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POPULATION AND SEX DIFFERENCES IN ANTIPREDATOR RESPONSES OF BREEDING FATHEAD MINNOWS (*Pimephales promelas*) TO CHEMICAL STIMULI FROM GARTER SNAKES (*Thamnophis radix* and *T. sirtalis*)

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Abstract—We conducted a predator bite survey on a population of fathead minnows (*Pimephales promelas*) considered to be under substantial predation pressure by western plains garter snakes (*Thamnophis radix*). Scarring, due to failed predation attempts by garter snakes and crayfish (*Orconectes virilis*), was observed significantly more often in breeding males than in breeding females and nonbreeding minnows. Likely, territorial nest defense under the edges of rocks along the water's edge, a habitat occupied by crayfish and frequented by snakes, caused the breeding males to be differentially vulnerable to predation. Under controlled laboratory conditions, breeding males from this population exhibited an antipredator response to chemical stimuli from live snakes (*T. sirtalis* and *T. radix*) significantly more often than breeding female minnows from the same population and breeding minnows of both sexes from a population that was presumed to be under lower predation pressure from snakes.

Key Words—Differential vulnerability, differential predation, alarm substance, cost of reproduction, learned predator recognition, predator avoidance, fathead minnow, *Pimephales promelas*, garter snake, *Thamnophis radix*, *Thamnophis sirtalis*.

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INTRODUCTION

Differences in predation intensity frequently result in interpopulation differences in antipredator behavior. Generally, individuals from populations in which predators are common exhibit stronger antipredator responses than individuals from populations that experience lower levels of predation. Interpopulation differences in antipredator behavior have been documented in many taxa, including spiders (Riechert and Hedrick, 1990), salamanders (Ducey and Brodie, 1991), snakes (Herzog and Schwartz, 1990), deermice (Hirsch and Bolles, 1980), and fishes (Giles and Huntingford, 1984; Huntingford, 1982; Magurran and Pitcher, 1987; Magurran and Seghers, 1990; Seghers 1973, 1974; Mathis et al., 1993). In Trinidadian guppy (*Poecilia reticulata*) populations, for example, there are differences in attack cone avoidance (Magurran and Seghers, 1990) and school cohesiveness (Seghers, 1973, 1974). European minnows (*Phoxinus phoxinus*) from pike-sympatric populations increase shoaling (Magurran and Pitcher, 1987) and numbers of inspection visits, following exposure to live or model pike (*Esox lucius*), compared to pike-allopatric populations (Magurran and Pitcher, 1987). Similarly, pike-sympatric fathead minnows (*Pimephales promelas*) show a greater increase in cover use and greater decrease in overall activity levels than pike-allopatric populations following exposure to visual or chemical stimuli from pike (Mathis et al., 1993). Huntingford (1982) and Giles and Huntingford (1984) documented that populations of three-spined stickleback (*Gasterosteus aculeatus*) with high predation risk showed more extreme fright responses than populations from low-risk sites.

Differences in predation pressure have also resulted in intrapopulation sex differences in antipredator behavior and predation vulnerability. For example, Huntingford (1982) showed sex differences in antipredator responses in three-spined stickleback. Males were found to be bolder than females when encountering predators. Sex-specific predation due to differential vulnerability has been documented in various taxa, including birds (Sargeant, 1972), mammals (Bergerud, 1971), and fishes (Britton and Moser, 1982). Sargeant (1972) demonstrated that differential vulnerability associated with nesting caused female dabbling ducks to be selectively preyed upon by red foxes (*Vulpes vulpes*). Male caribou (*Rangifer tarandus*) calves, due to their greater tendency to wander away from the protection of the herd than females, are more often captured by lynx (*Lynx canadensis*) (Bergerud, 1971). Likewise, Britton and Moser (1982) demonstrated that female mosquito fish (*Gambusia affinis*) are more vulnerable to capture by grey herons (*Ardea cinerea*) because they are larger and more conspicuous than males.

Breeding male fathead minnows associate with defined nesting areas, which they defend for 10–30 days (McMillan, 1972; McMillan and Smith, 1974). At a pond located on the University of Saskatchewan campus, breeding male minnows defend territories under the edges of rocks in shallow water. This territoriality in shallow water likely results in an increase in the frequency of interaction between breeding male minnows and snakes when compared to the frequency of interaction between either breeding females or nonbreeding fish and snakes. The snakes inhabit the areas around the water's edge, foraging for minnows exclusively in the shallow water rocks (personal observations). Breeding females and nonbreeding fish are primarily pelagic and do not frequent the shallow water rocks. Thus, the males' territoriality likely makes them more vulnerable to predation by the snakes. In addition, males become aggressive towards predators during the breeding season (McMillan, 1972), which is appropriate on the basis of parental care, but dangerous to the individual "care-giver." Breeding males, for example, often attack a person's hand that comes too close to their nest (personal observation).

Garter snakes (*Thamnophis* spp.) eat a variety of prey organisms (Burghardt et al., 1988), although individuals frequently specialize on one prey type (Carpenter, 1952; Drummond, 1983). During several hours of observations at the campus pond we observed western plains garter snakes (*Thamnophis radix*) catch and eat five breeding male fathead minnows, but in no instances did we observe breeding female or nonbreeding fish being caught.

In this study we examine whether breeding activities of male minnows make them more vulnerable to snake predation. We conducted a predator bite survey, testing the hypothesis that if breeding males are at higher risk to snake predation, then they should show significantly more signs of snake attacks than breeding females and both sexes of nonbreeding fish. Predator-induced injuries from which fish have recovered have been documented in fathead minnows (Smith and Lemly, 1986), three-spined stickleback (Reimchen, 1988, 1992), and whitefish (*Coregonus nasus* and *C. clupeaformis*) (Reist et al., 1987). In response to the increased risk of snake predation associated with nest defense, males may have developed a stronger antipredator response to snakes than females since increased predator avoidance ability in risk-prone individuals increases their probability of survival (Holtby and Healey, 1990; Johnsson, 1993). In laboratory experiments, we compared the intensity of antipredator responses of male and female minnows to chemical stimuli from snakes. We predicted that breeding male minnows should exhibit a stronger antipredator response to the chemical stimuli than breeding females.

Magurran and Seghers (1990) demonstrated that populations of Trinidadian guppies skilled in dealing with one type of predator do not necessarily cope well in unfamiliar predation situations. Minnows from Pike Lake (a population originating from the same drainage basin as the campus pond population) are preyed upon heavily by pike (Mathis et al., 1993) but, because of differences in minnow spawning habitat between Pike Lake and the campus pond (personal observations), it is expected that the Pike Lake minnows are exposed to lower snake predation pressure than the campus pond minnows. In the laboratory, we compared antipredator responses of breeding minnows from both the campus pond and Pike Lake to chemical stimuli from snakes, to test the hypothesis that males from the campus pond will show a more intense response to snake stimulus than males and females from the lake population.

METHODS AND MATERIALS

Predator Bite Survey

We used Gee's Improved Minnow Traps to capture 2609 minnows from the campus pond. They were transported to the laboratory and examined for evidence of predator-induced injuries. The minnows were maintained in three 160-liter tanks, and all uninjured fish were returned to the pond less than 24 hr after capture.

Prior to examination, we anesthetized the minnows with MS 222 (triciane methanesulfonate). Injuries were classified into three categories: (1) snake bites, which were symmetrical, rounded, rasplike lacerations or abrasions on both sides of the fish; (2) crayfish (*Orconectes virilis*) pinches, which were small, deep cuts on either side of the fish; and (3) other injuries, those that could not be classified in either of the aforementioned categories. For the purpose of statistical comparisons, we divided the minnows into three categories: (1) breeding males, (2) breeding females, and (3) other, nonbreeding fish not distinguishable as either sex on the basis of secondary sexual characteristics. We used a chi-square test (Siegel and Castellan, 1988) to compare the frequency of breeding male, breeding female, and nonbreeding fish with snake and crayfish bites.

Responses of Minnows to Chemical Stimuli from Garter Snakes

Collection and Maintenance. We collected fathead minnows from Pike Lake, an oxbow lake of the South Saskatchewan River in south-central Saskatchewan, and from the campus pond. We then transferred the minnows to the laboratory where they were maintained in separate 160-liter tanks at approximately 20°C on a 10 : 14 light–dark photoperiod and were fed daily with Nutrafin flake food.

Snake Stimulus Preparation. Prior to collecting the stimulus, two garter snakes [one western plains garter snake (*Thamnophis radix*), and one red-sided garter snake (*Thamnophis sirtalis*), both found locally] were each fed, twice a week, approximately equal volumes (range = 4–5 ml, measured by volumetric displacement in water) of previously frozen neonate mice (*Mus domesticus*). We fed the snakes a diet of mice, not fathead minnows, to ensure that alarm substance (AS) would not be ingested by the snakes. This eliminated the possibility that AS would label the predator as dangerous to naive prey (Mathis and Smith, 1993a,b). One glass bowl containing 900 ml of glass-distilled water was placed into a cage (50 × 30 × 25 cm) that contained both snakes. The snakes bathed and defecated in the water. After 72 hr, the water was removed, filtered through glass wool, pipetted into separate 12-ml polypropylene containers, and frozen at approximately −20°C. As a control stimulus, 12-ml units of glass-distilled water were frozen in separate polypropylene containers.

Testing Protocol. To test for population and sex differences in the responses of minnows to chemical stimuli from the snakes, we divided the minnows (all of which were in breeding condition) into four groups of 10 fish each: (1) campus pond males, (2) campus pond females, (3) Pike Lake males, and (4) Pike Lake females. We arbitrarily placed individual fish into separate Plexiglas acclimation tanks (45 × 45 × 20 cm). Fresh water passed through the acclimation tanks at a constant rate of approximately 250 ml/min, maintaining a constant depth of 4–5 cm. Each tank contained a floating circular shelter measuring 9 cm in diameter. The fish were maintained on a 10:14 light–dark photoperiod and were fed Nutrafin flakes daily. After a two-day acclimation period we transferred the minnows to test tanks. The flow rate in the test tanks was increased to approximately 500 ml/min. Acclimation and testing both occurred at a mean temperature of 19°C.

The test tanks were surrounded by an Opto-Varimex Aqua tracking meter (Columbus Instruments) which laid out a grid of light beams throughout the tanks. A microcomputer scanned the light beam grid for breaks at a frequency of 8 pulses/sec. Lemly and Smith (1986, 1987) described this testing apparatus in detail. Our system differed only in that outflowing water was discarded rather than recirculated. The computer recorded two measures of activity that typically decrease during a fright reaction in fathead minnows: (1) distance travelled (centimeters) and (2) number of stereotypic movements (Lawrence and Smith, 1989; Mathis et al., 1993; Chivers and Smith, 1993). A fright response in fathead minnows includes freezing and increased use of shelter, which the Opto-Varimex apparatus interprets as a decrease in distance traveled and number of stereotypic movements. By decreasing distance traveled and number of stereotypic movements, the minnows likely become less visible to visually oriented predators.

An observer stationed in an adjacent room, to avoid disturbing the test fish, injected chemical stimuli into inflowing water lines that passed through the observation room before entering the test tanks. We conducted experimental observations between 0800 and 1000 hr using control water. Trials lasted for 16 min, with 12 ml of control water stimulus being injected after 8 min. Injection of the test stimulus took approximately 10 sec. A second set of observations, on the same minnows, took place the same day between 1030 and 1230 hr using 12 ml of the snake stimulus. The procedure for injection was the same as for the water control. The minimum time between tests for an individual fish was at least 150 min.

The minnows' responses were quantified using the two measures recorded by the computer. The significance of the minnows' responses was determined by using a Wilcoxon-Mann-Whitney test (*Wx*; Siegel and Castellan, 1988) for each of the four groups of minnows. The two different stimuli were tested independently.

RESULTS

Predator Bite Survey. Out of 2609 campus pond minnows examined, 30 (1.15%) showed scarring from some type of predation attempt. Fourteen minnows were found with snake bite scarring, 13 minnows were found with crayfish pinches, and three minnows were found with scarring unlike those in the aforementioned categories (Table 1). Male minnows from the campus pond showed significantly more snake- and crayfish-induced injuries than females (χ^2 = 11.565, $df = 1$, $P < 0.01$; $\chi^2 = 7.07$, $df = 1$, $P < 0.01$, for snake and crayfish, respectively) and nonbreeding minnows ($\chi^2 = 47.56$, $df = 1$, $P < 0.001$; $\chi^2 = 22.75$, $df = 1$, $P < 0.001$, for snake and crayfish, respectively). These results suggest that breeding male minnows are more vulnerable to snake and crayfish predation than breeding female and nonbreeding minnows.

Responses of Minnows to Chemical Stimuli from Garter Snakes. In response to snake water, breeding male minnows from the campus pond decreased their distance traveled ($Wx = 65$, $m = 10$, $n = 10$, $P < 0.003$; Figure 1) and numbers of stereotypic movements ($Wx = 63$, $m = 10$, $n = 10$, $P < 0.002$; Figure 2) significantly more compared to exposure to the water control. In contrast, males from the Pike Lake population and females from both popula-

TABLE 1. NUMBER AND PERCENT OF BREEDING MALE, BREEDING FEMALE AND NONBREEDING MINNOWS FROM THE CAMPUS POND POPULATION WITH SNAKE- AND CRAYFISH-INDUCED INJURIES

	Minnows with snake bites		Minnows with Crayfish pinches	
	N	%	*N*	%
Breeding males	13/417	3.12	9/417	2.15
Breeding females	1/519	0.19	2/519	0.39
Nonbreeding fish	0/1673	0.00	2/1673	0.12
TOTAL	14/2609	0.54	13/2609	0.50%

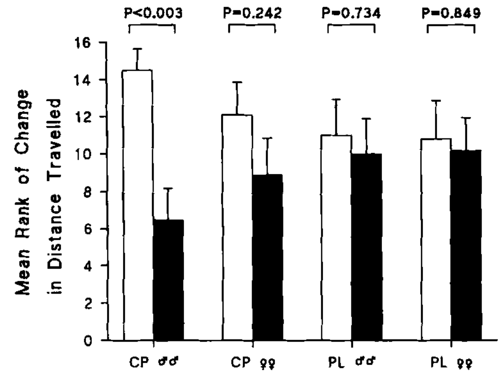

FIG. 1. Mean rank (±SE) of change in total distance traveled (cm) by campus pond (CP) male minnows, CP females, Pike Lake (PL) male minnows, and PL females following exposure to distilled water control (open bars) and chemical stimuli from garter snakes (solid bars).

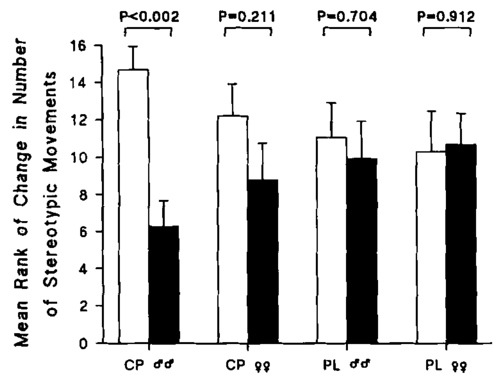

FIG. 2. Mean rank (±SE) of number of stereotypic movements by campus pond (CP) male minnows, CP females, Pike Lake (PL) male minnows, and PL females following exposure to distilled water control (open bars) and chemical stimuli from garter snakes (solid bars).

tions showed no significant decrease in their total distance travelled (cm) ($Wx = 100$, $m = 10$, $n = 10$, $P = 0.734$; $Wx = 102$, $m = 10$, $n = 10$, $P = 0.849$; $Wx = 89$, $m = 10$, $n = 10$, $P = 0.242$, for Pike Lake males, Pike Lake females, and campus pond females, respectively; Figure 1) following exposure to chemical stimuli from snakes compared to exposure to the water control. Likewise, males from the Pike Lake population and females from both populations showed no significant decrease in number of stereotypic movements ($Wx = 99.5$, $m = 10$, $n = 10$, $P = 0.704$; $Wx = 103$, $m = 10$, $n = 10$, $P = 0.912$; $Wx = 88$, $m = 10$, $n = 10$, $P = 0.211$, for Pike Lake males, Pike Lake females, and campus pond females, respectively; Figure 2) following exposure to chemical stimuli from snakes compared to exposure to the water control.

DISCUSSION

The results of this study suggest that breeding male minnows from the campus pond are more vulnerable to snake predation than breeding females and nonbreeding minnows. This difference likely results from a higher encounter frequency with snakes while defending breeding territories in shallow water. Our results also provide evidence that breeding male minnows are more vulnerable to crayfish predation, probably for the same reason. Such results indicate the cost of reproduction incurred by the individual parent responsible for providing prenatal care, as it may reduce the probability of the parent surviving to future breeding seasons (Gross and Sargent, 1985).

The use of two species of garter snakes in the preparation of the stimulus was not intentional; one snake was initially misidentified. Nonetheless, breeding males from the campus pond did recognize and display appropriate antipredator behavior to the combined chemical stimuli from the snakes, while female campus pond minnows and both male and female Pike Lake minnows did not. From our results we do not know whether the male campus pond minnows' response was a general response to garter snake stimulus or a specific response to western plains garter snake stimulus. Nevertheless, our results demonstrate intra- and interpopulation differences in antipredator behavior.

Our experimental design did not allow us to empirically test whether genetic population differences or learning was responsible for the observed interpopulation differences in ability to recognize snakes as predators. The two minnow populations originated from the same drainage basin and have been separated for only 35 years. This length of time may not be sufficient to develop genetic population differences in ability to recognize snakes. The two populations have not developed genetic population differences in their ability to recognize another predator, the pike (Chivers and Smith, 1994b; unpublished data). It seems likely that learning and not a genetic population difference is responsible for the observed difference in antipredator behavior.

Several mechanisms of learning predator recognition have been demonstrated for fathead minnows. Cyprinid fishes learn to recognize unfamiliar predators when alarm substance, a pheromone released by mechanical damage to epidermal alarm substance cells (ASCs) (reviewed in Smith, 1992), is presented in conjunction with chemical (Göz, 1941; Magurran, 1989; Chivers and Smith, 1994a) or visual (Chivers and Smith, 1994b) stimuli from an unfamiliar predator. Alarm substance ingested by a predator may chemically label the predator as being dangerous to naive prey (Mathis and Smith, 1993a,b). These mechanisms may not be responsible for the acquisition of predator recognition in the breeding male minnows from the campus pond as ASCs are reduced or eliminated in breeding male fathead minnows (Smith, 1973; Smith and Murphy, 1974). Scarring due to failed predation attempts indicates that a portion of the males attacked by the snakes survive the encounter. These individuals, through the encounter, should learn that snakes are dangerous predators. Since breeding territories are often located in close proximity to one another (McMillan, 1972), other breeding males may witness the snake attack and could learn to recognize garter snakes as dangerous. Pike-naive fathead minnows learn to respond appropriately to pike by observing the reaction of pike-experienced minnows (Chivers and Smith, 1994c). By having a few snake-experienced individuals within visual range, snake-naive males may also learn to regard snakes as potential predators by watching the antipredator reactions of the experienced minnows.

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