Chemically-Mediated Predator Inspection Behavior by Fathead Minnow (*Pimephales promelas*)

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ABSTRACT
In the absence of visual cues, information gained from chemically-mediated inspection behavior may serve to reduce predation risk by informing minnow shoals of the proximity of potential predators. Behavioral inspection of predator chemical cues implies that sensory modes other than visual detection may be important for assessing predation risk in shoaling fishes. To assess the potential for inspection behavior by fathead minnow (*Pimephales promelas*; FHM), we quantified the responses of FHM to chemical cues of a natural predator, northern pike, and chemical cues of conspecifics. The presence of northern pike odor elicited inspection-like behavior among FHM. In control treatments, FHM remained within vegetated cover, and no differences were observed in the number of individuals in open water areas. When northern pike odor was added, significantly more FHM were observed in the open water habitat, although FHM activity was similar to that of controls. This suggests that FHM respond to northern pike chemical cues via directional movement into the open water habitats. These results indicate that FHM acquire information about potential predation risk in the absence of visual cues and engage in seemingly high risk investigating activity.

INTRODUCTION
Predator inspection behavior involves the slow approach of prey towards a predator, interrupted by stationary pauses during which the prey is constantly fixated on the predator, and then immediate return to cover or to conspecifics (Pitcher et al. 1986, Pitcher 1992, Brown and Zachar 2002). Although risky, predator inspection behavior is believed to relay important information about the presence of a predator to conspecifics or to the predator (Godin and Davis 1995, Godin and Dugatkin 1996) or about the state of a potential predator (e.g., hunger level, activity, etc.; Dugatkin and Godin 1992b, Mathis and Smith 1993, Godin and Davis 1995). Such behavior is often exhibited either by shoaling fish or by single individuals (Pitcher 1993) and has been well documented among a variety of prey fishes (Dugatkin and Godin 1992a, Pitcher 1992). By participating in search behavior, bold individuals may 1) reduce potential predation risk (Godin and Davis 1995), 2) develop an aversion for a novel predator (Brown and Godin 1999a), and 3) be more attractive to females, inferring increased fitness associated with the behavior (Godin and Dugatkin 1996).

In most studies, inspection behavior is quantified by exposing prey to live predators or models of predatory fish (Pitcher 1993, Chivers et al. 1995), and such studies have revealed differences in the inspection behavior as the posture or behavior of the predators or models change (Licht 1989, Murphy and Pitcher 1997). Inspection behavior can change in response to individual experience (i.e., conditioned versus naïve; Magurran 1986, Magurran et al. 1993), physiological status (i.e., hunger level) of the individual.
(Godin and Crossman 1994), or the presence of other species of the same prey guild
(Pfeiffer 1963, Chivers and Smith 1994a).

Inspection behaviors are often quantified by combining both chemical and visual
cues (Pfeiffer 1977, Mathis and Smith 1993b). Considerably less work has examined
chemical cues alone, particularly for native predator-prey assemblages. Recently, Brown
et al. (2000) demonstrated that odors from a novel predator (convict cichlid [Cichlasoma
nigrofasciatum]) elicited inspection behavior in aquarium-reared glow light tetra
(Hemigrammus erythrozonus). When presented with odors from convict cichlids, tetras
approached in small groups or as singletons and generally remained near the tail of the
cichlid (Brown and Schwarzbaer 2001).

The role of chemically-mediated inspection behavior is likely an under-
appreciated component of anti-predator behaviors among shoaling fishes (Brown et al.
minnow (Pimephales promelas) to detect odors from a natural predator (northern pike
[Esox lucius]) is well documented (Mathis and Smith 1993, Mathis et al. 1993, Brown et
al. 1995, Chivers and Smith 1998). When presented with odor from northern pike,
fathead minnow generally shows greater shoal cohesion (Mathis and Smith 1993) as well
as dashing and freezing behaviors (Chivers et al. 1995). Whether fathead minnow
exhibits inspection behavior when confronted with northern pike odor has not been
quantified, but if it occurs, this behavior would be of adaptive significance in natural
predator-prey assemblages. In this study, we designed an experiment to quantify
inspection behavior by fathead minnows exposed to chemical cues of a natural predator,
northern pike.

**METHODS AND MATERIALS**

We obtained fathead minnows (FHM) and northern pike from a local lake (UTM
14 648106E 4907117N) in Brookings County, South Dakota, U.S.A. where pike are
known to feed on FHM (Strand et al. 2007). Minnows (N~250) were collected with a
seine, transported to the laboratory in aerated coolers, and maintained in four 114 L
aquaria that were connected to a filtered, re-circulating water system. Water temperature
was maintained at 12°C. A northern pike (457 mm total length) was collected with a trap
net, transported to the laboratory, and held for 48 h in a tank filled with 40 L of aerated
water at a temperature of 14°C. To maintain natural odors of the wild-caught pike, we
did not feed it during the holding period.

Water containing northern pike odor was obtained from 1 L of water from the
northern pike holding tank. This water was filtered through Whatman GF/C filters and
frozen in 100 mL aliquots until needed. Conspecific odor, meant to simulate injury and/or
predation by piscivorous fish (Brown et al. 1995), was prepared from 10 FHM. The
minnows were killed by cervical dislocation, macerated in a mortar and pestle, and added
to 500 mL of distilled water. The preparation was then filtered through a Whatman GF/C
(1.2 μm) glass fiber filter and frozen in 100 mL aliquots.

Fathead minnow behavior was assessed by replicating each of the following
treatments five times: 1) addition of pike odor, 2) addition of conspecific odors or 3)
addition of distilled water (i.e., control). For each trial, 12 minnows (X total length = 46
mm) were placed into one of three plexiglass test chambers holding 38 L of water and
allowed to acclimate for 24 h. FHM were fed twice -- 4 h after introduction to the test
chamber and 4 h prior to initiating the experiment. Food was 5-6 mg of thawed Daphnia
pulex. To simulate vegetative cover, artificial plants were attached to ceramic tiles and
placed in one end of the tank. To facilitate observations and distinguish vegetated areas
from the open water arena, a vertical line was drawn on the front of each test chamber,
marking the edge of the vegetation. An air stone was placed near the opposite end of the
tank to provide aeration and water circulation.
To begin a trial, each of three aquaria was randomly assigned one of the three treatments. Treatments were administered through a funnel located near the back of the test chambers approximately 3 cm below the air stone (Brown et al. 2000). Although we did not quantify water dispersal dynamics in the tanks, related studies with similar test chambers have shown that chemical cues disperse throughout the tank within 30 s and produce a concentration gradient that remains for up to 10 min (Brown et al. 2000). To minimize visual disturbance to FHM, cardboard was placed around the back and sides of each test chamber.

A video camera was positioned 0.5 m from each tank to record FHM behavior. We defined inspection behavior as described in Brown et al. (2000), where inspection involved a directed movement away from vegetation and towards the odor source. Thus, we considered inspection behavior to be the movement of singletons or groups of fish from the vegetation into the open water arena. A FHM was considered in open water when it was at least one body length (~46 mm) past the line marking the border between vegetation and open water. From the video footage, we quantified the number of FHM in open water at 30 s time intervals for 2 min following application of a treatment (n=4 observations per trial). Data were analyzed using a two-way analysis of variance (ANOVA) with time and treatment as grouping factors (SAS 2003). Observations of startled fish were excluded from the analysis so that fish counted in open water areas were those that quietly and deliberately left the vegetation. To evaluate the influence of chemical cues on activity patterns of FHM, we indexed activity by randomly choosing a single fish and recording the total number of times it crossed grid lines (4 x 8, 34 x 36 mm cells) drawn on a video monitor. Activity measurements were recorded for 2 min following treatment application and compared across treatments using ANOVA.

RESULTS AND DISCUSSION

Mean number of FHM in open water varied similarly with time (F_{3,59} = 0.39, P > 0.05) and time by treatment (F_{6,59} = 0.40, P > 0.05) but differed across treatments (two-way ANOVA; F_{2,59} = 6.37, P = 0.003; Fig. 1). In control trials, all FHM remained within artificial vegetation following addition of distilled water. When pike odor was added to the tank, more FHM (\bar{x} = 1.00 fish) were observed in the open water area compared to controls (\bar{x} = 0 fish) or the conspecific odor treatment (\bar{x} = 0.25 fish; Tukey’s, P < 0.05). For FHM exposed to conspecific odors, we found no difference in the mean number of fish in open water compared to distilled water controls (Tukey’s, P > 0.05). Activity level was similar among FHM exposed to pike odor, conspecific cues, or distilled water controls (ANOVA; F_{2,12} = 0.69; P > 0.05), implying that inspection behavior of FHM was characterized more by directed movements out of vegetation than by an increase in activity level.

In turbid or densely vegetated areas, fathead minnows may not be able to rely solely on vision to detect motionless predators before passing within striking distance. In the absence of visual cues, information gained through chemically-mediated inspection behavior may provide advantages to shoaling minnows by signaling the proximity of a potential predator. This ability to detect chemical substances may have important implications for minnow survival, particularly in environments inhabited by ambush-type predators such as northern pike. A minnow’s ability to use chemosensory cues for predator recognition has been shown to be an important predator avoidance adaptation (Chivers and Smith 1998).

When examining the adaptive significance of search behavior, studies suggest that prey inspectors may 1) assess the threat from a distance and continue foraging if no impending threat is sensed (Godin and Crossman 1994) or 2) recognize a potential threat from a distance and begin anti-predatory behavior if a threat is sensed (Brown et al. 2000). Individuals that participate in inspection-like behavior also have reduced attack
rates by the inspected predator (Godin and Davis 1995). Inspection behavior may relay important information to conspecifics of possible threats (Murphy and Pitcher 1997) and as a result increase the number of individuals displaying anti-predator behaviors (Dugatkin and Godin 1992a, Murphy and Pitcher 1997)

![Bar chart showing number of minnows over time](chart)

**Figure 1.** Mean (+S.E.) number of fathead minnows in the open water arena of experimental tanks following addition of conspecific odor (open bars) or northern pike odor (hatched bars). Horizontal line represents distilled water control where no minnows were observed in the open water arena.

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**LITERATURE CITED**


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