ANTIPREDATOR RESPONSE TO INJURY-RELEASED CHEMICAL ALARM CUES BY CONVICT CICHLID YOUNG BEFORE AND AFTER INDEPENDENCE FROM PARENTAL PROTECTION

by

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Summary

Injury-released chemical alarm cues are released when predators attack aquatic prey. These cues are generally released only in this context and as such, conspecific alarm cues form an important component of risk assessment. Minnows (Ostariophysi, Cyprinidae) possess a well-developed chemical alarm system. However, minnows do not respond to conspecific injury-released alarm cues until 30 to 50 d post-hatch. Non-ostariophysan fishes respond to chemical alarm cues with antipredator behavior but the ontogeny of this behavior is not known for any species. Here, we test convict cichlids (Acanthopterygii: Cichlidae), a species known to respond to alarm cues as adults. Convict cichlid parents care for their eggs and defend their developing young from predators for 4 to 6 weeks. In our experiment, we tested the ontogeny of antipredator response to chemical alarm cues in young convict cichlids well within and just beyond the size range typically defended by parents. We found that small convict cichlid young of a size typically defended by parents engaged in area avoidance and grouping behaviors in response to alarm cues and did so as effectively as young that would typically be independent of parental care.

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Introduction

For antipredator behavior to be effective, the context and timing of the behavior must be triggered by a relevant cue from the external environment (Wisenden, 2000). External cues are perceived by any of several sensory modalities. In aquatic environments, chemical cues serve as important indicators of predation risk (Smith, 1992; Dodson *et al.*, 1994; Chivers & Smith, 1998; Kats & Dill, 1998; Wisenden, 2001a). Injury-released chemical alarm cues reliably inform prey species of appropriate context and timing for antipredator behavior because they indicate the presence of a predator of conspecifics (Smith, 1992; Chivers & Smith, 1998; Wisenden, 2001a).

Fishes in the superorder Ostariophysi (*i.e.* minnows, characins, catfishes, suckers) possess specialized epidermal cells that contain an alarm 'substance', known also as *Schreckstoff* (von Frisch, 1938, 1941; Pfeifer, 1977). The ontogeny of the proliferation of alarm substance cells (ASCs) and responses to this cue have received little attention. In one study, European minnows *Phoxinus phoxinus* develop ASCs at about 51 d post hatch (Pfeiffer, 1974). In another study, ASCs developed at 20 d post-hatch in zebrafish (Cyprinidae: *Danio rerio*) but an alarm response to the cue did not occur until 32 d post hatch (Pfeiffer, 1963). In a third study, also on zebrafish, ASCs appeared at about 31 d of age but zebrafish did not respond to alarm cues until about day 52 (Waldman, 1982). One possible reason for the delay in cell formation could be that very young cyprinids may be better served (evolutionarily) to instead allocate resources to growth to outgrow predators or to increase the breadth of diet items available to them. Delay in the behavioral response could be because very young minnows lack receptors to detect the cue, or lack the muscular and skeletal development required to produce effective antipredator behavior.

Cichlid fishes are in the superorder Acanthopterygii and do not possess specialized epidermal alarm substance cells (Pfeiffer, 1977), however, like many non-Ostariophysan species (Chivers & Smith, 1998) adult cichlids respond to injury-released chemical cues of conspecifics with antipredator behavior (Jaiswal & Waghray, 1990; Wisenden & Sargent, 1997). The ontongeny of the behavioral response to alarm cues has not been investigated in any non-Ostariophysan fish species.

Cichlids are unusual among fishes in that they exhibit prolonged care of their eggs and free-swimming young (Keenleyside, 1991). Cichlid eggs are relatively well endowed with yolk and undergo much larval development before exogenous feeding begins. Convict cichlids, a Central American cichlid species with biparental care of its offspring, form monogamous pair bonds, and jointly prepare a spawning site in an excavated cavity (Wisenden, 1995). Eggs hatch in about 3 d with large yolk sacs and poorly differentiated fins. Young at this stage cannot swim. They remain heaped in little pits prepared by the parents. Continuous movements of the caudal fin produce water currents to ventilate the pit but do not result in locomotion. Young at this stage form a trembling mass and are referred to as 'wrigglers'. After about 3 d at the wriggler stage, young have sufficiently absorbed yolk and developed their fins to be capable of swimming. The brood of about 80 young (Wisenden, 1994) then forms a dense shoal hovering above the substrate and traveling along the bottom in search of food. Both parents station themselves over the mobile brood and fiercely defend the brood from predators. The young are about 5 mm in standard length (SL) when they first become free swimming, and they reach about 10 mm SL before becoming independent from parental care (Wisenden & Keenleyside, 1992). Defense of free-swimming young continues for 4 weeks or more in this species (Wisenden, 1995). The young, especially at early stages of development, are completely dependent upon their parents for protection against predators. Although adult convict cichlids respond to conspecific chemical alarm cues (Wisenden & Sargent, 1997), it is not known if the young recognize and respond to these cues. If they rely entirely on their parents for risk assessment and protection against predators, then one might predict that young smaller than 10 mm SL (pre-independence) should show no response to chemical alarm cues.

Materials and methods

A breeding colony of convict cichlids, *Archocentrus 'Cichlasoma' nigrofasciatus*, is maintained in our laboratory, originating from fish obtained from a commercial supplier. All fish were maintained at 27°C on a diet of commercial flake food. Young convict cichlids were obtained from breeding pairs set-up in 74-L and 190-L aquaria. Offspring from 6 broods, from 6 different pairs of adults, contributed to the data in this study. Individual young were tested only once.

Groups of young convict cichlids were placed into a square test arena 45.5 cm square (Fig. 1). A 190-L reservoir tank was elevated about 30 cm adjacent to the test arena. Two plastic airline hoses (internal diameter = 5 mm) siphoned 27°C dechlorinated tap water from the reservoir tank at a rate of approximately 600 $\mathrm{ml \cdot min^{-1}}$ each. Siphons from the reservoir

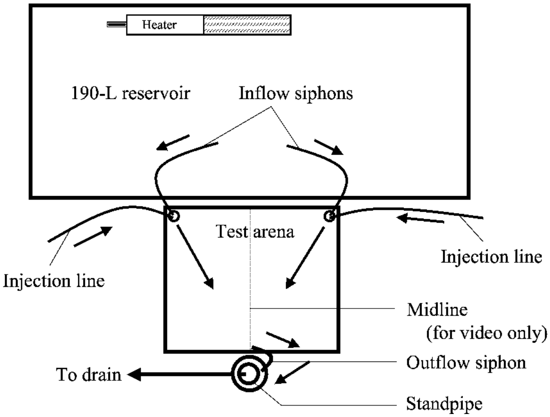

Fig. 1. Top view of test apparatus (not including video camera and tripod). An elevated reservoir containing 190 L of heated dechlorinated tap water contained two airline tubes that siphoned water to two adjacent corners of the test arena. Test stimuli were added to one side or the other. Water entering the test arena exited the opposite side of the test arena via a siphon to drain. The test arena water divided on video playback of trials along the midline shown.

were started a few minutes before test fish were added to the test arena. Water entered the test arena in two adjacent corners via a vertical rigid plastic tube (internal diameter = 11 mm) that directed water flow into each inflow corner of the test arena. Separate airline tubes used for injecting test stimuli were wedged into each of the rigid plastic tubes receiving one of the inflow siphons. Water exited the test arena via a single siphon hose (internal diameter = 8 mm) mid way along the opposite side into a second vessel. A standpipe in the second vessel maintained water depth in the test arena at 5 cm. Thus, volume of the test arena was approximately 10.35 L. Water overflowing the standpipe went to the drain (Fig. 1).

Five same-sized sibling offspring were placed in the test arena and allowed 15 min to acclimate. Several drops containing freshly hatched brine shrimp *Artemia* were added to the test arena to accelerate the acclimation process. We recorded fish behavior for one min of pre-stimulus behavior before the addition of test cue. Then for one min, we carefully injected 10-ml of test cue. The injection line was flushed with 60 ml of water obtained from the reservoir before the trial began. This was followed by one min of post-stimulus behavioral recording.

We used two test cues. One test cue was injury-released chemical alarm cues from young convict cichlids. A stock solution of alarm cue was prepared by placing individual convict cichlids (mean ± SE = 10.08 ± 0.26 mm SL, $N = 20$) in a mortar and quickly crushing it to a fine pulp with a pestle. We added 5 ml of dechlorinated tap water and poured the suspension through a wad of polyester fiber to remove suspended tissue fragments. We repeated this process 20 times and rinsed the polyester wad with an additional 100 ml of dechlorinated water to bring the final volume of the stock solution to 200 ml. Alarm cue was aliquoted into 10-ml doses and frozen at −20°C until needed. Stimulus dose per trial was 10 ml therefore stimulus strength represented the approximate amount of alarm cue released by a single donor fish. The cue simulated the type and strength of cue that would result in a typical predation event. The flow-through design of the experiment allowed alarm cue to enter and leave the test arena in a way similar to natural situations. The second test cue was 10 ml of dechlorinated tap water to control for the effect, if any, of cue introduction. Water was passed through a fresh wad of polyester fiber and frozen in 10-ml aliquots at −20°C until needed. We used dechlorinated tap water as a control because convict cichlids exhibit antipredator behavior in response to cues from injured conspecifics but not to those of injured *Gambusia* (Wisenden & Sargent, 1997), indicating that convicts react to species specific cues and not simply to injured cues from any fish species. During trials, the side of the test arena that received the test cue was always the side that contained the fewest test animals. Overall use of left and right side inflow tubes was balanced over the experiment and among treatments (overall: 18L/13R; small size + cichlid cue: 5L/4R; small size + water cue: 7L/5R; large size + cichlid cue: 2L/2R [side not recorded for one trial]; large size + water cue: 4L/2R).

All trials were recorded on Hi8 analog videotape by a camcorder positioned directly above the test arena. Area use was calculated by counting the number of young at 10-s intervals present in the half of the test arena that received the test cue (Fig. 1). We recorded shoal cohesion at 10-s intervals by recording the number of groups among the 5 test animals separated by more than one body length. Area avoidance and increase in group cohesion are common antipredator behaviors (Kats & Dill, 1998). We predicted that if young convict cichlids recognize conspecific alarm cues as dangerous then (1) the cue-side area would be avoided in response to alarm cues but not in response to water, and (2) the number of groups would decrease if alarm cues cause an increase in shoaling behavior.

Young used in each test were either small (6.15 ± 0.122 mm SL, $N = 21$ trials) and well within the size range guarded by parents (5-10 mm SL, Wisenden & Keenleyide, 1992) or large (12.20 ± 0.371 mm SL, $N = 11$ trials) and beyond the typical size at independence from parental care. Among trials on small young, 9 trials used cichlid cue and 12 trials used water. Among trials on large young, 5 trials used cichlid cue and 6 trials used water. Young convict cichlids depend on their parents for protection from predators and therefore may not be attentive to chemical indicators of risk. If this is the case then we predicted that small young would not avoid or engage in other antipredator behavior in response to conspecific alarm cues.

Change in behavior was scored for each trial by subtracting the average of the pre-stimulus observations from the average of the post-stimulus observations. Thus, each trial produced a single datum for change in cue-side area use and for change in grouping behavior in response to the test cue. We used a 2 × 2 ANOVA with cue type and fish size as category predictor variables to test their independent and interacting effects on area use and grouping behavior.

Results

Young of both size groups avoided the cue-side area when injury-released chemical alarm cues were added to the test arena (ANOVA: Cue type: $F_{1,28} = 10.10$, $p < 0.01$; Fish size: $F_{1,28} = 1.79$, $p = 0.19$; Cue type × Fish size: $F_{1,28} < 0.01$, $p =$ NS; Fig. 2). Fish appear to be attracted to the cue-side area when water was the test cue (Fig. 2) but this is an artifact of the non-random test procedure. The test cue was always added to the side of the test arena with the fewest animals present. Evidence of this can be seen

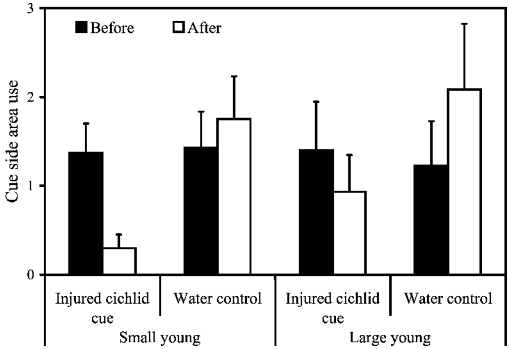

Fig. 2. Mean (± SE) change in cue-side area use before and after the addition of water or cichlid alarm cues.

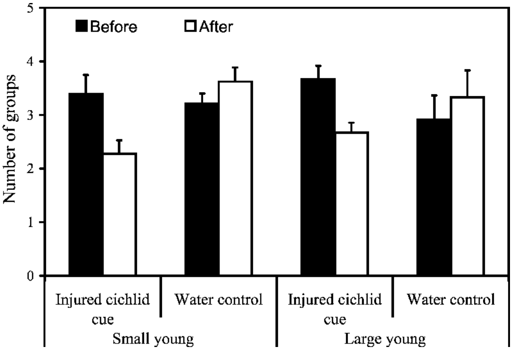

Fig. 3. Mean (± SE) change in the number of groups in response to test stimuli. A reduction in the number of groups indicates an increase in shoaling behavior.

by the mean pre-stimulus cue-side area use of only 1.37 ± 0.21 (mean ± SE, $N = 32$) instead of the expected 2.5 ($t = 5.41$, df = 31, $p < 0.01$). An increase in cue-side area use in water trials indicates random movement and an absence of an aversive effect of the stimulus introduction procedure. A further decrease in cue-side area use in alarm cue trials is a conservative test indicating a strong avoidance of the cue-side area.

Young convict cichlids of both size groups significantly increased shoaling behavior in response to conspecific alarm cues as evidenced by a decrease in the number of groups (ANOVA: Cue type: $F_{1,28} = 29.14$, $p < 0.01$; Fish size: $F_{1,28} = 0.04$, $p = 0.84$; Cue type × Fish size: $F_{1,28} = 0.04$, $p =$ NS; Fig. 3).

Discussion

Young convict cichlids exhibit antipredator behavior in response to injury-released chemical alarm cues from conspecifics. The capacity to detect and respond to these cues is present soon after the young become free-swimming and well before the size at which the young become independent from parental care. The behavioral response was manifest as avoidance of areas associated with alarm cues and by an increase in shoal cohesion. Shoaling is an adaptive antipredator response generally, but especially for young under the care of parents. Shoaling behavior concentrates the young into a small area that can be more effectively defended by the parents (McKaye *et al.*, 1992; Wisenden & Keenleyside, 1995; Wisenden, 2001b). Area avoidance by itself might increase the distance between the young and their parents and thus, increase the young's vulnerability to predation. The behavioral response of young with attendant parents was not recorded in our experiment. The attraction of young to parental fish (de Gannes & Keenleyside, 1992), especially in times of danger, would almost certainly take precedence over an area avoidance response. Parents flick their pelvic fins in response to perceived danger as a visual alarm signal to their young (Cole & Ward, 1969; Shennan *et al.*, 1994). The interaction between the parent's assessment of predation risk and the independent risk assessment by young is an area for future research.

Skin extract does not elicit antipredator behavior from zebrafish less than 30 days old (Pfeiffer, 1963; Waldman, 1982). Small convict cichlid young used in this study were less than 7 d free-swimming, or about 10 d post-hatch. Although adult convict cichlids recognize conspecific skin extract as an indicator of predation risk (Wisenden & Sargent, 1997), they are not known to possess analogous epidermal cells (Pfeiffer, 1977). The chemical bouquet that alerts adults to danger appears to already present in the young used to make our test stimuli (about 10 mm SL). Convict cichlids are able to use olfactory cues to assess predation risk at a very early stage of development. Thus, development of olfactory assessment occurs before their physical ability to flee (Wisenden & Keenleyside, 1992). Fleeing as an antipredator strategy is constrained by dependence on parental care (can not flee area) but also by motor development. A similar developmental sequence could be the case for minnows. Minnows younger than 30 d may detect conspecific alarm cue but lack the physical ability to do much about it. It may be best for young minnows to maximize food intake instead of minimizing predation risk.

The rate of the onset of full antipredator competence in convict cichlids plays an important role in their reproductive behavior. About 30 percent of convict cichlid broods in Costa Rican streams adopt young from neighboring broods and parents show a strong preference for adopting young that are similar in size or smaller than their own young (Wisenden & Keenleyside, 1992; see Wisenden, 1999 for review). Small young are less able to flee predators (Wisenden & Keenleyside, 1992). When a predator attacks a brood of mixed-sized young, the smallest, weakest young experience differential mortality (Wisenden & Keenleyside, 1992, 1994).

Co-evolution of egg number and egg size is intertwined with parental care (Wisenden, 2001b). The primary function of parental care in fishes is brood defense. Ontogeny of antipredator competence therefore determines duration of brood care and inter-spawning interval. Ontogeny of antipredator competence is influenced by how females allocate reproductive resources. When resources are allocated to few, relatively large eggs, there should be an increase in antipredator competence of the young upon independence but a concomitant increase in the duration of care and a reduction in fecundity. Understanding the ontogeny of antipredator competence in species with parental care should shed light on the co-evolutionary trade-offs between egg size, egg number and care.

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