SYMPOSIUM

Foraging Behavior Delays Mechanically-Stimulated Escape Responses in Fish

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Synopsis Foraging and the evasion of predators are fundamental for the survival of organisms, but they impose contrasting demands that can influence performance in each behavior. Previous studies suggested that foraging organisms may experience decreased vigilance to attacks by predators; however, little is known about the effect of foraging on escape performance with respect to the kinematics and the timing of the response. This study tested the hypothesis that engaging in foraging activities affected escape performance by comparing fast-start escape responses of silver-spotted sculpins Blepsias cirrhosus under three conditions: (1) control (no foraging involved), (2) while targeting prey, and (3) immediately after capture of prey. Escape response variables (non-locomotor and locomotor) were analyzed from high-speed videos. Responsiveness was lower immediately after capturing a prey item compared with the other two treatments, and latency of performance was higher in the control treatment than in the other two. Locomotor variables such as maximum speed, maximum acceleration, and turning rates did not show statistical differences among the three groups. Our results demonstrate that foraging can negatively affect two fundamental components of the escape response: (1) responsiveness and (2) latency of escape, suggesting that engaging in foraging may decrease an individual’s ability to successfully evade predators.

Introduction

In fishes, escape responses usually consist of a C-start maneuver, which involves the unilateral contraction of the body musculature into a “C” shape (stage 1), followed by a propulsive stroke of the tail in the opposite direction of the initial contraction (stage 2), allowing fish to avoid a sudden danger in the environment (Domenici and Blake 1997). C-start performance can be evaluated on the basis of non-locomotor (e.g., responsiveness, directionality, and latency of escape) and locomotor (e.g., turning rate, speed, and acceleration) components (Domenici 2010a, 2010b). C-start escape responses are not stereotypic and can differ in aspects that may significantly influence an individual’s success in evading predators (Eaton et al. 2001; Scharf et al. 2003; Marras et al. 2011). Typically, C-start escape responses are triggered by the Mauthner cells, which ensure short latencies (of the order of 10–20 ms) and fast turning rates (Eaton and Hackett 1984). However, responses not mediated by Mauthner cells can also occur, although with longer latencies (Eaton et al. 2001; Kohashi and Oda 2008).

Although reaction distance is one of the most fundamental variables that affect preys’ vulnerability (Godin 1997; Scharf et al. 2003; Walker et al. 2005; Domenici 2010a, 2010b), little is known about the context-dependency of other components of escape behavior, such as the swimming kinematics and latency in response to a mechanical stimulation. Foraging is one of the potential factors affecting escape behavior, along with the presence of conspecifics or predators, and proximity to a refuge, among others (Ydenberg and Dill 1986; Godin 1997; Langerhans et al. 2004; Domenici et al. 2008;...
Domenici 2010b). Foraging and the evasion of predators impose different challenges (Ydenberg and Dill 1986), so that individuals that are attacked while feeding face the “feed-or-flee” dilemma (i.e., feed and risk being eaten or flee and lose resources). Previous work found that foraging was associated with prey allowing predators to approach more closely, and being less responsive to them (Krause and Godin 1996), resulting in greater vulnerability to predators. However, C-start escape latencies and kinematics of fish attacked while foraging have not been investigated thus far.

Understanding how feeding may modulate escape performance will provide insight into the dynamics of predator–prey interactions and the ecological significance of locomotor and non-locomotor aspects of escaping (Scharf et al. 2003; Domenici 2010b). Here, we test whether engaging in behaviors related to foraging can influence C-start escape performance of the silver-spotted sculpin, Blepsias cirrhosus (Pallas 1814), by comparing escape responses exhibited in the absence of foraging behavior with those performed while focusing on a prey item, prior to and after capturing it.

Materials and methods

Collection and maintenance of specimens

Forty-five adult silver-spotted sculpins, B. cirrhosus (family Cottidae), were collected using a beach seine between June and July 2009 at Jackson Beach, Friday Harbor (WA). Fish were transported to Friday Harbor Laboratories (FHL), a research facility of the University of Washington, Seattle, and were acclimated to aquaria with recirculating seawater (12–16°C) for at least 4 days prior to testing.

Blepsias cirrhosus can be found from the intertidal zone to depths of 37 m (Miller and Lea 1972), and feeds primarily on nekton or benthic crustaceans (Kolpakov and Dolganova 2006), so a diet of dock shrimp Pandalus danae was used. All animal care and experimental protocols were in accordance with guidelines approved by the Institutional Animal Care and Use Committee at the University of Washington (IACUC protocol # 3018-09) for the use and care of research animals at FHL.

Description of treatments

Individuals were randomly divided into three groups (C: control, n = 15; BF: before feeding, n = 12; and AF: after feeding, n = 15); and tested according to each group’s protocol. For the two experimental treatments that involved foraging behaviors; a freshly euthanized P. danae shrimp was attached to a transparent wire for 30 s to simulate prey. The control treatment (C) analyzed escape responses triggered in the absence of prey.

The protocol for the experimental treatments was as follows: a single prey was placed approximately one fish-body-length from the place at which the stimulus would be dropped, allowing the fish to approach. In the BF treatment, the stimulus was dropped before the fish captured the prey (i.e., when the individual’s mouth was less than one-body-length from the prey). In the AF treatment, fish were stimulated immediately after they had captured prey.

For all treatments, the mechanical stimulus was released when the fish was approximately 1–2 body-lengths away (range: 0.083–0.248 m), and at least 0.5 body-length from the sides of the tank, so as to eliminate wall effects (Eaton and Emberley 1991). Stimulus distance (D_{stim}) was measured as the distance between the center of the stimulus and the center of mass (COM) of the fish.

Comparisons between the individuals in C and BF allowed us to assess the effects of the fish focusing their attention on the prey on escape performance while comparisons between BF and AF analyzed the effects of having successfully captured a prey on escape performance. Individuals were starved for at least 2 days prior to testing to ensure that satiation did not influence the analysis (Sass and Motta 2002).

Recording escape responses

Prior to testing, both sides of the fish were marked dorsal to the COM between the two dorsal fins with 6 × 6 mm² Spectrolite reflective tape to allow tracking with an automated data analysis program (DLTDataViewer3) in MATLAB (v2009a). The COM was determined on a subsample of euthanized fish (n = 2) using procedures described by Domenici and Blake (1991). Marked individuals were then placed in the filming tank and allowed 30 min to acclimate. Preliminary observations showed that individuals resumed normal behaviors in about 5 min after being introduced into the tank. Of the 45 B. cirrhosus that were collected, 42 fish were successfully filmed and analyzed. The sculpins that were analyzed ranged from 0.087 to 0.163 m total length, with an average of 0.131 ± 0.002 m. Total length was not significantly different between the C, BF, and AF groups (ANOVA: F = 0.941, P = 0.399).

The stimulus consisted of a 50-ml plastic centrifuge tube containing a 100-g brass weight, and attached to a thin rope. The stimulus was dropped down a black tube, which was suspended into the
filming tank and 0.5 cm above the water’s surface, in order to avoid visual stimulation (Dadda et al. 2010). A small mirror was placed near the water’s surface by the black tube in order to identify the frame in which the stimulus hit the water; that frame was used as a reference for calculating latency of escape. The filming tank was lined with a grid of $5 \times 5$ cm$^2$ to facilitate calibrating and digitizing the videos. A screened hole between the filming and outer tanks allowed for fresh seawater to be circulated throughout the apparatus. All videos were recorded using a high-speed Fastec Imaging Sportscam camera running at 250 Hz and subdivided based on whether or not fish responded to a startling stimulation.

The COM, tip of the snout, and midpoint of the stimulus tube were digitized from high-speed videos using DLTdataViewer3 routines (Hedrick 2008) in MATLAB (vR2009a).

**Description of the variables analyzed**

For videos in which an escape response was observed, escape performance was assessed on the basis of both non-locomotor and locomotor variables (Domenici 2010b). Non-locomotor variables for each treatment included: responsiveness (proportion of individuals responding), directionality (proportion of C-bends directed away from the stimulus [Domenici and Blake 1993]) and latency of escape (time between the onset of the stimulus touching the water’s surface and the first visible response by the fish). Responsiveness was determined for all individuals, but only those that escaped were analyzed for the other variables. Stimulus distance ($D_{si}$) was also measured in order to control for potential effects of differences in that variable among the three treatments.

The variables related to maneuverability included average turning rate (ATR; average angular velocity of the anterior part of body [head to COM] while turning during stage 1) and maximum turning rate (MTR; maximum angular velocity of the anterior part of the body while turning during stage 1). Variables for performance over distance and through time were based on the displacement of the COM and included average cumulative distance (CD) traveled following stimulation, maximum speed (MS), and maximum acceleration (MA) during a fixed time (0.06 s; determined to be the average duration of the sum of stage 1 and stage 2 for our data). Data on speed and acceleration were derived from calculation of CD by using five-point smoothing techniques on the raw data (Lanczos 1956). Speed prior to the escape response ($U_{prior}$) was measured in all trials, using data from the COM that was digitized from the high-speed videos, in order to control for potential differences in activity before stimulation. The average speed prior to the escape responses was not significantly different between treatments ($F_{(2,24)} = 0.295, P = 0.747$), suggesting that activity prior to escaping was not affected by the presence of food. ATR was measured in 15 trials of routine swimming on fish that were analyzed for escape responses, in order to provide a baseline for comparing escape responses with spontaneous behavior.

**Statistical analysis**

Differences between groups for locomotor variables were analyzed with parametric ANCOVA’s with Tukey’s post-hoc tests, using the standard length of fish and the speed prior to escape ($U_{prior}$) as covariates. Group differences for latency were also tested with an ANCOVA using the previous covariates as well as the stimulus distance ($D_{si}$: distance between the stimulus to the COM) as another covariate. Categorical data (directionality and responsiveness) were compared using chi-squared tests. Statistical analyses were conducted using “R,” “SigmaStat,” and “Statistica.”

**Results**

Fish exhibited significant differences between treatments for non-locomotor components of the escape response. Although the majority of the fish escaped in response to mechanical stimulation in C (73%) and in BF (83%), only 43% of AF individuals exhibited this behavior (Fig. 1A). Responsiveness was significantly different among treatments ($\chi^2 = 6.28, P < 0.05$). Subsequent chi-squared analysis among treatments shows that AF differs significantly from both C ($\chi^2 = 5.09, P < 0.05$) and BF ($\chi^2 = 7.49, P < 0.05$), while C and BF did not differ from each other ($\chi^2 = 0.56, P > 0.05$). Directionality did not differ among treatments ($\chi^2 = 2.24, P > 0.05$).

Latency showed significant differences among treatments after controlling for the covariates ($F_{(2,21)} = 4.146, P = 0.030$). The post-hoc Tukey test indicated that C was significantly different from the feeding treatments (C-AF: $P < 0.001$, C-BF: $P < 0.001$), but the feeding treatments were not different from each other ($P = 0.845$). Thus, BF and AF had longer latencies than did C (Fig. 1B).

Locomotor aspects of the escape responses did not differ among the three treatments (Fig. 2): ATR ($F_{(2,22)} = 2.004, P = 0.159$), MTR ($F_{(2,22)} = 1.091, P = 0.353$), CD ($F_{(2,22)} = 3.256, P = 0.058$), MS
The ATR for routine turns and escape responses were significantly different (two-tailed t-test: $t = -16.534$, df = 37.998, $P < 0.001$), and there was no overlap between turns during routine swimming ($32-696\,\text{s}^{-1}$) and escape responses ($1065-2790\,\text{s}^{-1}$) (Fig. 3).

**Discussion**

Our findings suggest that foraging activities affect two important components of the escape response: responsiveness and latency (Fig. 1). Individuals in AF exhibited lower responsiveness (Fig. 1A), which may result in reduced success in evading predators, since the lack of a response would most likely allow the predator to successfully capture the prey. Previous work shows that responsiveness was the most important factor affecting the success of fish larvae to escape, accounting for 86% of the variation in potential for escape (Fuiman et al. 2006). Interestingly, the lowest responsiveness was found in the AF treatment, suggesting that fish that have ingested a prey may be physically constrained or inhibited from escaping due to limitations in the rate of neurological processing of multiple sources of information (Dukas 2004). Occasionally, fish (∼14%) in the AF treatment released the shrimp after completing an escape response, supporting the idea that the ability of fish to perform multiple tasks (i.e., feeding and escaping) is limited.

Foraging decreases latency of performance by increasing the delay in escaping following stimulation...
to about 61.60 ± 6.48 ms (BF) and 57.33 ms ± 8.62 (AF), compared with 24.73 ± 4.85 ms (C) (Table 1).

This suggests that focusing on, and capturing, prey may alter the timing of an individual’s response to an attack. Escape latencies of C-starts can be extremely fast (10–50 ms) (Domenici 2010a) and can determine whether or not a fish is captured in a predator–prey gambit in which high speeds and fast reactions are at a premium, and life or death may be a matter of a few milliseconds (Catania 2009). Therefore, an increase of the order of about 30 ms, such as the one we found, may have important consequences for survival. This increase is likely to be due to the negative effect of multitasking on an individual’s ability to perform certain behaviors (Krause and Godin 1996; Kaby and Lind 2003), suggesting a general pattern of decreased performance during multitasking such as that due to simultaneous feeding and fleeing. For instance, Krause and Godin (1996) found that guppies (Poecilia reticulata) responded with a longer reaction distance when foraging, possibly as a result of the fish’s attention being taken up by searching for food and by feeding (Krause and Godin 1996).

Based on the speed of their model predator, the differences in timing of the responses observed in control versus foraging fish can be estimated to be of the order of 1–2 s. Such differences in time are likely to be due to different degrees of attention being paid to the surroundings and/or limits in the visual field caused by a head-down foraging posture (Krause and Godin 1996). Our results are based on mechanical stimulation and show differences that are of the order of 30 ms, which are more likely to be due to different neural controls triggering the escape response, such as Mauthner cells versus non-Mauthner cells, although the possibility that other mechanisms related to delays in the sensory processing cannot be excluded.

Our work shows that the ability to perform an anti-predator response can be limited when another task, such as pursuing or capturing a prey, is under

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Before feeding</th>
<th>After feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_0$ (m)</td>
<td>0.122 ± 0.009</td>
<td>0.182 ± 0.013</td>
<td>0.157 ± 0.016</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>24.727 ± 4.849</td>
<td>61.600 ± 6.483</td>
<td>57.33 ± 8.620</td>
</tr>
<tr>
<td>ATR (s$^{-1}$)</td>
<td>1632.100 ± 122.698</td>
<td>2017.435 ± 120.719</td>
<td>2026.272 ± 236.917</td>
</tr>
<tr>
<td>MTR (s$^{-1}$)</td>
<td>2221.706 ± 136.174</td>
<td>2534.568 ± 127.876</td>
<td>2515.692 ± 308.997</td>
</tr>
<tr>
<td>MS (ms$^{-2}$)</td>
<td>1.273 ± 0.076</td>
<td>1.184 ± 0.054</td>
<td>1.096 ± 0.145</td>
</tr>
<tr>
<td>MA (ms$^{-2}$)</td>
<td>47.027 ± 3.919</td>
<td>42.754 ± 4.483</td>
<td>41.911 ± 2.948</td>
</tr>
<tr>
<td>CD (m)</td>
<td>0.050 ± 0.002</td>
<td>0.049 ± 0.002</td>
<td>0.042 ± 0.003</td>
</tr>
</tbody>
</table>

Fig. 3 Frequency distribution of turning rates (ATR) during routine turns (white bars) and during escape responses (black bars).
way. Because multitasking can be more easily accomplished by lateralized fish, e.g., when foraging while in the presence of a predator (Dadda and Bisazza 2006), it would be interesting to test the possibility that the escape response of lateralized fish may be less affected by foraging activities than those of non-lateralized ones. Lateralized fish have indeed been found to exhibit shorter latencies than non-lateralized ones, possibly because the latter tend to possess greater complexity in the neural control of their responses that could result in a higher threshold for triggering Mauthner cells (Dadda et al. 2010). Further work on the effect of multitasking on sensory and locomotor performance of fish is necessary in order to further unravel the complex relationship between the need to forage and the need to respond to attacks by predators. Furthermore, it would be interesting to include the duration of the starvation period as another variable to be analyzed in future studies, since it has been reported to be an aspect that influences the decision of whether to flee or feed (Godin and Sproul 1988).

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