabolic rates for the decision-making behaviour of animals is discussed.

Keywords: three-spined sticklebacks; refuge use; predation; food deprivation; metabolic rates

1. INTRODUCTION

Refuge use in animals provides an excellent but under-exploited phenomenon for the study of trade-offs between the benefits of predator avoidance and the costs of lost feeding opportunities (Sih 1997). The longer an animal stays hidden in a hole or crevice, the smaller the chances that the predator will still be there on emergence, but the larger also the energetic costs. This scenario has given rise to a number of studies looking at how long predators wait for prey to emerge (Johansson & Englund 1995), how emergence time of prey is affected by its nutritional state (Dill & Gillett 1991; Koivula *et al.* 1995) and how the emergence strategy of prey is influenced by fluctuating resources in the environment (Dill & Fraser 1997). Another factor that can have an important influence on emergence times of prey is body size (Dill & Gillett 1991). Studying barnacles, *Balanus glandula*, of four different size classes, Dill & Gillett (1991) found that smaller individuals showed longer hiding times than large ones. This seemed counter-intuitive at first because smaller barnacles have higher metabolic requirements per unit of weight (Wu & Levings 1978). However, Dill & Gillett (1991) suggested that lost feeding time may be less costly to smaller barnacles because they might be able to recoup the cost of hiding in part by their relatively higher cirral beat frequencies. In fish we have a different situation, with both weight-specific metabolic requirements and locomotion (and thus foraging) being more costly to

small individuals, the latter being due to a relatively larger drag coefficient (Wootton 1994). Therefore we should expect small fish to emerge sooner from their refuge than large ones. However, smaller fish also experience higher predation pressures than larger individuals, a mechanism that selects for the opposite trend, i.e. for smaller fish to have longer hiding times (see Sogard (1997) for a review). Whether or not small individuals should hide for longer than large ones should depend on the relative importance of these two selective forces.

In our first experiment, we investigated the relationship between body length and weight loss of three-spined sticklebacks (*Gasterosteus aculeatus*) ranging from approximately 25–55 mm to estimate the length-related metabolic costs of different food deprivation periods. Larger fish were expected to have higher absolute weight losses but smaller relative ones (absolute weight loss divided by initial fish weight). In a second experiment, the refuge use of fish was investigated under three different conditions: control, food deprivation and predation. We predicted that both small and large fish should increase and decrease their hiding times in response to predation and food deprivation, respectively. Furthermore, the difference in hiding times between food deprivation and control treatments, and predation and control treatments was used to compare the relative importance of food deprivation and predation threats for fish of different body lengths. However, no directional prediction for the relationship between hiding time and body length could be made beforehand because this would require knowledge of the relative importance of the selective forces that have shaped strategies of refuge use in fish.

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2. MATERIAL AND METHODS

Several hundred male and female three-spined sticklebacks (*G. aculeatus*) were collected from Adel Beck in Leeds (UK) in December and January 1998 and kept for about one month at 12 °C and 16L:8D before they were used in the experiments. The fish were fed on freeze-dried chironomids to satiation once a day. Sticklebacks were chosen for this study because they seek refuge under stones or in vegetation when a predator approaches (Krause, personal observation). The most commonly observed predators of sticklebacks in our study area were brown trout, *Salmo trutta*; grey heron, *Ardea cinerea*; and kingfisher, *Alcedo atthis*.

(a) Weight loss

Fish (standard body length: 25–57 mm; body weight: 0.15–2.11 g; condition factor *K*: mean $\pm$ s.d. = 1.1 ± 0.14) were kept in groups of three per tank (40 × 20 × 16 cm, L × W × H) at 12 °C water temperature. Weight measurements of fish ranging from 25–57 mm were taken before and after a food deprivation of one day ($n=69$), two days ($n=54$) and three days ($n=33$). Fish were measured on a digital balance to 10^{-4} g and repeated measurements were taken of each fish on each date to check for measurement errors. Absolute and relative weight loss (absolute weight difference divided by initial weight), which included the weight of the gut contents, were calculated. One fish died of fungus in each of the two-day and three-day treatments so that sample sizes were 53 and 32, respectively. Experiments by Pascoe & Matthey (1977) showed that sticklebacks can sustain food-deprivation periods of 2–3 weeks before any mortalities occur. Therefore food-deprivation periods of 1–3 d (used in this study) should increase the motivation of fish to forage but should not present a serious starvation risk for fish. Each fish was used only once in a food-deprivation trial and different individuals were used in trials of different duration of food deprivation.

(b) Refuge use

Fish of a continuous body length range (standard body length: 19–53 mm) were used. The experimental tank (85 cm × 9 cm × 55 cm, L × W × H, 5 cm water depth) was made out of opaque, grey plastic. One corner of the tank provided a refuge which consisted of a small polystyrene float (figure 1a). The fish was introduced to the refuge via a clear plastic cylinder that was placed through a hole in the polystyrene float (figure 1b) so that the test fish was inside the refuge after release. We chose this way of introducing fish after a number of pilot trials demonstrated the advantages of this method in causing less stress to the fish. Lines on the bottom of the tank marked every 5 cm as reference points for how far the fish emerged from the refuge.

At the beginning of each trial, a single stickleback was transferred from its holding tank to the plastic cylinder in the test tank and left for three min to settle. The cylinder was then pulled up via a remote pulley system to release the fish (figure 1b). The cylinder was pulled upwards by 4.5 cm to remain in contact with the water surface, which avoided potential disturbances caused by pulling the cylinder fully out of the water (Tegeder & Krause 1995). After release, the following variables were measured: (i) time it took for the fish to appear on the edge of the refuge; (ii) time to appear fully from the refuge for the first time (full body out from under the float); (iii) time spent outside the refuge after first appearance until return to the refuge; (iv) maximum displacement from refuge measured in

Figure 1. (a) Top-view of the experimental apparatus showing the refuge (radius 8 cm), the plastic cylinder (elliptical shape: 6 × 2.5 cm) and the seven zones marked out on the tank bottom. (b) Side-view illustrating the remote pulley mechanism used to release the test fish and the predator model used to frighten the fish. (A) Monofilament line, (B) predator model, (C) remote-pulley system, (D) plastic cylinder, (E) refuge and (F) test fish.

terms of zones (see above); and (v) duration of second period in the refuge. Response variables (i) and (ii) were not independent from one another and therefore we also calculated (ii)–(i), which is the time a fish spent in the refuge after its first appearance on the edge. Observations of test fish were made from a hide.

Three treatments were used: control, predation threat and food deprivation. In the control treatment, we used the above procedure without any modifications. In the predation treatment, a model of a kingfisher was suspended from a monofilament line and moved over the test tank, simulating the attack of an aerial predator (figure 1b). Kingfishers were frequently observed hunting sticklebacks in the area where we collected our fish and should therefore present an appropriate predator. The predator model was released from behind a hide and moved directly over the plastic cylinder containing the test fish. The predator stimulus was provided immediately prior to the release of the test fish. In the third treatment, fish were starved for two days prior to testing. We used a total of 30 fish and each fish was tested once under each of the three treatments (over a period of

6d) in random order, giving 90 trials altogether. To allow for retesting of the same individuals under different treatment conditions, fish were kept singly in small numbered plastic containers (10 cm in diameter) for the duration of the data collection period. Tests were separated by two-day gaps to avoid habituation effects and to allow fish sufficient time to recover from the food-deprivation treatments. Pilot trials showed that two days were sufficient for fish to regain their initial weight.

At the extreme ends of the body length range (25–30 mm and 45–50 mm) we additionally tested 16 fish each to be able to compare the response of small and large fish (in contrast to the continuous body length range of the previous trials, see above) to food-deprivation and predation treatments. Fish were tested under the same three treatments as above and randomly assigned to a treatment order. The experimental protocol was the same as above but only the time of first emergence from the refuge was recorded.

Given the nature of the refuge, searching for food and hiding were not mutually exclusive activities in this experiment. However, the size of the refuge was so small that sticklebacks, which are visual predators (FitzGerald & Wootton 1993), would be able to scan the area for food very quickly. Therefore fish should leave the refuge after a few seconds unless they were genuinely using it as a refuge (this was confirmed in pilot trials using different refuge sizes). No food was present in the test tanks. Both male and female sticklebacks were used. However, fish were collected in winter and kept at low temperatures, which means that they were not sexually active during the experiments.

(c) Relationship between weight loss and refuge use

In a final experiment, we selected 11 fish of approximately equal body length (36–40 mm) to test for a relationship between individual weight loss and reduction of hiding time in the absence of body length differences. Fish were tested in a control treatment, after a food deprivation of 2 d and a second control treatment one week after the first. Fish were weighed after each treatment and only the time of first emergence from the refuge was recorded.

3. RESULTS

(a) Weight loss

The general relationship between body length and body weight is shown in figure 2a. The most dramatic changes in absolute and relative weight loss were observed during the first two days. After that, fish continued to lose weight but the increments were smaller (figure 2b,c). Absolute weight loss significantly increased and relative weight loss significantly decreased with body length for all three food-deprivation periods (figure 2b,c). There was a significant difference between the slopes of the regression lines (ANCOVA: absolute weight loss: $F_{2,148}=17.7$; $p<0.001$; relative weight loss: $F_{2,148}=22.4$; $p<0.001$) and the gradients were all significantly different (apart from fish in the two-day and three-day treatments) from one another (post hoc Tukey tests in all cases: $p<0.001$) (figure 2b,c).

(b) Refuge use

Larger fish spent more time hiding and less time exploring outside the refuge than small fish in all three treatments (figure 3). The only exceptions are

Figure 2. (a) Relationship between body length and weight in three-spined sticklebacks: $n=69$, $r^2=0.96$, $p<0.001$. Relationship between body length and (b) absolute weight loss: after 1 day, $n=69$, $r^2=0.51$, $p<0.001$; after 2 days, $n=53$, $r^2=0.32$, $p<0.001$; after 3 days, $n=32$, $r^2=0.371$, $p<0.001$ and (c) relative weight loss: after 1 day, $n=69$, $r^2=0.42$, $p<0.001$; after 2 days, $n=53$, $r^2=0.373$, $p<0.001$; after 3 days, $n=32$, $r^2=0.34$, $p<0.001$; as a function of the duration of food deprivation.

Figure 3. Individual sticklebacks of different body lengths were released and took shelter under a polystyrene float. The behaviour of 30 fish was observed under three treatments: control, simulated predator attack and food deprivation for two days, and the following variables were measured: (a) appearance at the refuge edge, predation: $r^2=0.17$, $p=0.026$; control: $r^2=0.32$, $p=0.001$; food deprivation: $r^2=0.44$, $p<0.001$; (b) first full emergence, predation: $r^2=0.71$, $p<0.001$; control: $r^2=0.84$, $p<0.001$; food deprivation: $r^2=0.83$, $p<0.001$; (c) time between first appearance on edge and emergence, predation: $r^2=0.51$, $p<0.001$; control: $r^2=0.68$, $p<0.001$; food deprivation: $r^2=0.65$, $p<0.001$; (d) time spent outside the refuge, predation: $r^2=0.05$, $p=0.25$; control: $r^2=0.31$, $p=0.001$; food deprivation: $r^2=0.06$, $p=0.20$; (e) duration of return to the refuge, predation: $r^2=0.44$, $p<0.001$; control: $r^2=0.41$, $p<0.001$; food deprivation: $r^2=0.46$, $p<0.001$. y denotes log time (s) for each of the three experimental treatments.

the food-deprivation and predation treatments regarding the time spent outside the refuge, where no significant relationships with body length were found (figure 3d).

Treatment slopes were significantly different for the appearance of fish at the edge of the refuge (repeated-measures ANCOVA, $F_{2,84}=16.34$, $p<0.001$; figure 3a). Pair-wise comparisons showed that the food-deprivation data were significantly different from both the control and the predation data (post hoc Tukey tests, both $p<0.01$). No significant difference between the slopes of the control and predation data was found (Tukey test, $p>0.05$). The latter showed a significant difference between the elevations of the regression lines ($t_{56}=5.19$, $p<0.001$).

Time until first full appearance from the refuge showed the strongest positive correlations with body length among all response variables for all three treatments (figure 3b). Emergence times varied between a few seconds for small fish and up to 34 min for large ones and doubling the body length from 20 mm to 40 mm was on average associated with more than a tenfold increase in hiding time. A significant difference between the treatment slopes was found (repeated-measures ANCOVA, $F_{2,84}=29.72$, $p<0.001$). Multiple comparisons of the slopes showed that all gradients were significantly different from one another (Tukey tests: $p<0.001$). Therefore no test regarding elevations was carried out. However, an additional test showed that large fish (45–50 mm) changed their hiding time more strongly in response to the predation treatment than that of the food deprivation (calculated as percentage change relative to the control treatment). Whereas, small fish did not (Wilcoxon test: large fish, $n=16$, $z=3.20$, $p=0.001$; small fish, $n=16$, $z=-0.77$, $p=0.43$; see also figure 3b).

Treatment slopes were significantly different for the time spent between appearance on the edge and emerging from the refuge (repeated-measures ANCOVA, $F_{2,84}=13.97$, $p<0.001$). Pair-wise comparisons showed significant differences between the slopes of the predation and control data (Tukey test, $p<0.001$) and food-deprivation and predation data (Tukey test, $p<0.001$; figure 3c). Furthermore, there was a significant difference in the elevations between food-deprivation and control data ($t_{56}=2.09$, $p<0.05$).

For the time spent outside the refuge we found significantly different slopes between treatments (repeated-measures ANCOVA, $F_{2,84}=4.05$, $p<0.01$; figure 3d). Pair-wise comparisons showed that only the slopes of the control and predation data were significantly different (Tukey test, $p<0.01$). A significant difference between the elevations of the food-deprivation and predation data was found (Tukey test, $p<0.05$) but not between the control and the food-deprivation data ($t_{56}=1.19$, $p<0.05$).

No difference in the median zone was found between the three different treatments (Kruskal–Wallis one-way ANOVA, $p=0.219$). A negative correlation was observed between zone and body length when data from all three treatments were pooled (Spearman's $\rho=-0.403$, $n=90$, $p<0.01$).

For the second visit to the refuge, slopes were significantly different between treatments (repeated-measures ANCOVA, $F_{2,84}=5.67$, $p<0.01$, figure 3e). The only pair that differed significantly from one another were the

food-deprivation and predation data (Tukey test, $p<0.05$). We found a significant difference between the elevations of control and predation data (Tukey test, $p<0.05$) but not between control and food-deprivation data ($t_{56}=0.86$, $p>0.05$).

(c) Relationship between weight loss and refuge use

There was a significant relationship between the individual ranks of the emergence times between the first and second control treatment indicating that individual behaviour of fish is highly repeatable (Spearman's $\rho=0.85$, $n=11$, $p<0.001$). Relative weight loss was significantly correlated with the relative reduction in emergence time, calculated as food-deprivation data/control data for each fish (Spearman's $\rho=0.55$, $n=11$, $p<0.05$). As a control, we tested for influences of both body length and (initial) body weight on relative weight loss and relative reduction in hiding time but no significant correlations were found (all tests: Spearman's $\rho<0.29$, $p>0.19$).

4. DISCUSSION

Irrespective of body length, sticklebacks responded to predation and food-deprivation treatments by increasing and decreasing their emergence times respectively, relative to the control treatment. Furthermore, the time spent outside the refuge was longer when fish were food deprived compared to fish in the predation treatment (but no difference was found between the control and the food-deprivation data). These trends are consistent with results from previous studies (Dill & Gillett (1991) on barnacles; Koivula *et al.* (1995) on birds; and a theoretical model by Sih (1992); see also Dill & Fraser (1997) on the importance of short-term resource fluctuations).

Small sticklebacks generally showed shorter hiding times than large fish but greater relative increases and decreases in response to predation and food-deprivation treatments than large fish. This result suggests that smaller fish are subject to both higher predation and starvation risks than large ones, a result that is further supported by our finding that relative weight loss was greater in small individuals. Reviews by Sogard (1997) and Fuiman & Magurran (1994) further support the idea that smaller fish have greater predation risks than larger ones because many fish predators are gape-limited. Large fish changed their hiding times more strongly in response to the predation treatment than the food deprivation one, whereas in small fish the magnitude of the changes were similar in response to both predation and food-deprivation treatments. This difference between small and large fish may explain why large fish hide longer than small ones. Although at lower risk from predators on emergence, large fish can afford to be 'extra' cautious because of the low cost of lost feeding opportunities. Small fish, on the other hand, are forced to emerge early despite the high predation risk because of their high energy demands.

A more detailed insight into the importance of predation threats and food deprivations can be gained by looking at figures 3a (first time at the edge) and 3c (time spent between first appearance at the edge and first full emergence from the refuge). In figure 3a, a significant

difference between the slopes of the control and food-deprivation treatments was found but not between the control and the predation treatments. The relatively greater importance of food to small fish probably motivated them to start moving around and swim to the edge of the refuge disproportionately earlier than large fish. However, since fish in the refuge were safe from potential predators irrespective of body length differences, no difference between small and large fish in the relative increase in hiding times due to the predation treatment was observed. Evidence for the latter was only found in figure 3c (see difference in slopes between control and predation treatment) when the fish emerged, exposing itself to potential predators. The above trends indicate that body length-related differences in metabolic demands became relevant to the fish immediately after release (from the plastic cylinder) whereas body length-related differences in predation risk only became important when the fish emerged from the refuge (but not before).

The slope of the relationship between hiding time and body length was less steep for the second visit to the refuge (compared to the first one). Small fish spent about the same time in the refuge as before (which is probably close to the minimum time for an area that size) but large fish strongly reduced the duration of their visits. Also, treatment differences appeared to have been reduced or disappeared altogether. This suggests that the importance of the polystyrene float as a refuge decreased in the course of the trial as fish explored more of the tank without being disturbed.

The hiding strategy of individual fish (of the same length) was highly repeatable and strongly related to their percentage weight loss. Differences in relative weight loss in the absence of body length differences can be the result of a number of factors. First, it is possible that individuals inherently differ in their metabolic rates. Second, differences in relative weight loss could be related to differences in the activity of fish during the food-deprivation period. Increased activity at the beginning of a food-deprivation period has been described in cyprinid fishes (Wieser 1991; Wieser *et al.* 1992) and can be functionally interpreted as increasing the likelihood of encountering food. Therefore, those individuals that become most active to search for food would naturally also be the first ones to emerge from the refuge in doing so. This potential link between variation in activity (increased food searching) and weight loss could be an interesting topic for further experiments.

A strong contrast was found in the ability of small and large fish to cope with food deprivation. Absolute weight loss in sticklebacks was greater in large individuals than small ones but relative weight loss was greater in small fish. This is consistent with the general principle of lower maintenance costs per body mass unit with increasing body weight in teleost fishes (Meakins 1975; Brett & Groves 1979; Wootton 1994). The fact that (small) fish continued to lose substantial amounts of weight after the first day of food deprivation indicates that the observed weight loss was not just due to the evacuation of gut contents. Relative weight loss over two days was about 11% in small fish (25 mm) and about 5% in large fish (50 mm) and the corresponding reductions in hiding time were 70% and 21%, respectively. It would be interesting

to test whether a similar difference in relative weight loss of 6% would produce a similar reduction in hiding time when compared for large fish that were subject to different durations of food deprivation.

Relative weight loss as measured in this study gives some indication of the energetic requirements of fish of different length. However, more detailed studies are needed to look at the specific components of weight loss, such as the rate of lipid and protein turnover (Weatherley & Gill 1987; Collins & Anderson 1995). The ability to survive longer periods without food not only depends on the metabolic rate but also on the ability to store energy and control its allocation during periods of resource limitation. These processes are likely to be body length-dependent as well and are expected to have important implications for the decision-making behaviour of fish.

Our results have important consequences for the experimental design of studies that compare the behaviour of fish of different body length (see Peuhkuri 1997, 1998). Food deprivations of the same duration affect fish of different size classes differently and therefore do not necessarily have the standardizing effect they are meant to have by applying the same treatment. As a result, behavioural differences between small and large fish could be a consequence of different physiological constraints rather than a differential response to their social environment (as concluded by Peuhkuri (1997)).

Average trial durations were 6, 5 and 13 min for control, deprivation and predation treatments with a range of 1–36 min (all treatments combined). We estimated that disturbances (appearance of predators, non-predatory overflying birds, etc.) occur several times per hour at Adel Beck so that fish would rarely be completely undisturbed for periods substantially longer than about 30–40 min. These observations suggest that the trial durations used in our experiments correspond reasonably well to the rate at which fish get disturbed in the wild.

Our experiments highlight the importance of body length-related metabolic constraints in the context of behavioural decisions of animals. Fish provide a particularly suitable study system for this relationship because they continue to grow throughout their lives (Wootton 1994).

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