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CROSS-REACTION TO SKIN EXTRACT BETWEEN TWO GOBIES, *Asterropteryx semipunctatus* AND *Brachygobius sabanus*

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Abstract—Two gobies that possess alarm pheromones were tested for cross-reactions to water extracts of injured members of the other species in the first such cross-reaction test conducted in the Family Gobiidae. *A. semipunctatus* reacted to extract from injured *Brachygobius sabanus* with the same bobbing and reduction in activity as it shows in response to conspecific extract. However, *B. sabanus* showed a feeding response, including increased activity, to extract from injured *A. semipunctatus*. Even a one-way cross-reaction suggests some degree of homology between the alarm pheromone systems of the two species.

Key Words—Alarm pheromone, Schreckstoff, alarm substance, goby, *Asterropteryx semipunctatus*, *Brachygobius sabanus*, Gobiidae, fish behavior.

INTRODUCTION

In the alarm pheromone systems of ostariophysan fishes (Schutz, 1956; Pfeiffer, 1977; Smith, 1986) and percid darters (Smith, 1979, 1982), the members of the taxonomic group that share the chemical alarm system show some degree of cross-reaction between species or even between genera or families. Although there is often some reduction in response with increasing taxonomic distance, the presence of any cross-reaction probably indicates homology between the alarm systems of the members of a taxonomic group. Such homology of response to a common active compound occurs in the ostariophysan fishes (Schutz, 1956; Pfeiffer, 1982).

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Two species of gobiid fishes, *Asterropteryx semipunctatus* (Smith, 1989) and *Brachygobius sabanus* (Smith and Lawrence, 1992) have now been reported to show a defensive response (fright reaction) to chemicals from injured conspecifics. Six other gobiid species, *Gobius niger*, *G. paganellus*, *Pomatoschistus microps*, *P. minutus* (Pfeiffer, 1977), *Gnatholepis anjerensis* (Smith, 1989), and *Coryphopterus nicholsii* (Hugie, personal communication) have been tested and apparently do not show a fright reaction to chemicals from injured conspecifics. The question thus arises, do *A. semipunctatus* and *B. sabanus* react to each other's skin extract? If they do cross-react, it may indicate that there is a homologous component to goby alarm pheromones. If they do not cross react, it would support, but not confirm, the hypothesis that chemical alarm signaling has arisen independently in two gobiid species. We compared cross-reactions between the two species using the same observers and criteria as had been used in the original studies of each species.

METHODS AND MATERIALS

Asterropteryx semipunctatus were collected from patch reefs in Kaneohe Bay, Oahu, Hawaii, by scuba divers and snorkelers using plastic bags or hand nets. Those *A. semipunctatus* that were used as receivers for skin extract from *Brachygobius sabanus* were kept in running seawater in holding tanks at the Hawaii Institute of Marine Biology, then transferred to 38-liter observation aquaria in groups of three. They were allowed at least seven days of acclimation before observation. The holding and observation aquaria were maintained between 24° and 27°C under a 12:12 hr light–dark photoperiod. During this time, the fish were fed with live plankton collected in Kaneohe Bay. *A. semipunctatus* that were used as extract donors were transported by air to the Department of Biology, University of Saskatchewan, where they were maintained in artificial seawater at 24°C under a 12:12 hr light–dark photoperiod and fed live and frozen brine shrimp and commercial flake food. Extract receivers included 12 males (mean total length 34 mm), 13 females (mean total length 37 mm), and two small fish that could not be sexed (mean 22 mm). Extract donors were four males (mean 28 mm) and six females (mean 25 mm).

Brachygobius were purchased from commercial pet suppliers in Calgary, Alberta, and Kaneohe and Aiea, Hawaii. Our specimens (ROM 56628) were tentatively identified by R. Winterbottom of the Royal Ontario Museum as *Brachygobius sabanus* Inger, 1958. There are some inconsistencies involving the degree of scalation of the operculum and pelvic-fin length of our specimens and the description of the type specimens (collected in North Borneo). The original source of the fish is unknown. A sample of the receivers, eight males (mean total length 25 mm) and two females (mean 27 mm), was measured. The nine donors included four females (mean 27 mm) and five males (mean 24 mm).

B. sabanus were kept in brackish water prepared by mixing 37 liters of freshwater with 1 liter of seawater. At the Hawaii Institute of Marine Biology they were fed live brine shrimp and marine plankton and kept under a 12:12 hr light–dark photoperiod between 24° and 27°C. At the University of Saskatchewan they were kept at 27–29°C under a 12:12 hr light–dark photoperiod and fed live whiteworms and live brine shrimp. They were observed in groups of four and were allowed at least seven days of acclimation before being observed.

All observations were conducted using 38-liter tanks. The observer sat quietly in full view of the fish and recorded their behavior using a mechanical counter. The behavior of *B. sabanus* receivers was also recorded on videotape for later analysis of swimming time. The observation tanks in Hawaii, housing *A. semipunctatus* receivers, were equipped with seawater inlet tubes and the chemical stimuli were injected into the inlet. The observation tanks in Saskatoon, housing *B. sabanus* receivers, were equipped with power filters and the chemical stimuli were injected into the inlet tube running from the filter to the tank. The arrangement of sand and shelters was the same as in the original studies of alarm pheromone responses in the two species (Smith, 1989; Smith and Lawrence, 1992).

In the standard test procedure, the fish were observed for 15 min; then the chemical stimuli were injected into the inlet tube, and the fish were observed for an additional 15 min. The totals for the two 15-min observation periods were compared for each tank of fish. This procedure was repeated nine times for *A. semipunctatus* receivers and 10 times for *B. sabanus* receivers, each time with a different group of fish. Each group was used only once. Since the fish in a tank cannot be considered independent, comparisons were made on the basis of group totals. Following each test, food dye was injected into the system and the time from injection to dispersal through the tanks was recorded as an estimate of the time from stimulus injection to the reception of the stimulus by all fish in the tank. The mean time for the dye to enter the tank was 22 sec, with 181 sec for full dispersal in the *A. semipunctatus* tanks and 10 sec for entry and 32 sec for dispersal in the *B. sabanus* tanks.

A. semipunctatus are negatively buoyant, benthic fish. They move episodically rather than continuously, with movements separated by intervals when they remain stationary. Each time a fish changed position, it was counted as making one move. Bobbing behavior was also counted, in which the anterior part of the goby rose slowly off the bottom, apparently by extension of the pelvic fins, then settled to the bottom again. A fish could move without bobbing and bob without moving. In *A. semipunctatus*, bobbing occurs in response to visual and olfactory stimuli from predators (Smith, 1989; Smith and Smith, 1989). *B. sabanus* are often negatively buoyant and can move about episodically in much the same manner as described above, but they can also swim in midwater, maintaining neutral buoyancy with their swim bladder. As well as counting moves, we recorded the total swimming time, the time in a 15-min observation period when one or more fish was swimming, for *B. sabanus*. Agonistic encounters were also counted, as in Smith and Lawrence (1992). *B. sabanus* was never observed to bob.

Chemical extracts of injured fish were prepared by killing the donor with a blow to the head, placing it in a clean disposable Petri dish, cutting it several times on each side with a scalpel, rinsing the fish in tank water and drawing up the extract into a clean syringe. *A. semipunctatus* donors were cut 25 times and rinsed with 7 ml of water, and the extract was drawn into a 5-ml syringe. *B. sabanus* donors were cut 50 times, rinsed in 10 ml, and the extract was drawn into a 10-ml syringe. These procedures mimic the original studies for each type of receiver. Extracts were used within 20 min of preparation. Control stimuli, water, and extracts of nongobiid fishes were tested in the original studies of each species and were not repeated here.

The results of each test were analyzed with a Wilcoxon matched-pairs, signed-rank test (Siegel, 1956) comparing the total number of acts, or minutes of swimming, before stimulus introduction with the total after introduction, for each group of fishes.

RESULTS

A. semipunctatus responded to skin extract from *B. sabanus* with decreased activity in eight of the nine trials; the mean change was −15% (Figure 1). There were no bobs during the prestimulus interval but one group performed 63 bobs and another 20 bobs in the poststimulus interval. These two groups showed substantially greater activity reduction than the other groups, −40% and −42%, respectively. Qualitatively, the response of the *A. semipunctatus* resembled the response shown to conspecific extract (Smith, 1989).

B. sabanus responded to skin extract from *A. semipunctatus* with increased moves in all 10 trials; the mean change was +86% (Figure 1). Swimming time did not change significantly, but the number of agonistic encounters increased in eight of 10 groups, by an average of 123%. This response was opposite to the response of *B. sabanus* to conspecific extract and more closely resembled a feeding response (Smith and Lawrence, 1992). Other, qualitative resemblances to a feeding response included increased biting at the substrate, moving out of shelters, and attacking snail shells.

DISCUSSION

A. semipunctatus responds to extract from injured *B. sabanus* with the same sort of antipredator response that it shows in response to extract from injured conspecifics or stimuli from predators (Smith, 1989). The response

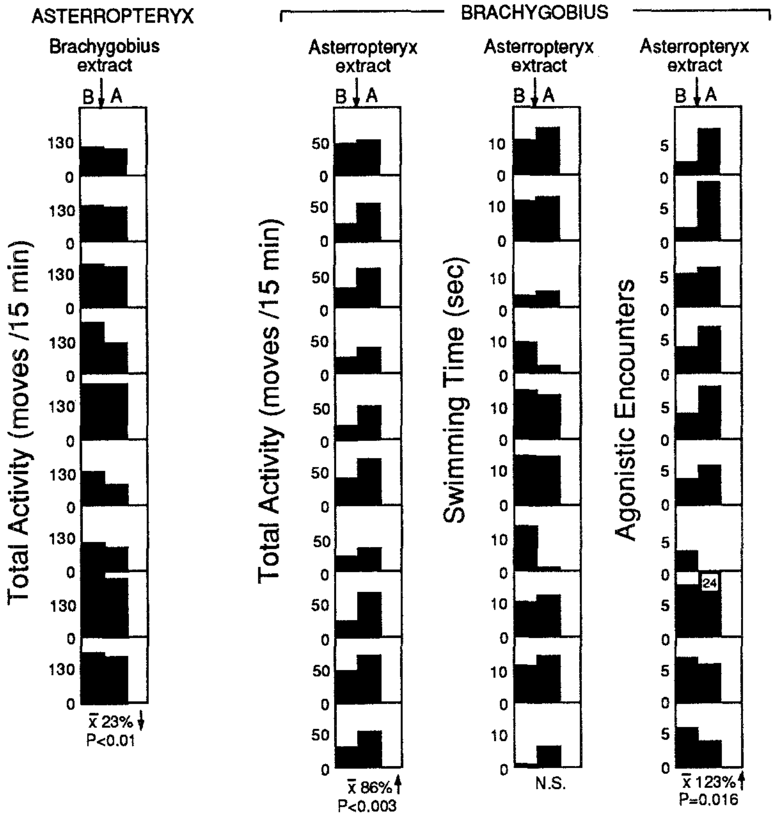

FIG. 1. Behavioral responses of gobies to skin extracts from heterospecific fishes. Values plotted are the number of moves by *Asterropteryx semipunctatus* in 15-min intervals before (B) and after (A) exposure to extract from injured *Brachygobius sabanus* and the number of moves, total swimming time, and number of agonistic encounters by *B. sabanus* in 15-min intervals before and after exposure to extract from injured *A. semipunctatus*. P = probability based on Wilcoxon matched-pairs, signed-rank test, N.S. = not significant.

seems slightly weaker than the response to conspecific extract—a mean decrease in moves of 23% with bobbing in six tanks of 10 (Smith, 1989)—but this is reasonable considering that the two species are in different genera. Schutz (1956) catalogued several examples of declining cross-reaction with phylogenetic separation in the alarm system of ostariophysan fishes. The presence of two very strong reactions to *B. sabanus* extract with approximately 40% activity reduction and frequent bobbing reinforces the conclusion that *A. semipunctatus* responds to *B. sabanus* extract with an antipredator response. In contrast to these results, control tests by Smith (1989) had demonstrated that *A. semipunc-*

tatus showed small, about 5%, nonsignificant increases in activity and no increase in bobbing when given control water or water that had contained uninjured conspecifics as a stimulus. Extract from a nongobiid fish, the Mexican molly *Poecilia mexicana*, induced an 18% increase in activity but no bobbing, and extract from *Gnatholepis anjerensis*, a goby that lacks alarm pheromone, led to a similar 18% increase in activity (Smith, 1989).

B. sabanus responds to extract from injured *A. semipunctatus* as if it were a feeding stimulus. In control tests conducted by Smith and Lawrence (1992), *B. sabanus* responded to extract of an injured nongobiid, the guppy *Poecilia reticulata*, with significant increases in moves, swimming, and agonistic encounters. This response was characterized as a feeding response because it resembled the response to brine shrimp extract. The response to conspecific extract was a reduction in moves, swimming, and agonistic activity, associated with moving to shelters. The response of *B. sabanus* to *A. semipunctatus* extract differed from the feeding responses described by Smith and Lawrence (1992); responses were directed more to the bottom of the tank, whereas the response to guppy extract and brine shrimp extract was directed more to the surface or midwater. The significance of this observation in not clear, although cyprinodonts are usually surface fish while gobies are often benthic.

A possible explanation for this one-way cross-reaction between two gobiid species that have been shown to possess alarm pheromones is that the two species share active components in their chemical alarm signal but that *B. sabanus* is more selective in avoiding "false alarms" from injured heterospecific fishes. This selectivity might be favored if *B. sabanus* shared its environment with other pheromone-bearing gobies that were subject to attack by predators that do not prey on *B. sabanus* or if *B. sabanus* preyed on other gobies, perhaps by fin nipping. *A. semipunctatus* does not respond indiscriminately to all goby skin extracts since it responds with feeding behavior to skin extract from injured *Gnatholepis anjerensis*, a sympatric goby (Smith, 1989). Our results suggest that there are homologies between the two gobiid alarm pheromone systems. The phylogeny of gobies is not clear, and it is therefore difficult to fit our results into any phylogenetic pattern.

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