Variability in troglomorphic adaptations of a Mexican cavefish, *Poecilia mexicana,* from Tabasco, Mexico

Michael Dieterich[a] and Kathleen H. Lavoie[b] (**Faculty**)

[a]Center for Earth and Environmental Science
[b]Department of Biological Sciences
SUNY Plattsburgh, Plattsburgh, NY

ABSTRACT

The cavefish *Poecilia mexicana*, earlier known as *Poecilia sphenops*, is a live-bearing toothcarp or molly found only in Cueva de las Sardinas (also known as Cueva de Villa Luz or El Azufre), in Tabasco, Mexico. The cave ecosystem is based on mixed energy inputs from sulfur springs and chemolithotrophic baceteria, bats, and skylights. The rich food base supports an amazing population density of the cavefish. Earlier studies reported that the fish showed increasing troglomorphic adaptations in physical characteristics and behaviors as you went deeper into the cave. Data presented on eye size reduction show considerable variation and overlap of data by location sampled, and no statistical analyses were done. We sampled from some of the same areas as earlier studies and from the most remote sites in the cave. Our results show considerable variation in troglomorphy of eye reduction from all locations, and no statistical difference with fish from any part of the cave. Evolutionary pressure to develop troglomorphy may be reduced in this cave because of the rich food base, or hybridization with surface forms may not be limited by physical location within the cave.

Key Words: cavefish, Mexico, troglomorphic adaptations.

INTRODUCTION

Organisms that are restricted to the cave environment show strong parallel (for related organisms) and convergent evolution (for unrelated organisms) in morphology that often results in a progressive elongation of body form and appendages and an increase in sensory structures with reduction of eyes and body pigment. These changes are collectively referred to as troglomorphy (Christiansen 1961).

A unique sulfur-rich cave called Cueva de las Sardinas (or Cueva de Villa Luz or El Azufre) located near the village of Tapijulapa, Tabasco, Mexico, has an extensive population of *Poecilia mexicana* (formerly known as *Poecilia sphenops*), a live-bearing toothcarp or molly. Surface populations of the same species are known from several locations. The cave is about 2 km in extent, made up of large chambers interconnected by a stream (Hose and Pisarowicz (1999). The cave is informally divided into a downstream portion (before the crawlway) with abundant skylights and lower levels of toxic gases, and upstream (beyond the crawlway) which has fewer skylights. The cave presents unique challenges to researchers since the atmosphere is often quite toxic, especially in upstream sites, with areas of high H_2S (sometimes more than 300 ppm), and occasional areas of low oxygen, high CO_2, and high CO encountered. In addition, microbial metabolism of H_2S results in production of sulfuric acid that frequently drips from the ceiling and walls. The cave stream, while milky-white due to suspended sulfur, maintains a pH close to neutral.

Previous studies have suggested that there is increasing troglomorphic adaptations among the fish in different parts of the cave. Breder (1943) first reported the existence of a complete integration from the surface stream form to the fully blind and white cave form (all fish are eyed to some extent). Gordon and Rosen (1962) and Peters and Peters (1968) completed an analysis of features of the cavefish, and reported that the eye size of the cave molly is significantly reduced along a gradient from larger eyed fish downstream to smaller eyed fish upstream within the cave. They agreed with Breder that the present cave population was a hybrid between the epigean and cavernicolous forms of the molly and suggested that more remote sites might yield cave fish with even greater eye reduction. These results were recently summarized by Pfarzfall (2001). We agree that there is a real reduction in eye size between the surface and cave forms, but noted that the data presented on eye reduction show considerable variability at each sample site, often with complete overlap of data, and no statistical analyses of the data were ever done. Our results show great variability in the degree of eye reduction, but no statistical difference with location within the cave.

METHODS

Sample locations for our study are shown in Figure 1. More accessible sites are Sale Grande and the downstream site at Ragu. The most remote sites are upstream. Perched Pool is at the top of a 1.5 m waterfall, Other Buzzing is beyond a very low crawlway, and the Bat Room is the furthest site from the entrance. (There is one even more remote site in the cave, referred to as Beyond Yellow Roses (a difficult crawl through submerged areas beyond the Other Buzzing Passage). We were unsuccessful in three attempts to collect fish from this site due to extremely high levels of H_2S that forced us to evacuate the area in 2001 and 2002.)

Figure 1. Outline map of the passages in Villa Luz. Sample sites and number of fish measured from each location: RG = Ragu (n=8), SG = Sale Grande (n=8), PP = Perched Pool (n=8),.OB = Other Buzzing Passage (n=11), and BR = Batroom (n=11). Map provided by Bob Richards. (For a detailed map, see http://www.caves.org/pub/journal/PDF/V61/v61n1-Hose.pdf)

Cavefish were collected in June 2001. At each location we selected the largest fish available, and caught them using hand nets. Fish were held in a bucket of stream water with a battery-powered air bubbler. Data were collected by photography using a Polaroid Spectra camera with a close up lens. A three-person team was used during the photography; a handler, a photographer and data recorder, and a hand model wearing a latex exam glove (unpowdered) and holding a metric ruler. The handler netted individual fish from the bucket and placed them on their side beside the ruler on the hand model glove. Fish were photographed from a set distance and the location and date marked on the back of each Polaroid photograph. The fish were placed in a second bucket with a bubbler to ensure that no fish was photographed twice. Once all fish were photographed, they were released at the point of capture. No attempt was made to separate fish by gender.

When analyzing the photographs, the location information was covered to eliminate researcher bias and the photos randomly ordered. Measurements were made to the nearest 0.1 mm using calipers and a metric ruler. Length was recorded from the snout of the fish to the base of the dorsal fin. The lens width was measured horizontally across the eye. Head height was measured from the top of the head above the eye to the bottom of the head. Measurements were made in reference to the ruler in the photograph and then converted to actual measurements. Data were analyzed for mean, standard deviation, variance and significance by location using a one-way ANOVA with SPSS.

RESULTS AND DISCUSSION

The ratio of fish length (cm) to eye diameter (mm) is shown in Figure 2 by sampling location. All of the data from each sample site shows considerable variation and overlap. The ratio of head height (mm) to eye diameter (mm) is not shown, but resulted in even more complete overlap of variability from each site, with fish from Sale Grande showing the lowest range. Differences by location are not significant for length:eye ($P>0.498$, d.f. = 4) and height:eye ($P>0.860$, d.f. = 4). A comparison of length:height was significantly different ($P >0.001$, d.f. = 4) and reflected the smaller sized fish caught at the downstream Ragu site. Smaller fish downstream were also reported by Langdecker *et al.* (1996), and thought to be maintained by the regular ritual removal of fish (Hose 2001) from the downstream part of the cave by local peoples.

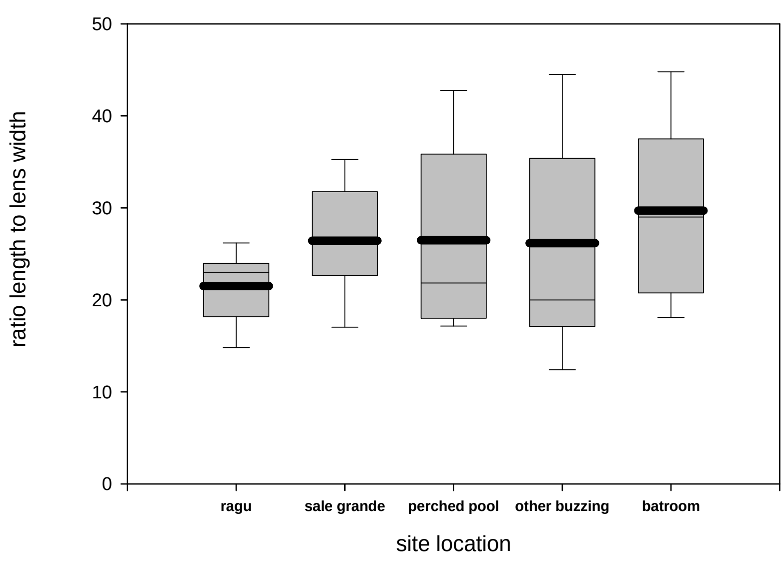

Figure 2. Boxplot of the ratio of fish length to eye width by location going deeper into the cave (see Figure 1). The shaded box delimits the middle quartiles. The heavy bar is the median and the thin bar the mean if it differs from the median. Whiskers delineate the range of data at each site.

Troglomorphy is proposed to develop by two models. The energy economy model applies to animals in typically low-energy cave ecosystems (Poulson and Lavoie 2001) There would be evolutionary pressure to develop characteristics that would save on energy use, such as reduced metabolism or reduction in eye size and pigment. The second model is one of neutral mutations where there is no selective pressures to retain characteristics, such as eye size or pigmentation, and these are gradually lost over time. Neither model is exclusive and both may work over time to result in troglomorphic adaptations. In the case of *Poecilia mexicana*, the fish are in an unusually food-rich environment, so the energy economy model is not likely to be operating. There may also be much more movement of fish throughout the cave and less hybridization with surface forms than suggested by earlier researchers. A genetic analysis of the fish from different locations in the cave would be useful in understanding the troglomorpic adaptations of *Poecilia mexicana*.

ACKNOWLEDGEMENTS

We thank Dee Blank (SUNY-Plattsburgh) and Katri Laukkanen (Chapman University) for assistance with field studies and Dr. Thomas Wolosz for statistical advice. This study was partially funded by a grant from the SUNY Research Foundation for travel (MD). The Polaroid Corporation donated the Polaroid Spectra Camera used in this study.

LITERATURE CITED

Breder, C.M., Jr. 1943. Problems in the behavior and evolution of a species of blind cave fish: Transactions of the New York Academy of Science Series II 5(7):168-176.

Christiansen, K. 1961. Convergence and parallelism in cave entomobryinae. Evolution 15(3): 288-301.

Gordon, M.S. and D. E. Rosen. 1962,. A cavernicolous form of the Pocilid fish *Poecilia sphenops* from Tabasco, Mexico: *Copeia* 2: 360-368.

Hose, L.D. 2001. "*La Ceremonia de la Pesca*" in Cueva de Villa Luz, Mexico: National Speleological Society News 59(2): 221-223; 246.

Hose, L.D. and J.A. Pisarowicz. 1999. Cueva de Villa Luz, Tabasco, Mexico: Reconnaissance study of an active sulfur spring cave and ecosystem. Journal of Cave and Karst Studies 61 (1):13-21.

Langdecker, T.G., H. Wilkens, and J. Parzefall. 1996. Studies on the trophic structure of an energy-rich Mexican cave (Cueva de las Sardinas) containing sulfurous water. Mémoires de Biospéologie Tome XXIII: 121-125.

Parsefall, J. 2001. A review of morphological and behavioural changes in the cave molly, *Poecilia mexicana*, from Tabasco, Mexico: Environmental Biology of Fishes 62: 263-275.

Peters, N. and G. Peters. 1968. Zur genetischen interpertation morphologischer (GestzmaBigkeiten) der degenrativen evolution: Zeitschrift fuer die Morphologie und Oekologie der Tiere 62: 211-244.

Poulson, T.L., and K.H. Lavoie. 2001. Chapter 12. The trophic basis of subsurface ecosystems. In: Ecosystems of the World 30: Subterranean Ecosystems. Eds. H. Wilkens, D. C. Culver and W.F. Humphreys. Elsevier. pp 231-249.