Proceedings of the Indiana Academy of Science
(1991) Volume 100 p. 155-160

STARTLE RESPONSE AND CAUSES OF BURYING BEHAVIOR IN CAPTIVE EASTERN SAND DARTERS, *AMMOCRYPTA PELLUCIDA* (PUTNAM)

Thomas P. Simon
Indiana University-Northwest
Department of Biology
Gary, Indiana 46204

ABSTRACT: Three hypotheses have been proposed to explain the burying response of darters in the genus *Ammocrypta*: 1) elusive escape response to predators, 2) ambush of prey, and 3) conservation of energy. These three hypotheses were tested using *Ammocrypta pellucida* by varying current velocity, food resources, and the actions of a model predator in an aquarium. Darters, when buried, remained so for an average of 8.6 hours and did not emerge either to feed or when partially uncovered by increasing current velocities. Rapid current velocities caused fewer darters to bury. The startle response of sand darters was characterized by a rapid ascent to the water surface, by fluttering across the atmosphere-water interface, and by using the pectoral fins to wedge themselves partially out of the water. This form of startle behavior would be advantageous for confusing predators and for breaking visual contact between the predator and prey. A response in the energy conservation experiments represented resting behavior. The number of darters resting while buried decreased with increased habitat complexity.

INTRODUCTION

The burying behavior of the sand darters (genus *Ammocrypta*) has been speculated to be a direct result of elicited stimuli. Jordan and Copeland (1877) thought burying in *Ammocrypta* was an escape response to avoid predators. Trautman (1981) argued that *Ammocrypta* buried themselves to ambush potential prey. Williams (1975) proposed that burying was to conserve energy.

Daniels (1989) studied the mechanics behind burying behavior in a series of experiments run to test these three hypotheses. He concluded that burying behavior is used to maintain the darters' position on the relatively homogeneous sand beds on which it lives. Daniels also found that burying behavior could be exhibited at any time and during any season. He was unable to validate any of the three hypotheses during any of his trials, and he suggested that these factors might not be the only ones operating.

The current study was conducted to check the observations of Daniels (1989), to determine what stimuli elicit the burying response, and to propose a hypothesis that explains the relationship between these stimuli and the burying response in *A. pellucida*.

METHODS AND MATERIALS

This study grew out of a larger study on the reproduction and early life history of *A. pellucida* in the Tippecanoe River near Winamec in Pulaski County, Indiana. Specimens were collected using a 4.5 m seine and transferred to a 44 L aquarium for observation. Only ten fish were utilized for these experiments. Temperature was not controlled in the aquarium and varied between 19° C and 22° C during the summer trials and between 8° C and 10° C during the winter trials. Photoperiod was regulated to simulate natural conditions: 16 hours of light/ 8 hours of darkness in the summer trials and 10 hours of light/ 14 hours of darkness in the winter trials.

Circular water velocity was varied through the use of different aquarium pumps. Slow flows were attained using a Whisper power filter C (125 gallon/hour), moderate flows were attained using a Supreme Superking power filter (400 gallon/ hour), and rapid flows were attained by using a small Tecumseh Products Little Giant submersible pump Model 2MDSC (510 gallons/hour). During the trials, the fish were allowed to acclimate for 4 hours, at which time the fish were observed during dawn, day, dusk, and night trial periods. The duration of observation depended on the trial. Fish were maintained in captivity from June 1986 to April 1987.

Two experiments were conducted to test whether *A. pellucida* utilized burying behavior to conserve energy in flowing water. Darters were exposed to three different current velocities using power filters. If the relative number of exposed darters did not change significantly with different current velocities, current velocity could be rejected as a stimulus for burying behavior. Current velocity and the number of exposed darters were noted at the beginning of each trial. Visible darters were counted after ten minutes of exposure.

To test whether burying is a form of resting behavior, the relative position of darters under the lowest current velocity was recorded under dawn, day, dusk, and night conditions. The position of buried fish was recorded at 0.25, 0.5, 1, 2, 4, 6, 12, and 24 hours after burial. Burial for longer periods of time would indicate that a darter was resting. The exact position of each buried fish was marked with a grease pencil on top of the glass hood. The darter's position was verified by viewing the fish through the glass bottom of the aquarium.

Another test was run to determine if a sudden movement or switching a light source on and off could elicit burying behavior. If the exposed darters suddenly buried themselves due either to my presence or to a sudden change from dark to light, burial behavior could be attributed to a startle response. At night, a flashlight was used to ascertain the position and number of exposed fish before the lights were turned on. Under daylight conditions, the fish were observed from behind a screen. Then, either the vibration of the screen or my sudden appearance were used to determine if exposed fish buried. A daylight test involved three quick movements from behind the screen, followed immediately by recording the position of each fish.

In order to test the hypothesis that darters buried to avoid predators, a 200 mm long, wooden model of a largemouth bass (*Micropterus salmoides*) was constructed and attached to a wooden dowel rod. The model was manipulated to test the darters' response to a predatory form and aggressive approach. If the darters did not bury, when confronted by a predatory form, the hypothesis that burial behavior lessened predation could be rejected. The test was conducted from behind a screen pierced by a small hole through which the fish were observed. The model was placed in the aquarium near the water surface and moved around the tank. Then, a single darter was selected, and the model was moved rapidly towards it. The test was repeated until the selected fish either buried or swam away twice.

The hypothesis that *A. pellucida* is an ambush predator and buries to await its prey was also tested. Two types of prey were introduced into the power filter jet to disperse them into the aquarium. The prey items were a tubificid black worm and thawed adult brine shrimp. To accept this hypothesis, exposed fish would have to be observed burying themselves before attacking the prey items, or buried fish would have to be observed ambushing the prey items. The number of exposed darters was counted prior to the addition of food items, and observations were made at two-minute intervals during both the day and night trials. The thrust of the power filter pushed the food items down toward the surface of the substrate, where they drifted along the bottom. However, once the tubificid worms settled out, they attached in the sediment, allowing the darters' response to a stationary, sedentary prey to be tested. Tests were also conducted after various lengths of food deprivation. Food was withheld from the test fish for 24, 48, or 96 hours. The experiments were repeated to determine if food deprivation either enhanced or negated the original observations.

RESULTS

Once the darters acclimated to each of the three current speeds, there was usually little or no change in the number of darters above the substrate within treatment (paired t-test, $p < 0.05$). At slow current velocities, 45% of the darters remained above the substrate, while at moderate and rapid current velocities, 37% and 75% of the darters did not exhibit burying behavior, respectively. The response to high current velocities was significantly different from the response to slow and moderate current velocities (paired t-test, $p < 0.05$).

Experiments designed to test whether burying behavior was a resting response to conserve energy were carried out only at slow current velocities. Darter position was recorded during dawn, day, dusk, and night trial periods. No significant differences ($chi^2 = 0.01$, $p < 0.05$, $df = 3$) were observed in the number of fish buried during the four time periods. Darters usually remained buried for an average of 8.6 hours. The fish remained buried 100% of the time in test durations of 2 hours or less, 68% for 4 hours, 32.1% for 6 hours, 25.8% for 12 hours, and 17.2% for 24 hours. Since the majority of buried darters remained so for at least 4 hours, this suggests that burying is a resting response (paired t-test, $p < 0.05$).

In the experiments designed to test darter response to predators, burial behavior could not be forced. In all but three of thirty trials, the darters swam away, when approached by the model. In three of the encounters, the darters remained motionless until the model touched them. After contact, the fish responded by darting away. No instances of burial were observed in fish confronted by the model bass.

Startle response experiments did not elicit the expected results. In 20 tests, no burying was induced. During night tests, 95% of the individuals did nothing, when the lights were turned on. Eighty percent remained motionless, when sudden movements were made outside the aquarium. In day tests, sudden movements caused each fish to swim obliquely towards the surface, to flutter across the water surface, and to wedge itself in the corner of the aquarium with its large pectoral fins. Fish remained partially (anterior portion of body to mid-torso) out of the water for up to 5 seconds before falling back into the water and sinking to the bottom. The darters were never injured and appeared to have complete control over this response.

When prey were introduced into the aquarium, no significant difference between the number of exposed and buried darters ($chi^2 = 0.01$, $p < 0.05$, $df = 1$) before and after their introduction was noted. Each darter usually faced into the current, and when a prey organism drifted near enough, the darter arched its back and struck the prey with its head pointed downward at approximately a 30° angle. In treatments utilizing live, sedentary prey, each darter usually foraged by creeping up to the organism, arching its back, and then plunging its snout into the gravel substrate. When successful (63.5% of the time), the darter quickly drew the worm into its mouth and pumped its operculum to force negative pressure into its branchial region. Under no circumstances (40 treatments) did the darters bury themselves to ambush drifting or sedentary prey. Current velocity and time of day did not affect the mode of feeding.

Food deprivation did not change foraging behavior. Darters that were completely buried did not emerge to forage even after 96 hours of deprivation. Partially exposed (i.e., snout and head) darters usually emerged from the substrate to feed, when prey was observed. Individuals that were not aware of the presence of food did not emerge.

DISCUSSION

The three hypotheses proposed to explain burying behavior in *Ammocrypta pellucida* (Jordan and Copeland, 1877; Williams, 1975; Trautman, 1981) suggest that the behavior is elicited by a stimulus. Daniels (1989) carried out the first tests of these hypotheses, and he suggested that sand darters bury at any time, during any season, and may remain buried for up to 24 hours. He was unable to elicit the burying response on a consistent basis in any of the tests he conducted.

On the basis of my tests, predator avoidance can be rejected as a stimulus for burial. Neither a visual cue from the model bass nor an alarm reaction from light or vibration caused burial. The model bass did not elicit a burying response in 30 trials. The model caused the darter to swim away when approached, verifying Daniels' (1989) observations. At night, fish on the substrate were not disturbed by visual and vibrational stimuli. During the day, each darter responded using an escape response similar to that of other known prey species (Aronson, 1951; Barlow, 1972; Goodyear, 1973; Baylis, 1982). Multiple prey individuals swimming obliquely up the water column, fluttering across the surface, and leaving the water would confuse the predator and break visual contact between the predator and prey (Keenleyside, 1979). These results suggest that *A. pellucida* is not nocturnal and is not easily disturbed while resting.

The prey ambush hypothesis was also rejected as a stimulus for burial.

Spreitzer (1979) and Hendricks (1985) noted that eastern sand darters lie buried with only their eyes exposed. Based on this observation, they hypothesized that darters ambush their prey. Diet studies (Forbes and Richardson, 1920; Spreitzer, 1979) showed that eastern sand darters feed on components of the benthic macroinvertebrate community. Daniels (1989) demonstrated that sand darters did not ambush drifting prey. This study supports Daniels' conclusion. However, benthic tubificid worms were not ambushed by buried darters, as Daniels had speculated. Food deprivation did not cause buried individuals to emerge from the sediment to feed.

The use of increasing filter pump flows to simulate greater stream velocities also confirmed Daniels' (1989) work. After acclimatization, little difference in darter position was observed. Increasing the rate of flow caused the darters to remain above the substrate rather than to bury. This observation was not unreasonable, because the scouring or deposition of sediment at extremes of flow rate would lessen the chance that a buried *A. pellucida* would survive during environmentally harsh conditions. Predicted fish response would be to seek nearshore margins or obstructions to block the sheer stress exerted on them.

Burying behavior appears to be a resting response used during occupation of homogeneous sand habitats. This conclusion is supported by two of the experiments. First, startle response experiments during night conditions did not elicit any response. Instead, the darters maintained their position and were unaffected while resting. Second, the length of time the darters remained buried in the same physical location was not related to feeding strategy or predator avoidance.

Burial for average periods of 8.6 hours apparently conserves energy while the darter is resting. Burial behavior as a resting response serves to explain the randomness of the behavior. Observations of complete burial and situations in which the head and snout were exposed correspond to long and short resting periods.

Spreitzer (1979) and Hendricks (1985) observed sand darters with the anterior portion of their heads exposed, but Daniels (1989) did not. During these tests, darters were observed with the main portion of their bodies buried but with different amounts of their heads exposed. In 40 cases where a portion of the head was exposed, the entire head, including the opercle, was exposed 59% of the time; just the eyes and top of the head were exposed 23% of the time; and only the snout was exposed 18% of the time. In none of the observations were the dorsum of the body, caudal peduncle, or caudal fin exposed. Darters, which remained in the sediment for longer than 1 hour, were usually completely buried. Partial burial was usually temporary (less than 1 hour). However, on two occasions, darters with the tip of their snouts exposed remained buried for 2 hours. Short term burial may be an ectothermal temperature-regulating mechanism (Daniels, 1989). Temperatures below the substrate surface would be cooler during the summer and warmer during the winter.

ACKNOWLEDGEMENTS

I would like to thank Robert Daniels for elaborating on his observations during discussions at recent American Society of Ichthyologists and Herpetologists Meetings. Nancy Garcia and Ron Coleman assisted in collecting the specimens from the Tippecanoe River.

LITERATURE CITED

Aronson, L.R. 1951. Orientation and jumping behavior in the gobiid fish, *Bathygobius soporator*. Amer. Mus. Novit. 1486: 1-22.

Barlow, G.W. 1972. The attitude of fish eye-lines in relation to body shape and to stripes and bars. Copeia 1972: 4-12.

Baylis, J.R. 1982. Unusual escape response by two cyprinodontiform fishes, and a bluegill predator's counter strategy. Copeia 1982: 455-457.

Daniels, R.A. 1989. Significance of burying in *Ammocrypta pellucida*. Copeia 1989: 29-34.

Forbes, S.A. and R.E. Richardson. 1920. The fishes of Illinois. Illinois Dept. Registration Education, Springfield, Illinois, 357 pp.

Goodyear, C.P. 1973. Learned orientation in the predator avoidance behavior of mosquitofish, *Gambusia affinis*. Behavior 45: 191-223.

Hendricks, M.L. 1985. Eastern sand darter. *In:* H.H. Genoways and F.J. Brennan (Eds.), *Species of special concern in Pennsylvania,* pp. 182-184, Spec. Publ. Carnegie Mus. Natur. Hist. No. 11, 232 pp.

Jordan, D.S. and H.E. Copeland. 1877. The sand darter. Amer. Natur. 11: 86-88.

Keenleyside, M.H.A. 1979. Diversity and adaptation in fish behavior. Springer-Verlag, New York, 310 pp.

Spreitzer, A.E. 1979. The life history, external morphology, and osteology of the eastern sand darter, *Ammocrypta pellucida* (Putnam, 1863), an endangered Ohio species (Pisces: Percidae). Unpubl. M.S. Thesis, Ohio State University, Columbus, Ohio, 248 pp.

Trautman, M.B. 1981. The fishes of Ohio. Ohio State University Press, Columbus, Ohio, 782 pp.

Williams, J.D. 1975. Systematics of the percid fishes of the subgenus *Ammocrypta*, genus *Ammocrypta*, with descriptions of two new species. Bull. Alabama Mus. Natur. Hist. No. 1, 56 pp.