

Eye histology of the Tytoona Cave Sculpin: Eye loss evolves slower than enhancement of mandibular pores in cavefish?

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Despite the presence of caves in northern latitudes above 40–50°N that would typically be considered suitable environments for cave-adapted fish, stygobiotic fish are absent from these locations (Romero and Paulsen 2001; Proudlove 2001). One factor that likely hindered the distribution of cavefish in these areas was the migration of polar ice sheets during the Wisconsinan Period, which occurred approximately 20,000 years ago. The glaciers covered the majority of the Northern Hemisphere until about 12,000 years ago, making many caves in the region uninhabitable for fish until the period ended (Flint 1971).

Presently, the northernmost cave-adapted fish in the world is the Nippenose Cave Sculpin of the *Cottus bairdi-cognatus* complex (Espinasa and Jeffery 2003) (Actinopterygii: Scorpaeniformes: Cottidae), found at 41° 9' N, in caves of the Nippenose Valley, in Lycoming County, Central Pennsylvania. In some taxonomic databases and the genetic data repository GenBank, this taxon referred to as *Cottus sp.* 'Nippenose Valley' (Pennsylvania Grotto Sculpin). Here, we discuss a second population different from Nippenose Cave Sculpin. We refer to this population from Tytoona Cave, Pennsylvania, as the Tytoona Cave Sculpin.

The Nippenose Cave Sculpin population exhibits troglomorphic characteristics, such as reduced pigmentation, wider heads, longer pectoral fins and more abundant and wider mandibular pores when compared to surface sculpin. Of interest to the current study is that while the Nippenose Cave Sculpins still retain eyes, they have degenerated retinas and reduced optic nerves. In some specimens, the photoreceptor layer, outer nuclear layer and outer plexiform layer of the retina are absent in large parts of the eye (Espinasa and Jeffery 2003).

After the Nippenose Cave Sculpin was discovered, a second population of cave-adapted sculpin was found in Tytoona Cave in Central Pennsylvania and assigned to *Cottus bairdi* (Espinasa et al. 2013), based on 16S rRNA DNA sequences. Tytoona Cave Nature Preserve, which is owned by the National Speleological Society (NSS), is found between the cities of Tyrone and Altoona in Blair County, Pennsylvania. Tytoona Cave is the 19th longest cave in Pennsylvania with a passage length of 1140 m. The cave has a flowing stream, which emerges at Arch Spring. A detailed description of the cave and map of Tytoona/Arch Spring caves can be found in White and White (2012) and on the Tytoona Cave Nature Preserve Management Plan website (<http://www.caves.org/preserves/tcp/mp-tcp.html>).

Tytoona Cave Sculpin are much less troglomorphic than the Nippenose Cave Sculpins (Espinasa et al. 2013). Their eyes, pectoral fins and mouths appear to be as large as those of their surface counterparts. They have the same number of cephalic lateralis pores (part of the lateral line system) and their pigmentation levels do not appear to be significantly reduced (**Figure 1**). Nonetheless, they were considered to be cave-adapted due to the presence of ovigerous females, a lack of evidence for starvation and primarily because the cephalic lateralis pores are significantly larger than those of similar-sized surface fish (Espinasa et al. 2013). Larger pores may correlate to an enhancement in mechanosensory ability, which would allow cavefish to thrive better in a dark environment.

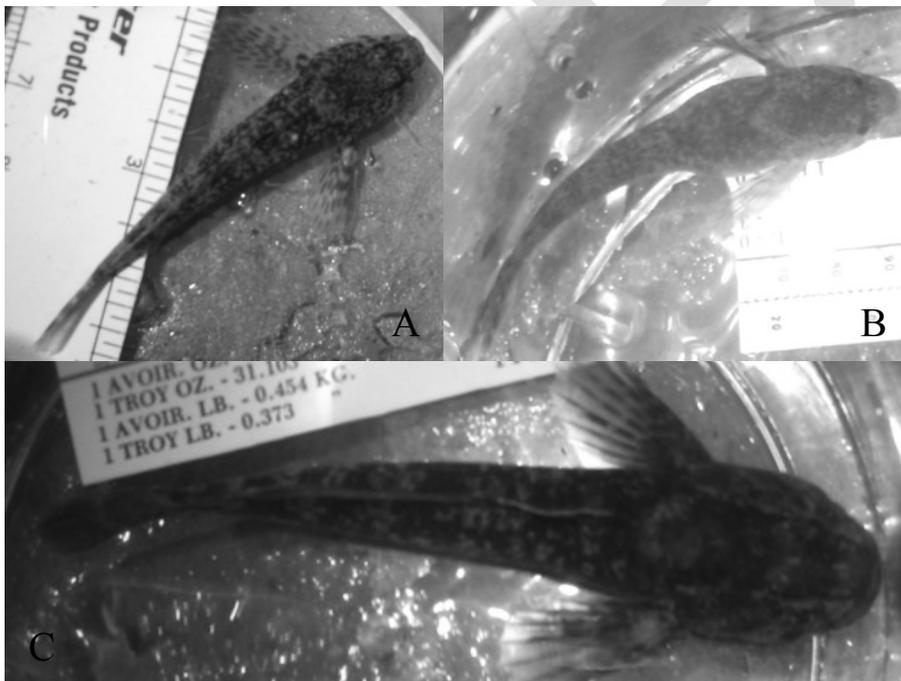


Figure 1. Variability in pigmentation and color patterns in the Tytoona Cave Sculpin. While most cavefish pigmentation levels are within the range of surface fish (A and C), some may be less pigmented (B). Specimen B is also an ovigerous female, as seen by the distended abdomen (from Espinasa et al. 2013).

For generations, biologists seeking cave-adapted organisms have emphasized the collection and study of eyeless organisms; however, which is most strongly favored in cave evolution, being blind or being eyeless? Many cave organisms are thought to be derived from surface individuals that have accidentally been swept into the cave, because they have eyes, and thus have been disregarded. For many years, this was the case of the Nippenose Cave Sculpin. It was not until their retinas were described as being degenerate that they were recognized as a stygobiont. Could it be that the retinas in Tytoona Cave Sculpin are also degenerate while the rest of the eye remains? Genetic studies in *Astyanax* cavefish have shown the existence of two independent developmental units within the eye that can give rise to large lenses combined with undifferentiated retinas or of well-developed retinas combined with rudimentary lenses in hybrids (Wilkens 2010). Since there are reports of cavefish in the wild that have well developed anterior eye chambers and lenses, but have reduced retinas (Espinasa et al. 2001), we were interested in examining the histology of the eye of the Tytoona Cave Sculpin. The hypothesis was that while Tytoona Cave Sculpin may outwardly have eyes as large as surface sculpin, they could have reduced retinas.

After conducting a preliminary capture-recapture study and determining that the population has a healthy size of hundreds or potentially even thousands of individuals, a single cave specimen was sacrificed and preserved in 10% formalin, in agreement with the Preserve Management Chair. Sampling occurred on 31 March 2012. Surface sculpin were also collected from the stream that emerges at Arch Spring. Eyes were excised using a scalpel and forceps under a dissection microscope. The samples were embedded in paraffin and were then stained with hematoxylin and eosin after being sectioned.

Both surface and cave specimens had well-formed lenses. As seen in **Figure 2b**, the following retinal layers were evident in surface specimens: the ganglion (1), inner plexiform (2), inner nuclear (3), outer plexiform (4), outer nuclear (5), rods and cones (6), and dark pigmented epithelium (7). The cave specimen had all the aforementioned layers that were very similar to the surface samples, with the exception of one characteristic; there was a clear difference in the width of the rods and cones layer, giving the appearance of an extra layer between the cones and rods and the pigmented epithelium (**Figure 2a**).

This “extra layer” is due to normal retina movements in response to light and does not reflect a structural difference between cave and surface fish (Espinasa and Jeffery 2006). Teleosts living in surface habitats employ a series of orchestrated changes in their retinal photoreceptor and pigmented epithelium cells known as retinomotor movements to adjust to bright or dark conditions. During the day, cells in the pigmented epithelium disperse their pigmented granules into long apical projections that interdigitate with the outer photoreceptor segments, shielding them from excessive light and reducing the extent of photopigment bleaching (King-Smith et al. 1996). Likewise,

during the day, the cones are positioned closer to incoming light than the rods (Cavallaro and Burnside 1988). At night, the situation is reversed; cones and rods exchange places and pigment granules aggregate toward the base of the pigmented epithelium (Cavallaro and Burnside 1988). Thus exposed, photoreceptor cells are able to catch the maximum amount of available photons.

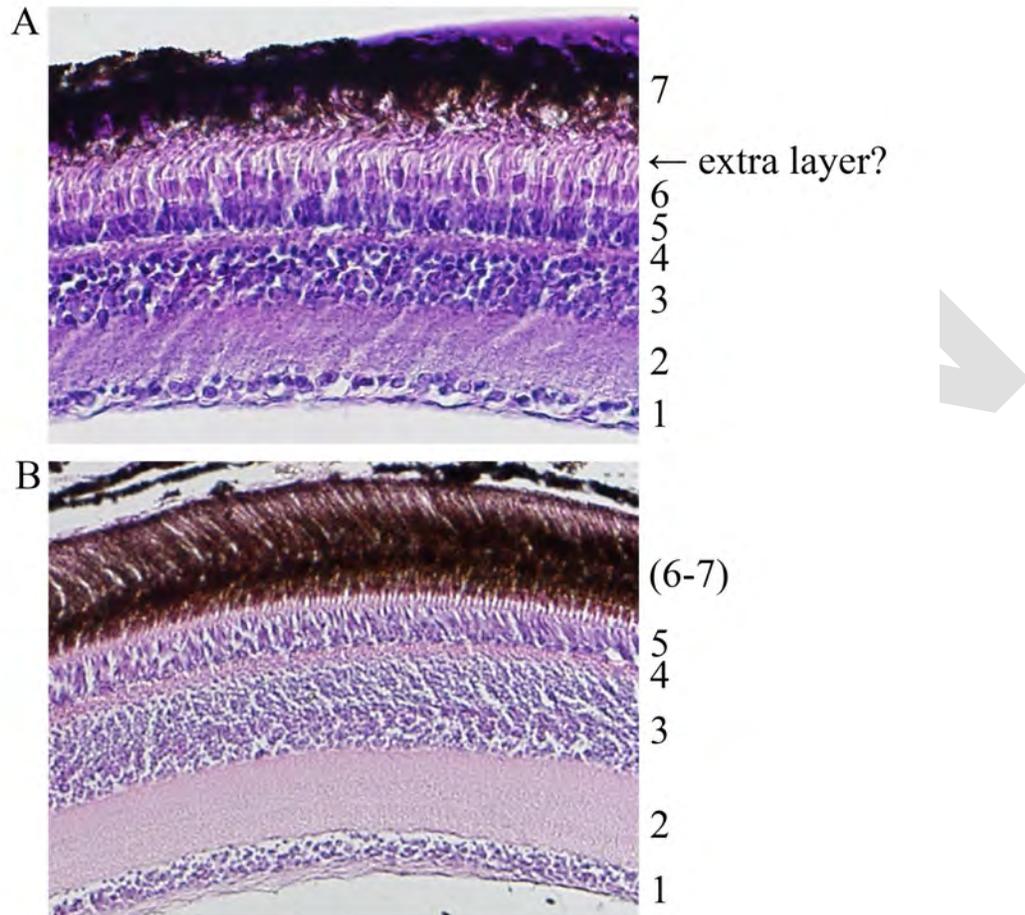


Figure 2. Histology of the retina: Tytoona Cave Sculpin (A) and surface sculpin (B). Notice the apparent extra layer between the rods and cones (6) and the dark pigmented epithelium (7) in the Tytoona Cave Sculpin.

In conclusion, there is no distinguishable structural difference between the retinas of the Tytoona Cave Sculpin and the surface population. Morphologic differences are equivalent to those seen in eyes of specimens sacrificed and fixed in the day or at night, or in our case, in the darkness of a cave. The retinal tissue of the cave specimen showed no signs of degeneration or differentiation in comparison to the surface specimens. A study with salamanders collected in caves had previously shown that apparent retinal differences between cave and surface specimens may simply be the result of comparing eyes adjusted to the light or to the dark (Nacca 2009).

There are two hypotheses that attempt to explain the regressive evolution of eyes in cave-adapted organisms. The first hypothesis suggests that the loss of the organ somehow enhances efficiency or reshapes the morphology and physiology of the organism to better suit living in eternal darkness. Under this hypothesis, natural selection is the force driving evolution. The alternative hypothesis is that genes controlling the development of unnecessary structures are rendered effectively neutral in the dark environment. They are neither good nor bad, and so the forces of natural selection that once maintained these genes controlling eye development are relaxed and accumulate mutations, which impair their function. In essence, the two arguments are based either on natural selection or neutral mutation theory. The first occurs in a hastened fashion, while the latter progresses more slowly. The Tytoona Cave Sculpin would appear to be a model well suited to shed light on determining the role of selection during eye degeneration. Since cave colonization only became a viable option a mere 12,000 years ago, evolutionary time available may be a determining factor. Developing enhanced somatosensory systems such as the enlarged mandibular pores seen in Tytoona Cave Sculpin would appear to be intrinsically favorable and thus acted upon by selection. On the contrary, eye degeneration in Tytoona Cave Sculpin has yet to become apparent and thus supports that eye loss is controlled by a slower evolutionary force. It may be that the available time, restriction of gene flow, and/or strength of evolutionary forces have been sufficient enough to enhance mechanosensory systems, but have yet to regress eyes and pigmentation in the Tytoona Cave population.

As a final note, caution should be used to avoid making broad generalizations regarding the speed of constructive versus regressive evolution. For example, Culver et al. (1995) presented evidence that the rate of regressive evolution was *faster* than constructive evolution (antennal length) in the amphipod crustacean *Gammarus*. It is very likely that energy costs, pleiotropic effects, and developmental constraints in the invertebrate eye and antenna versus the vertebrate eye and mandibular pores are different, and thus, selective pressures and evolutionary speeds can be dissimilar. It is to be expected that different troglomorphic structures should have their own distinct evolutionary history, especially when comparing different cave animal models.

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