Environmental Biology of Fishes Vol. 10, No. 3, pp. 203–207, 1984
© Dr W. Junk Publishers, The Hague.

Behavior in an 'intermediate' population of the subterranean-dwelling characid *Astyanax fasciatus*

Aldemaro Romero
Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124, U.S.A.

Keywords: Cavernicoles, Cave colonization, *Astyanax fasciatus*, *A. mexicanus*, *Anoptichthys jordani*, *Brachyrhaphis rhabdophora*, Palo Verde, Costa Rica

Synopsis

A population of the characid *Astyanax fasciatus* was found living at the outflow of a subterranean source of water. Field observations revealed differences in behavior when compared with river populations. Affinity of this fish for the subterranean cavity was evidenced by behavior and distribution of individuals. Fish almost always carried bait into the subterranean cavity prior to feeding and also went into the cavity at night. Three factors may influence the affinity for the subterranean source of water: 1) protection against aerial predation, 2) the advantage to fishes of hovering in shade, 3) sheltering for reproduction. These observations suggest that cave colonization may take place actively rather than accidentaly, that behavioral changes may preced morphological ones during initial stages of cave entry, and that behavioral adaptations may occur quite rapidly.

Introduction

Although many examples exist of troglobitic (obligate) and troglophilic (facultative) cavernicoles whose epigean (surface) ancestor can be presumed, an important question remains to be answered: how does adaptation to cave dwelling occur? Several hypotheses have been suggested to explain structural reductions, such as blindness and depigmentation, that characterize populations that have become isolated and fully adapted to the subterranean environment. But how a species changes from epigean to troglobitic existence is still a matter of discussion; we usually have only the ancestral and the cave adapted forms but no intermediate stages to indicate how the changes occurred. Some theories of the evolution of cave populations assume accidental entry into caves resulting in permanent entrapment of the organisms. Other theories propose some directional (regressive) evolution on the assumption that cavernicolous animals represent 'dying phylogenetic lines' which seek refuge in caves (see Barr 1968 for review). Neither explanation has experimental confirmation.

A frequently cited example of cave adaptation from an epigean ancestor is the freshwater teleost *Astyanax fasciatus mexicanus* (Filippi 1853) (Characidae). This species is found as a surface (eyed and pigmented) form and as a cave (blind and depigmented) one. Because of its unusual characteristics, Hubbs & Innes (1936) described the cave form as a new genus and species *Anoptichthys jordani*. However, these two forms interbreed in both natural and experimental conditions, producing fertile hybrids with a phenotypically intermediate F_1 generation. Testcrossed F_2 individuals range from almost completely blind, depigmented fish to almost normal eyed, pigmented individuals (Sadoglu 1957, Peters & Peters 1973). Cytogenetic and electrophoretic studies also indicate that these two forms are conspecific (Avise & Selander 1972, Kirby et al. 1977). Both forms differ not only in their morphological characteristics, but also in their behavior. Contrary to the behavior of the epigean form, the troglobitic one does not school, lacks circadian rhythms (i.e. does not have rest periods like the epigean one), is not aggressive and produces an alarm substance but does not react to it (Breder 1943, Pfeiffer 1966, Schemmel 1980).

Schemmel (1980) showed that feeding behavior and morphological traits are genetically independent in *Astyanax*, which suggests that the rates of evolution of these two classes of characteristics may also be different. I have made preliminary observations on the behavior of a population of *A. fasciatus*, which is conspecific with the Mexican cave form (Wilkens 1970). These observations indicate that *A. fasciatus* can actively colonize a new subterranean-associated environment and, in a relatively short period undergo distinctive behavioral changes, whereas external morphological features remain unchanged.

Material and methods

During July 1981 and May 1982 I studied a fish assemblage of about 120 fishes consisting of two species, *Astyanax fasciatus* and the poeciliid *Brachyraphis rhabdophora* (Regan 1905) in one pool close to 'La Hacienda de Palo Verde', province of Guanacaste, NW Costa Rica. This pool, a man-made body of water formed in 1976, is under canopy shade and is supplied by water of subterranean origin. The outflow from this pool goes through a canal during the wet season to a second pool in an open area in a depression on a temporary road to 'La Hacienda'. The second pool dries out during the dry season. The fish in the second pool are all *A. fasciatus*. Some individuals of both species can be found in the canal connecting both pools. Observations made at the beginning of the wet season (May 1982) showed that the individuals in the second pool come from the first one. The origin of this fish assemblage is unknown even to the people who built a well in the first pool in 1978, but it is believed that fishes colonized the first pool after a heavy rainy season in that year. It is not surprising to find the two species under these conditions since *A. fasciatus* migrates at the end of the rainy season toward head-waters (Lopez 1980), while *B. rhabdophora* is a head-waters specialist.

Table 1. Number of individuals of both fish species per unit of time before and after bread was dropped into a quadrat. Fish density was determined by counting the number of fishes that entered through all sides of each of the 50 cm per 50 cm quadrats. Each count was conducted over a 5 minutes period.

	Number of fishes per quadrat per 5 min		Distance from the quadrat near edge to the entrace of the subterranean cavity (m)
	Before bread dropped	After bread dropped	
Quadrat I	148	197	1
Quadrat II	28	31	2.5
Quadrat III	4	5	3.5

Observations on schooling were made not only in the above mentioned pools, but also in a 1.5 per 5 meters man-made pool also close to 'La Hacienda' using videotape techniques (Sony black and white camera and portable Betamax video recorder). The videotapes of this and other kinds of behavior were later analyzed frame by frame in the laboratory using a Panasonic slow motion video recorder. Density of fishes was determined using 50 per 50 cm quadrats in three different locations in the pool that differed in distance from the cave mouth, by counting the number of fishes per unit of time, and relating the results to the distance to the subterranean chamber's entrance (Table 1).

Results

Schooling

As previously observed in *A. fasciatus*, the individuals of the open-area (and under any other open-area condition) almost always occurred in large schools (Bussing 1976). In contrast, individuals of both species in the first pool were rarely observed schooling; such schools were small (never more than 6 individuals) and of short duration (no more than 2 minutes). *B. rhabdophora* was never observed in schools.

When three groups of six individuals of *A. fasciatus* from the pool that was connected to the subterranean source of water were transfered to the 1.5 per 5 meters man-made pool in a open area they did not show any schooling behavior nor did they when the three groups (18 fish) were placed together in that pool for a 60 minutes period. Similar results were obtained with two groups of *B. rhabdophora*. However, when an equal number of *A. fasciatus* from river populations were placed in the artificial pool, they retained their schooling behavior. Six individuals of *A. fasciatus* and 12 of *B. rhabdophora* from the pool associated with subterranean waters were later brought to Miami in May, 1982 and since that time they have not shown any schooling behavior when placed either in small aquaria (50 l) or in a bigger (1.5 × 3 m) tank.

Fish morphology and distribution

Individuals of *A. fasciatus* from the pool associated with subterranean waters did not show any observable reduction in eye diameter or pigmentation (the two major structural reductions among cave organisms) when compared with individuals from river populations.

Videotape analysis showed that fishes of both species are comparatively larger and more abundant in the subterranean-associated pool near or within the entrance to the subterranean chamber (Table 1).

By skin diving I also observed that only large (>25 mm) *A. fasciatus* remained within the subterranean cavity during the day. Night observations using infrared light revealed that most individuals of both species were in the subterranean chamber from 1800 to 0615 h, during the end of the dry season (late May).

Table 2. Relationship of bread carrying to distance from subterranean chamber at which it was dropped.

	Day 1	Day 2	Day 3	Total
Number of times bread dropped <1 m from the mouth of the subterranean cavity	20	20	20	60
Frequency with which bread was carried into the cavity	20	19	20	59
Interval between the time the bread hit the surface of the water and it was carried into the subterranean chamber (seconds)	17.5	17	19.5	($\bar{x}$ = 18)
Number of times bread dropped >1 m from the mouth of the subterranean cavity	6	4	6	16
Frequency with which bread was carried into the cavity	1	0	0	1

Feeding behavior

In order to assess the feeding behavior of both species of fishes, I dropped several kinds of potential food (leaves, living and dead worms) on the surface of the pool associated with the subterranean source of water. Fishes of both species approached but did not eat the dropped objects. The fishes, which were usually close to the water surface moved away from the dropped objects as they sank.

Bread was used as an alternative bait. If the bread was fresh and could be easily broken into small pieces, individuals of both species ate it. But if the bread was dry, both species reacted by pushing pieces along the surface and into the mouth of the subterranean cavity. This behavior was observed in 59 of 60 instances when the pieces were dropped <1 m from the mouth of the subterranean chamber but only 1 in 16 instances when the pieces were dropped >1 m away from the cave (Table 2). When I placed a 8 cm diameter floating log between the drop point and the entrance of the subterranean chamber, fishes with the bread in their mouthes jumped over the log and entered into the subterranean cavity on all seven occassions.

The process of taking the pieces of bread, however, never took place immediately after the bread was dropped. Videotape analysis of this behavior shows that it takes a mean time of 18 seconds to carry the piece of bread into the subterranean chamber (Table 2).

Analysis of videotape recording of the above mentioned behavior showed that there was not any significant difference in behavior between *A. fasciatus* and *B. rhabdophora* and that the proportion of individuals of both species in the pool during the experiments was 3 to 2 respectively.

For each set of experiments, both species of fishes showed more activity after the first dropping of bread took place (Table 1).

Discussion

Caves are usually limited in food resources due to the lack of primary producers. However, since many different animal taxa have occupied cave environments to some extent (Barr 1968, Poulson & White 1969), active cave colonization is therefore probably advantageous although such advantages have never been demonstrated. In the present case the affinity that both species of fish showed for the subterranean chamber water may be explained by at least three factors which could operate together or independently as follows:

1) *Protection from aerial predators.* Piscivorous birds are known to visit Palo Verde in large number, especially during the dry season. *A. fasciatus* of the open area pool and rivers quickly react to any visual stimulus (e.g. shaking of arms above the surface of the water) by rapid changes in position and speed of individual fish or by sudden changes in the direction and form of the school. *A. fasciatus* of the pool associated with subterranean waters did not show such avoidance behavior as individuals nor did they try to form schools when transfered to the artificial pool in the open area or when presented with visual stimuli in any of the pools.

2) *Advantage to fishes of hovering in shade.* Most individuals, particularly intermediate and large fish of both species remained mainly in the shaded region created by the mouth of the subterranean cavity. This local overabundance could be explained by the relative visual advantage a shaded observer has over a sunlit observer in detecting approaching objects (Helfman 1981).

3) *Breeding site.* The subterranean cavity could be used as a secure breeding site, as has been described by Breder & Bird (1975) for the marine clupeid *Jenkinsia.* This is consistent with my observation that only large (>25 mm) and therefore sexually mature individuals of *A. fasciatus* (Bussing, personal communication) remained continuously at the subterranean cavity entrance. The reason I did not observe adult *B. rhabdophora* in the subterranean cavity could be either because observations were not made during breading season, a result of space competition by *A. fasciatus* or because they do not use subterranean cavities as breeding places at all.

Although Breder & Bird (1975) observed pigment changes in individuals of *Jenkinsia* that spent some time in caves, direct observations of captured individual showed that neither *A. fasciatus* nor *B. rhabdophora* display morphological indications of structural reduction, i.e. lost of pigmentation or reduction of eye diameter.

Conclusions

From these observations and field experiments, some preliminary conclusions can be offered concerning cave colonization by *Astyanax*: 1) Cave colonization can take place actively, and not by accident, as some cave-evolution theories claim, 2) behavioral changes may occur independently and prior to morphological ones during the cave-colonization process. No morphological change was observed in the *A. fasciatus* population of the pool associated with subterranean waters when compared with individuals from river populations, while the stability of the differences in schooling behavior is sustained by the lack of schooling in the cave associated population under a test condition in which the river population continued to school. The lack of structural reduction in *A. fasciatus* found in the cenotes (a subterranean environment) of the Yucatan Peninsula (Mexico) (Hubbs 1936) supports the idea that such morphological changes do not have to be present either before or right at the beginning of cave colonization; 3) given that the observations were made in a pool constructed four years previous to the observations, behavioral adaptation to the subterranean cavity must have occurred rapidly.

The extent to which the behavioral traits in the subterranean-associated population reflect genetic differences between this and non-subterranean populations has yet to be established. Demonstration would require further breeding experiments involving crosses between populations.

Since this is, probably, the first time that behavioral studies have been undertaken in 'intermediate' populations of a cave dwelling species-complex, further comparative studies with populations of other species under similar conditions will help in the understanding of the correlation between morphological and behavioral changes during the evolution of cave adaptation process.

Acknowledgements

I am grateful to the Department of Biology of the University of Miami, The Schneirla Foundation, and The Organization for Tropical Studies (OTS) for financial support, as well as to Gard Otis, coordinator of the OTS (1981–3) summer course, who showed me the pool associated with subterranean waters, to William Bussing for helpful discussion and identification of specimens, to Theodore Fleming and William Evoy for lending me the videotape equipment I used for the 1982 observations, and to Brian Partridge, Steven Green, John Heywood, Julian Lee, William Evoy and an anonymous reviewer who carefully read the manuscript and made valuable suggestions. This is contribution no. 90 from the Program in Ecology, Behavior, and Tropical Biology, University of Miami.

References cited

Avise, J.C. & R.K. Selander. 1972. Evolutionary genetics of cave-dwelling fishes of the genus *Astyanax*. Evolution 26: 1–19.

Barr, T.C. 1968. Cave ecology and the evolution of troglobites. pp. 53–102. *In*: T. Dobzhansky, M. Hecht & W. Steere (ed.) Evolutionary Biology, vol 2, North Holland Publishing Co., Amsterdam.

Breder, C.M. 1943. Problems in the behavior and evolution of a species of blind cave fish. Trans. N.Y. Acad. Sci. 5: 168–176.

Breder, C.M. & P.M. Bird. 1975. Cave entry by schools and associated pigmentary changes of the marine clupeid, *Jenskinsia*. Bull. Mar. Sci. 25: 377–386.

Bussing, W. 1976. Geographical distribution of the San Juan ichthyofauna of Central America with remarks on its origins and ecology. pp. 157–175. *In*: T.B. Thorson (ed.) Investigations of the Ichthyofauna of Nicaraguan Lakes, School of Life Sciences, University of Nebraska, Lincoln.

Helfman, G.S. 1981. The advantage to fishes of hovering in shade. Copeia 1981: 392–400.

Hubbs, C.L. 1936. Fishes of the Yucatan Peninsula. pp. 157–287. *In* A.S. Pearse, E.P. Creaser & F.G. Hall (ed.) The Cenotes of Yucatan, Carnegie Institution, Washington.

Hubbs, C.L. & W.T. Innes. 1936. The first known blind fish of the family Characidae: a new genus from Mexico. Occ. Pap. Mus. Zool. Univ. Michigan 342: 1–7.

Kirby, R.F., K.W. Thompson & C. Hubbs. 1977. Karyotypic similarities between the Mexican and blind tetras. Copeia 1977: 578–580.

Lopez, M.I. 1980. Migracion de la sardina *Astyanax fasciatus* (Characidae) en el rio Tempisque, Guanacaste, Costa Rica. Rev. Biol. Trop. 26: 261–273.

Peters, N. & G. Peters. 1973. Genetic problems in the regressive evolution of cavernicolous fish. pp. 187–201. *In*: L.H. Schroeder (ed.) Genetics and Mutagenesis in Fish, Springer-Verlag, New York.

Pfeiffer, W. 1966. Über die Vererbung der Schreckreaktion bei *Astyanax* (Characidae, Pisces). Z. Vererbungsl. 98: 97–105.

Poulson, T.L. & W.B. White. 1969. The cave environment. Science 165: 971–981.

Sadoglu, P. 1957. Mendelian inheritance in the hybrids between the Mexican blind cave fishes and their overground ancestor. Verh. dtsch. zool. Ges. Graz. 1957: 432–439.

Schemmel, C. 1980. Studies on the genetics of feeding behaviour in the cave fish *Astyanax mexicanus* f. *Anoptichthys*. An example of apparent monofactorial inheritance by polygenes. Z. Tierpsychol. 53: 9–22.

Wilkens, H. 1971. Genetic interpretation of regressive evolutionary process: studies on hybrid eyes of two *Astyanax* cave populations (Characidae, Pisces). Evolution 25: 530–544.

Received 11.5.1982 *Accepted 16.1.1983*