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Cavefish as biological models in the laboratory and in the wild

Cavefish have advanced to the forefront as excellent models for laboratory studies in developmental and evolutionary biology. The iconic Mexican characid, *Astyanax mexicanus*, which consists of a surface-dwelling morph and about 30 interfertile cave-dwelling morphs, has become a major contributor to behavior, sensory biology, trait evolution, and biomedicine (Jeffery, 2020). There are about 200 scientifically-described cavefish species distributed in karst areas on most continents (Proudlove, 2015). Similar to *A. mexicanus*, a small subset of other cavefish taxa coupled with related surface-dwelling species are now serving as additional laboratory models to address a variety of important biological problems. A prime example is the cyprinid genus *Sinocycloceilus*, which contains 75 closely related surface and cave species endemic to the karst massifs of southwestern China (Jiang et al., 2019, 2023; Ma et al., 2019). Comparative studies of Chinese and Mexican cavefish can be used to address a host of questions concerning the convergence and divergence of cave related traits. In the future, other Chinese cavefish species, such as the cave loach, as well as the more recently discovered European cavefishes, are likely to join the current group of excellent laboratory models. This special issue of Zoological Research presents the results of current research on these diverse cavefish species. In addition to recent research using cavefish models in laboratory studies, this issue also covers new research on cavefish in the wild.

The special challenges of living in dark environments have resulted in the evolution of distinct behaviors, and combinations of closely-related surface fish and cavefish can be used to obtain valuable insights into how such behaviors are regulated and how they evolved. In this issue, Zhang et al. (2023) examine stress behavior in three surface and three blind cave species of the Chinese loach *Triplophysa*. Their results suggest that cavefish have lost the stress response characteristic of surface species, and that this loss may be mediated by reduced activity of the hypothalamus-pituitary-interrenal axis, presumably as an energy conservation strategy. Other behaviors, such as Vibration Attraction Behavior (VAB; Yoshizawa et al., 2010), changes in feeding posture (Kowalko et al, 2013), and specialized larval prey capture (LPC) have evolved in *A. mexicanus* cavefish. In this issue, Espinasa et al. (2023a) report laterality differences in LPC, which are distinct from the previously reported laterality VAB differences in adult cavefish. In addition, LPC laterality was found to be exacerbated by low food abundance, suggesting a nutritional trigger for behavioral evolution in the dark cave environment. Thus, cavefish continue to provide important information on the evolution of behavior.

Understanding the phylogenetic relationships between closely related surface and cave species has been difficult to decipher due to recent divergences and possible introgressions. This has been an especially complex problem in *A. mexicanus*, in which cavefish are thought to have separated very recently from conspecific surface fish (Fumey et al., 2018). In this issue, Garduño-Sánchez et al. (2023) report an exhaustive phylogeographic study of *A. mexicanus* surface fish and cavefish populations using nuclear and mitochondrial markers, which support the conventional hypothesis that cavefish are comprised of two lineages, one centered in the Sierra de El Abra region and another in the Sierra de Guatemala region of northeastern Mexico. In another phylogenetic analysis, Luo et al. (2023) examined 39 species of Chinese surface and cave loaches and confirm previous taxonomies based on morphology. These phylogenetic studies further resolve and confirm taxonomic relationships in two of the most complex surface and cavefish assemblages.

Astyanax mexicanus cavefish have contributed to biomedical research by providing a comparative model for cardiac regeneration. As first discovered in zebrafish (Poss et al., 2002), surface fish are able to efficiently replace excised parts of the heart ventricle. In striking contrast, cavefish are unable achieve this feat, instead producing only scar tissue after ventricular surgery, a difference that provides a window to identify genes involved in heart regeneration (Stockdale et al., 2018). In this issue, Olsen et al. (2023) report that cavefish did not lose all of their regenerative capacities. Despite their inability to replace an injured heart, *A. mexicanus* cavefish can mount a robust skeletal muscle regeneration program based on the activity of satellite stem cells. Accordingly, the *A. mexicanus* model may be useful for studying the regenerative strategies displayed by different types of vertebrate muscle.

One of the most prominent contributions of the cavefish models has been to provide systems to study trait evolution. Although evolution is driven by environmental changes, some of the basic tenants of cavefish ecology, such as natural diet, population size, spawning time, and parasite load, are poorly understood. Early ecological studies of *A. mexicanus* by Charles Breder and associates at the American Museum of Natural History (Breder, 1942) were conducted in the dry season, approximately February to April in northeastern Mexico, when caves are most accessible, and focused on Chica Cave, which is atypical in many aspects from other

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Astyanax caves (Mitchell et al., 1977). Now, in a three-year study reported in this issue, Espinasa et al. (2023b) have filled gaps in the ecology of *A. mexicanus* cavefish through examining the stomach contents of larvae and adults as well as assessing reproductive output throughout the year in Pachón Cave, which is the source of the most frequently-studied cavefish laboratory population. They suggest that food preferences change between the post-larval and adult stages, implying distinct early and late survival tactics, and that larvae are present throughout the year with a likely spawning peak in January, the most food-rich month. This information will be important for future nutritional studies using natural dietary parameters.

Parasite load is known to drive evolution but has been neglected in most previous cavefish studies. In this issue, Santacruz et al. (2023) address this question in a detailed investigation of parasite taxa in 18 different *A. mexicanus* cavefish populations and their nearby surface conspecifics. The authors describe 13 parasite species distributed among the different cavefish that are a subset of the 27 parasite taxa found in all nearby surface fish populations. These studies set the stage for future explorations of the role of parasites in driving the evolution of cave related traits. Gut microbes are also likely to have vital functions in the adaptation of surface animals to cave habitats. Accordingly, Chen et al. (2023) compared the intestinal microbes in *Sinocyclocheilus* surface and cavefish species and found differences related to their specific habitats. The results of these two studies increase our understanding of the diversity of parasites and gut commensals in cavefish.

The identity of the earliest steps in cave colonization is a central question in the biology of cavefish as well as other cave adapted animals. Genetic background and phenotypic plasticity are two factors that are often cited as possible mediators of cave colonization. These factors can be distinguished by common garden experiments, in which specimens collected from nature are reared in the laboratory under standard conditions for one or more generations, and changes in their adaptive phenotypes are evaluated. When no changes in these phenotypes occur during laboratory rearing, then genetic control can be concluded, whereas if the phenotypes become gradually homogenized, plasticity is concluded. The common garden approach has been used by two studies reported in this issue. Working with recently introduced subterranean populations of *A. mexicanus* from the Edwards Plateau of Texas, Swanson et al. (2023) found that although most trait differences between the subterranean and surface populations were due to plasticity, a few, including the well-known wall-following behavior (Patton et al., 2010), are under genetic control. Also applying a common garden approach, Čupić et al. (2023) reported that molecular, morphological, and physiological differences expressed by surface and cave morphs of the Croatian cavefish *Lebistes karsticus* can be attributed to plasticity. The Čupić et al. (2023) study also documents the first cavefish species from the Dinaric Karst, a hot spot of cave animal diversity (Culver & Sket, 2000) that was previously lacking any described cavefishes. Together, these studies provide evidence that phenotypic plasticity may be a strong facilitator of cave colonization.

Breakthroughs in understanding cavefish biology have been mostly due to research conducted in laboratory settings, but there has been a recent shift to field studies with the realization that laboratory data will often need confirmation in the wild. Increased ecological studies will inevitably require more field collections, which brings conservation issues into sharp focus. In this issue, Legendre et al. (2023) describe field studies to determine the size of the *A. mexicanus* Pachón cavefish population. They estimate a very small population, much smaller than recorded in the last census conducted about 50 years ago (Mitchell et al., 1977). This study alerts us to the possibility of serious conservation issues for a major population of *A. mexicanus* cavefish. In this issue, Ma et al. (2023) also discuss the conservation efforts and ongoing research on Chinese cavefish, emphasizing the need for stricter control over collections and heightened attention to declining habitats. It is essential for cavefish researchers to actively address and prioritize these conservation issues to safeguard the long-term survival of these unique organisms.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

W.R.J. conceived the review and wrote the draft. All authors read and approved the final version of the manuscript.

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