Do Eastern Mosquitofish Exhibit Anti-predator Behavior towards Red Swamp Crayfish?

Emília Pereira Leite[1], Pedro Manuel Anastácio[3], Margarida Ferreira[2], Luís Vicente[2], and Alexandra Marçal Correia[1,*]

[1]*Universidade de Lisboa, Museu Nacional de História Natural, Departamento de Zoologia e Antropologia (Museu Bocage), Centro de Biologia Ambiental (CBA), R. Escola Politécnica, 58, 1269-102 Lisbon, Portugal*

[2]*Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Animal, Centro de Biologia Ambiental (CBA), Campo Grande, P-1749-016 Lisbon, Portugal*

[3]*Institute of Marine Research (IMAR), c/o Departamento de Ecologia, Universidade de Evora, R. Romão Ramalho, 59, 7000-671 Évora, Portugal*

Emília Pereira Leite, Pedro Manuel Anastácio, Margarida Ferreira, Luís Vicente, and Alexandra Marçal Correia (2005) Do eastern mosquitofish exhibit anti-predator behavior towards red swamp crayfish? *Zoological Studies* **44**(4): 513-518. Anti-predator behavior has been described in many freshwater fish species. Freshwater crayfish may have a great impact on prey species through behavioral changes. The eastern mosquitofish, *Gambusia holbrooki*, and the red swamp crayfish, *Procambarus clarkii*, are native to North America, and have been introduced worldwide, causing negative effects on aquatic ecosystems. The interactions between these exotic species are poorly known. In natural habitats *P. clarkii* consumes *G. holbrooki*, but it is not clear if the mosquitofish has anti-predator defenses against the crayfish. The main objective of this study was to determine if *G. holbrooki* exhibits anti-predator behavior in the presence of *P. clarkii*. To address this question, laboratory tests were performed in experimental aquaria, analyzing the following behavioral traits of the mosquitofish: activity, distance from the predator, and space use. The mosquitofish were generally active and mainly preferred open space in both controls and treatments. Results suggest that *G. holbrooki* does not display anti-predator behavior when exposed to *P. clarkii* under the laboratory conditions used in this study. http://zoolstud.sinica.edu.tw/Journals/44.4/513.pdf

Key words: Predator avoidance, *Gambusia holbrooki*, *Procambarus clarkii*, Activity, Space use.

Many invasive and native species are often integrated together in freshwater communities, and they develop complex interactions (Nyström and McIntosh 2003). The consequences of exotic species in aquatic systems are difficult to predict because the effects of introduced species vary according to their ecology, the environmental conditions, and the community composition of the ecosystem in question. Recently much effort has been made towards identifying and predicting the impacts of biological invasions (e.g., Parker et al. 1999, Kolar and Lodge 2001, Moyle 1999, Sakai et al. 2001, Heger and Trepl 2003, Simon and Townsend 2003), but few studies have examined how exotic species interact with each other in colonized environments. Such studies may offer excellent opportunities for understanding and managing invasiveness.

The red swamp crayfish, *Procambarus clarkii* (Girard, 1852), is native to northeastern Mexico and the southeastern US (Huner 1990), and it has been introduced worldwide. Its introduction into Portugal occurred in the late 1970s as a result of expansion of Spanish populations (Ramos and

Pereira 1981). Populations of *P. clarkii* increased without control, invading wetlands and most rice fields (Correia 1995). Because *P. clarkii* is omnivorous, it has a complex trophic role and has the potential to influence the structure of aquatic communities (Correia 2002 2003). The eastern mosquitofish, *Gambusia holbrooki* (Girard, 1859), is native to the East Coast of the US, but has been widely introduced in temperate and tropical regions worldwide through mosquito control programs (Cabral and Marques 1999). This species was introduced to the Iberian Peninsula in 1921, and in Portugal, it has invaded the lowest stream sections, wetlands, and coastal lagoons (Cabral and Marques 1999). Mosquitofish consume planktonic insect larvae and other invertebrates, as well as amphibian eggs and affect rare and endangered as well as economically important fish species through competition and by eating their eggs (Cabral et al. 2002). In Portugal, this species coexists with *P. clarkii* in several areas. The native range of both species overlaps in the US in southern Mississippi, southern Alabama, and Florida (Huner 1990, Wooten and Lydeard 1990).

It is well known that predators often induce life history, morphological, and behavioral changes in prey, in attempts to reduce their risk of predation (see Lima and Dill 1990, Lima 1998a b for reviews). In fact, there is extensive literature indicating that freshwater crayfish may exert great impact on prey species through behavioral changes (e.g., Rahel and Stein 1988, Turner et al. 2000, Bernot and Turner 2001, Nyström et al. 2001, McCarthy and Dickey 2002). Anti-predator behavior has been described in many freshwater fish species (see Lima 1998b for a review). According to the threat-sensitivity hypothesis, prey increase their anti-predator behavioral responses as the potential predation risk increases (Smith and Belk 2001). For instance in the presence of fish predators, mosquitofish avoid habitats with high predator risk (Winkelman and Abo 1993, Chick and McIvor 1997), and there is evidence that fish decrease their activity when exposed to predators (Rahel and Stein 1988, Lima and Dill 1990, Lima 1998a b, Bryan et al. 2002). Furthermore, Smith and Belk (2001) found that mosquitofish responded in an additive manner to several factors, e.g., the type of prey consumed by the predator, the predator hunger level, and the distance to the predator. In rice fields, *P. clarkii* consumes *G. holbrooki* (Correia 2003), but so far it is not clear if the mosquitofish has developed any anti-predator defenses against the crayfish.

The main objective of this study was to understand if *P. clarkii* has any effect on the behavior of *G. holbrooki*. This study tested 3 hypothesis regarding adaptive behavioral responses of mosquitofish to the presence of crayfish: 1) mosquitofish decrease their activity when exposed to crayfish; 2) mosquitofish maintain a certain distance from predators; and 3) mosquitofish use heterogeneous space for protection. Activity, distance from the predator, and space use by *G. holbrooki* were examined in the presence and absence of *P. clarkii* through laboratory tests in experimental aquaria. We expect that the information gathered will help obtain better insights into the global functioning of invaded aquatic ecosystems.

MATERIALS AND METHODS

Specimens of *G. holbrooki* and *P. clarkii* were collected in a 4 ha rice field, located 6 km SE of Salvaterra de Magos, at the lower River Tejo, Portugal (38°58'N, 8°45'W). Crayfish and mosquitofish of both sexes were collected with a dip net on 25 Sept. 2003. The total length of *G. holbrooki* and carapace length of *P. clarkii* were measured to the nearest millimeter. The most-common size classes were selected for the experiments.

Crayfish brought from the field were acclimated for 15 d in large aerated containers (60 x 40 x 40 cm, with adult and juveniles separated) under a 14:10 h light-dark period (Scheider et al. 1999). Mosquitofish were acclimated during 4 d in aerated glass aquaria (60 x 30 x 30 cm) under a 14:10 h light-dark period. Tap water treated to eliminate the chlorine was used, and it was changed every other day. Mosquitofish used in the experiments were fed TetraMin Tropical Fish Food daily, and carrots were fed to adult crayfish.

Experiments

The anti-predator behavior experiments were carried out in a glass aquarium (124 x 30 x 30 cm) divided by a 500 μm mesh into a middle section (test arena, 90 x 30 cm) and 2 end compartments (17 x 30 cm) (Fig. 1). The arena was marked transversally and longitudinally into 4 equal quadrants as illustrated in figure 1. The arena was covered with sand (to 1 cm deep) to provide a semi-natural substrate. Rice stems were placed upright in the 2 heterogeneous quadrants, with a density of 350 rice stems/m^2, similar to the density observed in the rice fields. Tap water treated to

eliminate the chlorine was added (to 10 cm deep). The aquarium external walls were covered with light-brown paper to avoid external visual effects.

Six mosquitofish (with an average total length of 28.7 ± 4.87 mm) were placed in the center of the arena and left to acclimate for 5 min. The position of *P. clarkii* was randomly assigned to one of the end compartments in both the treatment and control tanks. For the treatment, 1 *P. clarkii* (with an average carapace length of 50.6 ± 2.19 mm) was placed in the assigned end compartment after the acclimation period, whereas in the controls, no crayfish was added to the "assigned end compartment". The mesh dividing the end compartments allowed visual contact and unrestricted water and chemical flow into the arena. Crayfish created the chemical flow through use of their fan organs (see Breithaupt 2001 for further details). In pretests, the chemical flow was visualized by dropping 1 ml of methylene blue in the end compartment with the crayfish, and it took approximately 10 s for the dye to reach the arena. Following each experiment, the aquarium was drained, the sand and rice stems were removed, and the aquarium was thoroughly rinsed. The sand and rice stems were replaced before each experiment. Crayfish and mosquitofish were used only once in the experiments and then released.

Each experiment lasted 15 min and was filmed with a Sony Video HI8 pro camera for posterior analysis. The camera was placed 175 cm above the aquarium, just behind it, and the observ-er remained motionless behind the camera. Two experiments were recorded at the same time. Ten replicates of treatments (with crayfish) and controls (without crayfish) were carried out. The activity of *G. holbrooki* (active or inactive), distance (d) from the predator (close, d < 45 cm or far, d > 45 cm), and space use (open space or heterogeneous space) were recorded (Table 1), by continuous observations, using videotape analysis.

Statistical analysis

After the experiments, the average number of *G. holbrooki* and the percentage of time spent in each quadrant were calculated. Data from the behavior experiments were analyzed by means of a multiway frequency table of the variables (Sokal and Rohlf 1981). The software used was Statistica, version 6.0. The factors analyzed were the effect of crayfish (presence/absence), the activity of the mosquitofish (active/inactive), distance (close/far) from the predator, and space use by the mosquitofish (open space/heterogeneous space). The cut-off for significance was accepted at $\alpha = 0.05$.

RESULTS

In the behavior experiments, there were no significant differences of the effect (presence/absence) of the crayfish (Table 2), but there were
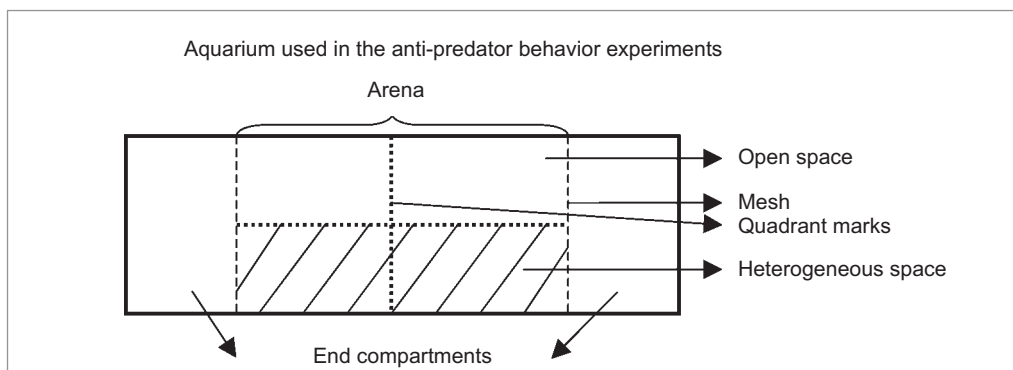

Fig. 1. Top view of the aquarium used for the behavior experiments.

Table 1. Behavioral traits of *Gambusia holbrooki* analyzed during the experiments

Activity	Distance (d) to *Procambarus clarkii*	Space use
Active	Close (d < 45 cm)	Open space
Inactive	Far (d > 45 cm)	Heterogeneous space

significant differences in space use, distance from the predator, and mosquitofish activity as shown in table 2. All combinations of variables showed non-significant differences (Table 2). The mosquitofish were mainly active and preferred the open space (Fig. 2). There was also a higher occupation of quadrants far from the end compartment "assigned to the predator" in both the control and treatment tanks.
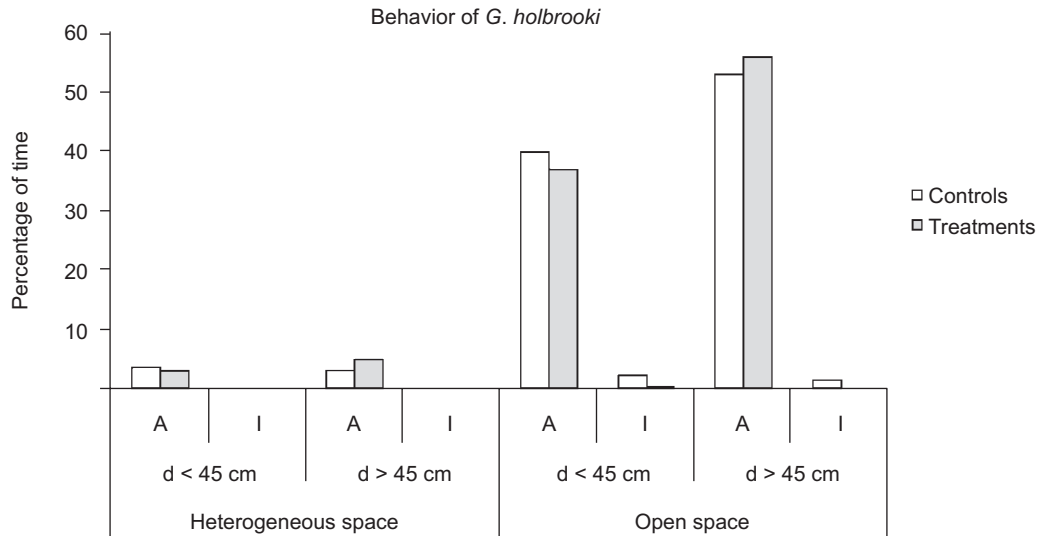
Fig. 2. Behavior of *Gambusia holbrooki*, in the presence or absence of the predator, *Procambarus clarkii*, under experimental conditions, according to the analyzed factors: activity, distance (d) from the compartment assigned to the predator, and space use. A, active; I, inactive.

Table 2. Results of the multiway frequency table of the behavior experiments. Factors analyzed were crayfish (presence/absence), space use by *Gambusia holbrooki* (open space/heterogeneous space), distance to the predator (d < 45 cm/d > 45 cm), and activity of *G. holbrooki* (active/inactive). Significant differences:*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

Effect	Tests of marginal and partial association				
	Degrees of freedom	Partial association χ^2	Partial association p	Marginal association χ^2	Marginal association p
Crayfish	1	0.027	0.870	0.0267	0.870
Space use	1	167.945	0.001***	167.9446	0.001***
Distance	1	5.011	0.025*	5.011	0.025*
Activity	1	226.849	0.001***	226.849	0.001***
Crayfish x space use	1	0.227	0.634	0.104	0.747
Crayfish x distance	1	0.299	0.584	0.360	0.548
Crayfish x activity	1	1.338	0.247	1.294	0.255
Space use x distance	1	0.072	0.788	0.121	0.728
Space use x activity	1	2.231	0.135	2.177	0.140
Distance x activity	1	0.536	0.464	0.664	0.415
Crayfish x space use x distance	1	0.094	0.759	0.071	0.789
Crayfish x space use x activity	1	0.376	0.539	0.369	0.544
Crayfish x distance x activity	1	0.024	0.878	0.002	0.961
Space use x distance x activity	1	0.116	0.734	0.105	0.746

DISCUSSION

Mosquitofish seem to rely on visual cues during exploratory behavior, but avoidance behavior is based on a combination of visual and chemical cues (Smith and Belk 2001). In this study, mosquitofish did not seem to exhibit anti-predator behavioral responses when exposed to visual and chemical stimuli of crayfish because treatment results did not significantly differ from those of the controls. It has been shown that predators may elicit a decrease in fish activity (Rahel and Stein 1988, Lima and Dill 1990, Lima 1998a b, Bryan et al. 2002), and that mosquitofish exhibit patterns of space selection by avoiding habitats with high predation risk (Winkelman and Aho 1996, Chick and McIvor 1997). However, the hypothesis regarding behavioral responses of mosquitofish to crayfish was not supported by the present results because in both treatments and controls, the mosquitofish were mainly active and preferred the open space. Mosquitofish also preferred distances at the far end of the compartment randomly "assigned to the predator", in both the treatment and control tanks, a fact for which no explanation was found.

Smith and Belk (2001) argued that the anti-predator behavior presented by mosquitofish depends on predator cues (e.g., hunger or type of diet) and on the type of response variables analyzed. In this study, crayfish could freely move within the end compartment, but they were satiated and had previously been fed carrots. For example, Smith and Belk (2001) observed that the distances maintained by mosquitofish from the predator varied according the level of hunger of the predator and type of prey consumed before the experiment. Mosquitofish maintained a greater distance with hungry (instead of satiated) and mosquitofish-fed (in opposition to chironomid-fed) predators. Other stimuli, such as chemical cues produced by stressed conspecifics, or by predators under different conditions, and tactile or other visual stimuli such as predator approximation, may also induce anti-predator behavior (Lima and Dill, 1990, Lima 1998b). It is possible that in the present case, crayfish did not elicit an anti-predator behavioral response from the mosquitofish because the predator was satiated and had not been fed prey conspecifics. Moreover, the crayfish was isolated from the mosquitofish, so it is possible that these fish perceived a small predation risk due to the presence of a barrier which prevented contact with the predator. In the context of the threat-sensitivity hypothesis, the time spent in predator avoidance strategies should balance the risk of predation because organisms that overreact to predators decrease their fitness (Lima 1998a b, Smith and Belk 2001). Thus if mosquitofish assessed that the predator threat was reduced, they should not be expected to exhibit anti-predator responses to the crayfish. Furthermore, in a group, as used in this study, the position of each member influences the probability of predator inspection and thus the elicitation of an escape response (Bisazza et al. 1999, Santi et al. 2001). However, further testing is necessary to assess if the lack of an anti-predator response by *G. holbrooki* to *P. clarkii* was also related to the group effect.

Our results indicate that when crayfish are isolated, satiated, and have a diet free of mosquitofish, they do not seem to elicit an anti-predator response from mosquitofish. Although *G. holbrooki* and *P. clarkii* coexist in southern Mississippi, southern Alabama, and Florida in the US (Huner 1990, Wooten and Lydeard 1990), an anti-predator response would only be beneficial when the energy and effort involved in that process compensate for the risk of predation. *Procambarus clarkii* is a polytrophic species that adjusts its diet to resource availability (Correia 2002 2003), being able to consume fish (goldfish (*Carassius auratus*) and *G. holbrooki*), but these prey are generally eaten in low proportions and in extreme conditions (Correia 2003). From the perspective of the threat-sensitivity hypothesis, it seems that tradeoffs between the crayfish predation risk and the loss of time and energy in predator avoidance by *G. holbrooki* result in a lack of anti-predator behavioral responses. In natural habitats, it is likely that prey fishes use multiple predator cues to access the predation risk, although it is not well known how prey integrate all the different sources of information (Smith and Belk 2001). Studies involving other predator cues (e.g., hunger level, type of diet, size, density, and contact) in assessing behavioral responses of *G. holbrooki* to *P. clarkii* are necessary to determine to what extent mosquitofish balance the risk of mortality with anti-predator responses.

REFERENCES

Bernot RJ, AM Turner. 2001. Predator identity and trait-mediated indirect effects in a littoral food web. Oecologia **129:** 139-146.

Bisazza A, A Santi, G Vallortigara. 1999. Laterality and cooperation: mosquitofish move closer to a predator when the companion is on their left side. Anim. Behav. **57:** 1145-1149.

Breithaupt T. 2001. Fan organs of crayfish enhance chemical information flow. Biol. Bull. **200:** 150-154.

Bryan SD, AT Robinson, MG Sweetser. 2002. Behavioral responses of a small native fish to multiple introduced predators. Environ. Biol. Fish. **63:** 49-56.

Cabral JA, JC Marques. 1999. Life history, population dynamics and production of eastern mosquitofish, *Gambusia holbrooki* (Pisces, Poeciliidae), in rice fields of the lower Mondego River valley, western Portugal. Acta Oecol. **20:** 607-620.

Cabral JA, C Mieiro, JC Marques. 2002. Role assessment of an exotic fish in rice fields of the lower Mondego River valley: life history, population dynamics, production and diet of eastern mosquitofish, *Gambusia holbrooki* (Pisces, poeciliidae). *In* MA Pardal, JC Marques, MAS Graça, eds. Aquatic ecology of the Mondego River basin. Global importance of local experience. Chapter 4.3, Coimbra: Imprensa da Univ. de Coimbra, pp. 363-380.

Chick JH, CC McIvor. 1997. Habitat selection by three littoral zone fishes: effects of predation pressure, plant density and macrophyte type. Ecol. Freshw. Fish **6:** 27-35.

Correia AM. 1995. Population dynamics of *Procambarus clarkii* (Crustacea: Decapoda) in Portugal. Freshw. Crayfish **8:** 276-290.

Correia AM. 2002. Niche breadth and trophic diversity: feeding behaviour of the red swamp crayfish (*Procambarus clarkii*) towards environmental availability of aquatic macroinvertebrates in a rice field (Portugal). Acta Oecol. **23:** 421-429.

Correia AM. 2003. Food choice by the introduced crayfish *Procambarus clarkii*. Ann. Zool. Fenn. **40:** 517-528.

Heger T, L Trepl. 2003. Predicting biological invasions. Biol. Invasions **5:** 313-321.

Huner JV. 1990. Biology, fisheries, and cultivation of freshwater crayfishes in the U.S. Rev. Aquat. Sci. **2:** 229-254.

Kolar C, DM Lodge. 2001. Progress in invasion biology: predicting invaders. Trends Ecol. Evol. **16:** 199-204.

Lima SL. 1998a. Nonlethal effects in the ecology of predator-prey interactions. What are the ecological effects of anti-predator decision-making? BioScience **48:** 25-33.

Lima SL. 1998b. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. Adv. Stud. Behav. **27:** 215-290.

Lima S, LM Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. **68:** 619-640.

McCarthy TM, BF Dickey. 2002. Chemically mediated effects of injured prey on behavior of both prey and predators. Behaviour **139:** 585-602.

Moyle PB. 1999. Effects of invading species on freshwater and estuarine ecosystems. *In* OT Sandlund, J Schei, Å Viken, eds. Invasive species and biodiversity management. Chapter 12. Dordrecht, Netherlands: Kluwer Academic Publishers, pp. 177-191.

Nyström P, AR McIntosh. 2003. Are impacts of an exotic predator on a stream food influenced by disturbance history? Oecologia **136:** 279-288.

Nyström P, O Svensson, B Lardner, C Brönmark, W Granéli. 2001. The influence of multiple introduced predators on a littoral pond community. Ecology **82:** 1023-1039.

Parker IM, JN Thompson, SG Weller. 2001. The population biology of invasive species. Annu. Rev. Ecol. Syst. **32:** 305-332.

Parker IM, D Simberlof, WM Lonsdale, K Goodell, M Wonham, PM Kareiva, MH Williamson, B Von Holle, PB Moyle, JE Byers, L Goldwasser. 1999. Impact: toward a framework for understanding ecological effects of invaders. Biol. Invasions **1:** 3-19.

Rahel FJ, RA Stein. 1988. Complex predator-prey interactions and predator intimidation among crayfish, piscivorous fish, and small benthic fish. Oecologia **75:** 94-98.

Ramos MA, TM Pereira. 1981. Um novo astacidae para a fauna portuguesa: *Procambarus clarkii* (Girard, 1952). Bol. Inst. Nac. Invest. Pescas **6:** 37-42.

Sakai AK, FW Allendorf, JS Holt, DM Lodge, J Molofsky, KA With, S Baughman, R J Cabin, JE Cohen, NC Ellstrand, DE McCauley, P O'Neil, IP Parker, JN Thompson, SG Weller. 2001. The population biology of invasive species. Annu. Rev. Ecol. Evol. Syst. **32:** 305-332.

Santi A, VA Sovrano, A Bisazza, G Valortigara. 2001. Mosquitofish display differential left-eye use during mirror image scrutiny and predator inspection responses. Anim. Behav. **61:** 305-310.

Simon KS, CR Townsend. 2003. Impacts of freshwater invaders at different levels of ecological organisation with emphasis on salmonids and ecosystem consequences. Freshwater Biol. **48:** 982-994.

Smith ME, MC Belk. 2001. Risk assessment in western mosquitofish (*Gambusia affinis*): do multiple cues have additive effects. Behav. Ecol. Sociobiol. **51:** 1021-107.

Sokal RR, FJ Rohlf. 1981. Biometry. 2nd ed. New York: WH Freeman, pp. 859.

Stelzer RB, GA Lamberti. 1999. Independent and interactive effects of crayfish and darters on a stream benthic community. J. North Am. Benthol. Soc. **18:** 524-532.

Turner AM, RJ Bernot, CM Boes. 2000. Chemical cues modify species interactions: the ecological consequences of predator avoidance by freshwater snail. Oikos **88:** 145-258.

Wilkelman DL, JM Aho. 1993. Direct and indirect effects of predation on mosquitofish behavior and survival. Oecologia **96:** 300-303.

Wooten MC, C Lydeard. 1990. Allozyme variation in a natural contact zone between *Gambusia affinis* and *Gambusia holbrooki*. Biochem. Syst. Ecol. **18:** 169-173.