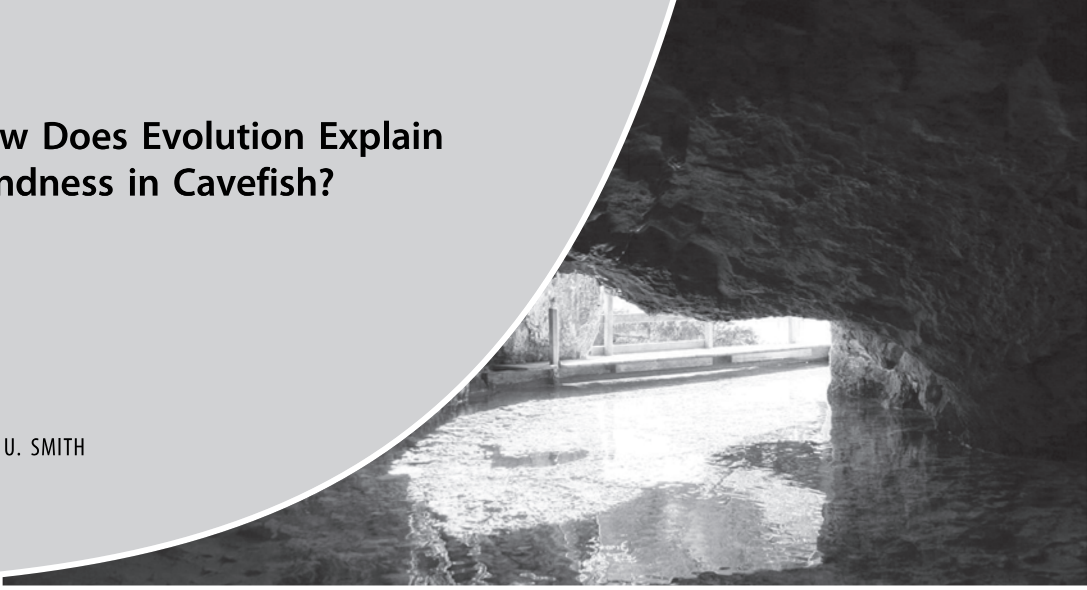
FEATURE ARTICLE

How Does Evolution Explain Blindness in Cavefish?



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ABSTRACT

*Commonly used evolution assessments often ask about the evolution of blindness in cavefish or salamanders, running speed in cheetahs, and/or the long necks of giraffes. Explaining the loss of function in cave animals, however, is more difficult than explaining evolution involving gains of function resulting from natural selection. In fact, the evolution of cavefish blindness is not yet well understood by scientists. This article presents the three current hypotheses for explaining the evolution of blindness in Mexican tetras (*Astyanax mexicanus*), related to the Next Generation Science Standards and the Advanced Placement curriculum.*

Key Words: *blindness; evolution; inheritance.*

○ Introduction

Measures of understanding of evolution often ask students to explain the evolution of blindness in cavefish or salamanders, running speed in cheetahs, and/or the long necks of giraffes (Bishop & Anderson, 1990; Demastes et al., 1995; Project 2061, n.d.; Settlage, Jr., 1994). These items are primarily used to identify student misconceptions, such as inheritance of acquired characteristics and evolution driven by need or "pressure" (Nehm et al., 2010). To the surprise of many, students typically have much more difficulty with the cavefish question than with cheetahs (Nehm & Ha, 2011), but students are not the only ones who have difficulty explaining the evolution of a loss of function (e.g., blindness) vs. a gain of function (increased running speed). Even Darwin (1872) got the explanation of blindness in cavefish wrong, attributing it to disuse: "As it is difficult to imagine that eyes, though useless, could in any way be injurious to animals living in darkness, I attribute their loss solely to disuse" (p. 110).

> *The evolution of different species with similar structures or functions in spite of their evolutionary ancestors being very dissimilar or unrelated is called "convergent evolution".*

The evolution of different species with similar structures or functions in spite of their evolutionary ancestors being very dissimilar or unrelated is called "convergent evolution" (Biology Online, 2016). The main question that has confounded biologists for years has been: How do so many different species that inhabit caves end up with very similar phenotypes—how do the phenotypes converge? In particular, how does blindness evolve in cavefish and in essentially every other species of animal whose life is spent in the dark (called "troglodytes" or "troglobionts")? The surprising answer is that we don't know the full story yet, but the mechanisms underlying the evolution of blindness in the Mexican cavefish have begun to be elucidated.

In the classroom, understanding how evolutionary biology could explain loss of function in the case of troglodytes provides an excellent opportunity not only for identifying student misconceptions, but also for understanding central concepts. These include: Disciplinary Core Ideas such as natural selection, adaptation, and the interplay of genetics and the environment ("evo-devo") promoted by the Next Generation Science Standards (NGSS Lead States, 2013; Lerner, 2000); as well as more advanced AP "Big Idea 1" (evolution), Science Practices 1 (using models), 3 (scientific questioning), 5 (using scientific explanations and theories), 7 (relating knowledge across scales, concepts and representations); and more specific concepts such as genetic drift, fitness, and homeotic genes (Biology Online, 2008, 2009, 2012). Troglodytes are an excellent case study for students to learn to explain the basic evolutionary question of how species change over time (adapt to shifts in their environment) (Table 1). The existence of vestigial structures such as the nonfunctional eyes of cavefish

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Table 1. Common troglodyte characteristics (Gross, 2012; McGaugh et al., 2014; Protas et al., 2007; Retaux & Caslane, 2013).

Characteristics
Blindness
Loss of pigmentation
Enhanced tactile & chemical senses (including taste)
Increased food finding ability
Increased starvation resistance
Reduced energy needs
Enhanced lipid storage
Reduced fecundity (larger, fewer eggs, etc.)
Slower, more efficient metabolism
Attraction to vibration
Loss of schooling
Loss of aggression
Dramatic sleep reduction
Shorter lifespans
Reduced genetic diversity
Smaller population sizes

is also an important component of the evidentiary support for the theory of evolution (Senter et al., 2015; Anonymous, 2011). Also, studying weird creatures that live in a dark world far beneath the surface can be fun because students often find these troglodytes interesting and motivating. To explain troglodyte evolution, first we need some background.

○ The Lives Of Troglodytes

The underground world of caves is largely unknown even though most of the world's unfrozen water (94%) is stored, not in the oceans, but underground (Culver & Pipan, 2009). There are nearly 50,000 caves in the U.S. alone (Retaux & Casane, 2013). So, what is life like in a cave, and what do we know about life there that might help explain the evolution of cave blindness? Of course, it is dark, and the lack of sunlight means the absence of photosynthesis. Therefore, there are no primary producers to convert light energy into biomass for consumption up the food chain. So how do organisms survive? What do they eat? How do they find mates? What kinds of animals are found there? Why are they all blind? And how did they get into the caves in the first place?

There is a surprising diversity of life in caves. Almost all the major phyla are represented, including fish, beetles, salamanders, shrimp, and spiders, among many others (Protas et al., 2011). There are 86 cavefish species alone (Jeffrey, 2009). The ancestors of most of these species that Darwin called "wrecks of ancient life" (Darwin, 1872, p. 112) were likely washed into the caves by spring flooding. The cave and surface (hypogean and epigean, respectively) populations were then separated when the water receded and connections between the two dried up. This example of the isolation of subpopulations has many parallels with the appearance of Darwin's finches (and other animals and plants) on the Galapagos Islands. Just as with Galapagos organisms, the first members of a species to arrive in a cave were likely few and varied little from their relatives left behind, left behind, but over time cavefish and surface fish subpopulations evolved differently (diverged) so that some sequences in the genomes of cavefish of the same genus are found to be different from their surface relatives (O'Quin et al., 2015). Of course, new spring rains sometimes bring new immigrants into a cave such that the population immediately after a rain actually consists of (sighted and blind) subpopulations.

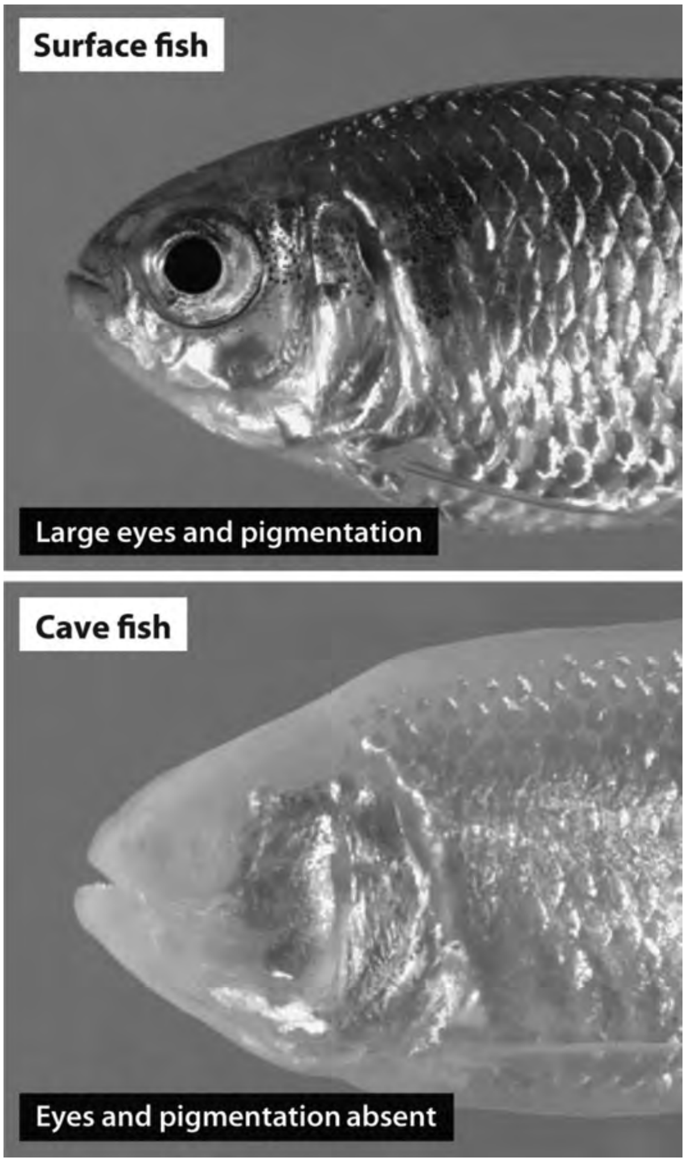

Figure 1. *Astyanax mexicanus* surface fish and cavefish. (Jeffery, 2009, Fig. 2. Used by permission.)

Probably the most studied of the troglodytes is *Astyanax mexicanus* (Figure 1) found in certain caves in northern Mexico (Figure 2), a tetra related to the common aquarium fish that students may be familiar with. In the absence of photosynthesis, they mostly survive on the film

Figure 2. Distribution map for *Astyanax mexicanus*.

of bacteria that break down bat and cricket guano (feces), although sometimes flooding brings in additional biomass food (Leighton, 2015; Gross, 2012). Darkness also means that finding mates is more difficult. Mating and sexual selection in most animal species is often based on coloration, but troglodyte species typically have no coloration. *Astynanax* (as-tie'-a-naks) individuals find mates through their enhanced tactile senses; they have greater ability to sense vibration. In fact, scientists capture these fish simply by putting a net in the water and vibrating it.

○ The Evolution of Blindness in *Astyanax mexicanus*

Most cavefish do in fact have tiny eye structures, but these eyes are sunken below the body surface. In many of these species, the initial development of the eye is relatively normal, but the eye structures degenerate (regress) and become nonfunctional as development proceeds.

As Darwin noted, the evolution of blindness by natural selection in cave animals is a conundrum. Natural selection only selects *for* traits that *enhance* survival to reproduction, explaining the *gain* of new structures, traits, and functions. How then can evolutionary theory explain the *loss* of function in structures that have no value to survival? In darkness, there is no advantage to having functional eyes, therefore there is no natural selection for better functioning eyes. The natural selection pressure is "relaxed," but there is, likewise, no obvious reason to select *for* the loss of sight. How can we explain this regressive evolution (the loss of useless characteristics over time) (Jeffrey, 2009)?

Three main hypotheses have been proposed to explain these examples of regressive evolution. According to the first hypothesis, eye loss is indeed caused by direct natural selection because there *is* an advantage to being eyeless in the dark. Studies have shown that maintaining eye tissue, especially the retina, and the related neural tissue comes at a high metabolic cost (Moran et al., 2015; Protas et al., 2007). Therefore, cavefish without eyes are at an advantage in this environment where energy sources (food) are scarce, because blind fish do not waste energy on these useless structures.

A second hypothesis employs the phenomenon of pleiotropy, that is, cases in which multiple phenotypic effects are caused by the same mutation in a single gene. There is, for example, evidence that one of the genes responsible for eye loss in cavefish also increases the number of taste buds on the ventral surface of the head, which helps cavefish find food more effectively (Gross, 2012). Natural selection for this increase in taste buds would, therefore, also promote blindness.

The third hypothesis is based on neutral mutation and genetic drift. All too often textbooks use the terms "evolution" and "natural selection" interchangeably, ignoring the importance of genetic drift. Genetic drift is "the process of change in the genetic composition of a population due to chance or random events rather than to natural selection, resulting in changes in allele frequencies over time" (Biology Online, 2008). Genetic drift differs from natural selection because observed changes in allele frequency are completely at random, not the result of natural selection for a trait. Genetic drift can have a relatively larger impact on smaller populations such as a typical population of cavefish. According to the neutral mutation and genetic drift hypothesis, therefore, normal mutation processes in a small population of cavefish sometimes produce neutral mutations (mutations that lead to phenotypic changes that natural selection does not act on), and in the absence of natural selection, totally random events can sometimes result in the increased frequency of such mutations over time. Such changes could include eye degeneration.

So, what's the right answer? What genetic evidence is there to support each of these hypotheses? As with so much in science, the answer is probably that these explanations are not mutually exclusive; it is likely that all three partially explain cavefish blindness. To understand that statement, we must have some further background on *A. mexicanus* genetics.

○ The Genetics of *Astyanax mexicanus*

Much is known about the genetics of this cavefish. The genome consists of more than a billion base pairs (NCBI, 2013). Unlike typical Mendelian traits, inheritance of eye structures and eyesight is polygenic, that is, determined not by a single gene but by many (e.g., genes related to eye structure, the lens, the retina, pigments, etc.) This is particularly easy to understand in the case of eye pigments, which are formed by a series of metabolic reactions, each reaction being catalyzed by a different enzyme and coded by a different gene, all of which are required to produce the final active pigment.

Some *A. mexicanus* genes have an additive effect and thus are called "quantitative" traits. For example, there are at least three genes that determine the extent of ossification (bone development) in the sclera (tough outer covering of the eye) in *A. mexicanus* (O'Quin et al., 2015). The genes have an additive effect; that is, when two (or three) of these genes are unmutated, more bone development in the sclera occurs than if only one is not mutated. Skin pigmentation in *A. mexicanus* is also an additive trait (Gross, 2012). In contrast, if the genes were Mendelian (each trait determined by a single gene), the elimination of any one gene product by mutation (in both alleles) would result in no scleral ossification; that is, the effect is qualitative: yes/no, tall/short, and so on.

Most of the genes discussed so far are likely "structural" genes; that is, their DNA sequences code for the sequences of amino acids in the resulting proteins. Cavefish eye development is also determined by "regulatory" genes, genes encoding products that regulate an entire developmental pathway, such as the well-known Antennapedia (*antp*) gene in Drosophila that produces an entire well-formed pair of legs on the head in the place of antennae (Figure 3).

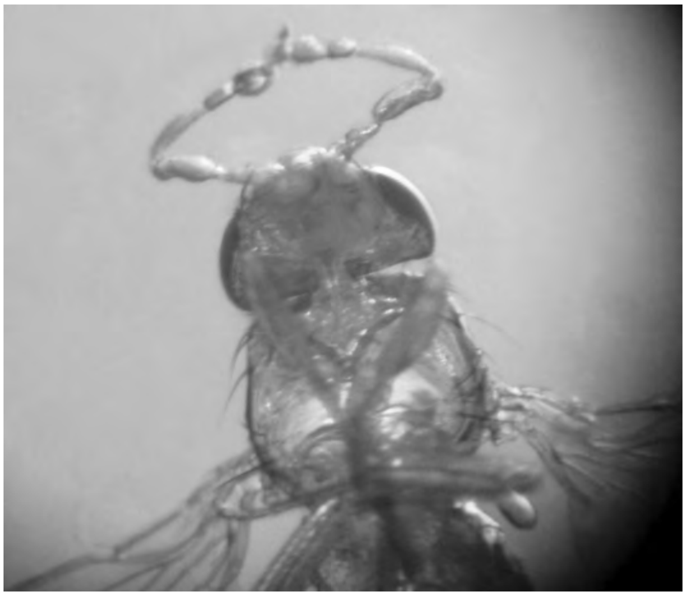

Figure 3. *Drosophila* with antp mutation. Note leg-like structures on the head.

As mentioned above, some genes involved in *A. mexicanus* eye development are also pleiotropic, that is, a single gene impacts more than one recognizable trait. For example, the overexpression of the genes sonic hedgehog (*shh*) and tiggy-winkle hedgehog (*twhh*)—which are both homeotic genes (genes that encode transcription factors that often control an entire developmental pathway) in *A. mexicanus*—results in degradation of the lens of the eye, but it also results in an increase in the size of the fish's jaws and in the number of taste buds on the lips (Retaux & Casane, 2013; Protas et al., 2007). In fact, such a gene would also be considered pleiotropic because it has multiple phenotypic effects (on the eye and the mouth). Note that regulatory genetic effects tend to be qualitative, not quantitative, that is, the mutation of a single gene, not many genes, is typically sufficient to determine the effect. Thus, *shh* is likely to have its effect "upstream" in development, that is, prior to the actions of the structural genes described above.

○ Evolution of Cave Blindness in *A. mexicanus*

It is easy to understand how pleiotropic genetic determination of eye development and sensory perception could explain cavefish blindness. Natural selection simply favors mutations that increase the number of taste buds, and the loss of eyesight is a coincidental byproduct—supporting both the natural selection and pleiotrophy hypotheses. Thus, degeneration of the eye is indirectly selected for. On the other hand, studies of the sequences of other genes related to the cavefish eye show high frequencies of substitutions in both coding and noncoding regions, which would support the genetic drift hypothesis (Retaux & Casane, 2013).

In summary, the most current proposed explanation is that the determination of sight in cavefish is a complex process with polygenic determination, involving pleiotropic genes with multiple effects as well as qualitative and quantitative, and structural and regulatory genes. The evolution of blindness in cavefish is best explained by a combination of all three hypotheses described above.

○ Evolution of Albinism in Troglodytes

It is important to note that not all losses of structure or function are determined in the same way. For example, reduced coloration in cavefish is another loss of function, and the determination and evolution of this trait has similarities to, but also differences from, the evolution of eyesight loss. Although there is not space to discuss this other commonly cited example in detail, pigment regression in *Astyanax* is also a polygenically determined trait involving both regulatory and structural genes. A prominent structural gene involved is melanocortin 1 receptor (*Mc1r*), which is involved in determination of the brown component of melanin (a pigment found in the skin). This gene and its functioning were recently discussed at length in this journal (Offner, 2013). Another gene involved is oculocutaneous albinism (*oca2*), which is known to cause albinism in a variety of vertebrates (Gross, 2012). In the case of pigmentation evolution, current data suggest greater support for the importance of the neutral mutation/genetic drift hypothesis than for the other two hypotheses discussed above (Jeffery, 2009).

○ Summary

The central tenets seem to be these:

1. Evolutionary science is both an experimental and a historical science; successful evolutionary explanations provide possible explanations (that must be consistent with the data), not "proven" answers. Explanations become increasingly tenable if they are supported by additional research (i.e., supported by the "weight of the evidence").
2. Evolution can be used to explain losses of functions or structures over geologic time.
3. Evolutionary theory includes not only natural selection but also genetic drift.
4. The exact mechanisms involved are not fully understood, but scientists are learning more and more about the evolution of cave blindness.
5. Sightedness in *A. mexicanus* is a polygenic trait, involving structural and regulatory genes, pleiotropic genes, and qualitative and quantitative genes.
6. Evolution of blindness in *A. mexicanus* can be explained by a combination of three hypotheses:
    a. The high cost of sight/direct selection hypothesis: Natural selection directly produces blindness in this species because there is advantage to not wasting energy on a useless and costly function and structure.
    b. The pleiotropy/indirect selection hypothesis: Sightedness is determined by individual mutations that code for more than one trait (e.g., increase in sensory perception and loss of eyesight); natural selection for one function necessarily results in increases the frequency of both outcomes. Gains

in sensory perception are beneficial and positively selected; loss of eyesight has no effect on selection.

c. The neutral mutation and genetic drift hypothesis: Many mutations that occur in the genes involved in sightedness are selection-neutral; that is, they are not affected by natural selection. The frequencies of these mutations can increase over time as a result of purely random events (genetic drift). As these mutations accumulate in genes related to eye structure and function over many generations, blindness results.

○ Assessment, Teacher Talk, Student talk, and Misconceptions

There is, of course, ample reason for using troglodyte blindness in assessing student understanding of evolution: misconceptions about evolution are common, and students often evidence these misconceptions when they try to apply their nascent understanding of evolution by explaining challenging cases such as this. Teachers, textbooks, and even journal articles are, likewise, not immune to these mistakes. In fact, experts who clearly understand the phenomena involved often use misconception language as a shorthand ("but we all understand what we really mean"). Although that may be true, it is also true that many learners do not understand the difference (Ryan, 1985). Table 2 presents some common misconceptions that are easily addressed by instruction about the evolution of *A. mexicanus* blindness, typical language that demonstrates each misconception, and the preferred language.

How then should teachers explain the evolution of blindness in *A. mexicanus*? The evolution of loss of function or structure in general? What misconceptions should we be on the lookout for in student explanations of these processes? How might the case of blindness in *A. mexicanus* be used effectively in introductory and/or AP biology?

Table 2. Selected evolution misconceptions.

Misconception	Typical phrase or statement evidencing this misconception	Preferable language	Counterexample from *A. mexicanus* evolution (or correct concept)
Each trait is influenced by one Mendelian locus.	"the gene for blindness"	Many traits are polygenetic—determined by more than one gene.	Sightedness involves multiple genes.
Individuals adapt to their environment (inheritance of acquired characteristics).	"The cavefish loses its eyesight because . . . "	Over many generations, the frequency of sightedness in a cavefish population decreases to zero because . . . "	Individual *A. mexicanus* fish washed into caves by the most recent rain do not become blind in a single generation.
Natural selection involves organisms trying to adapt.	"The fish are trying to get rid of what they don't need."	Individuals with beneficial traits reproduce more often and leave more offspring.	Evolutionary change in *A. mexicanus* is the result of natural selection, pleiotropy, and genetic drift, not individual effort.
Natural selection gives organisms what they need.	"The fish need to save energy so they lose their eyesight." "The fish lose their eyes because they don't need them."	Individuals with beneficial traits reproduce more often and leave more offspring.	Evolutionary change in *A. mexicanus* is the result of natural selection, pleiotropy, and genetic drift, not in response to need.
The fittest organisms in a population are those that are strongest, healthiest, fastest, and/or largest.	The fittest cavefish are those that are strongest, healthiest, fastest, and/or largest.	The fittest organisms are those that are best suited to survive and reproduce in a given environment.	Sighted fish are the fittest in pools above ground; unsighted fish with enhanced sensory perception, etc., are the fittest cavefish.
Evolution results in progress; organisms are always getting better through evolution.	Evolution strives for perfection. Humans are the peak of evolution.	Natural selection tends to increase the frequency of those mutations that enhance the potential to survive and reproduce.	Evolution in cave *A. mexicanus* results in LOSS of sight (regressive evolution).
Selective "pressures" directly force change in the species to occur.	The lack of light forced the ancestral fish to lose their eyesight.	Natural selection tends to increase the frequency of mutations that enhance the potential to survive and reproduce.	The environment affects the proportion of blind *A. mexicanus* by selecting the individuals most suited to the cave environment.

○ Brief Thoughts on Effective Classroom Use

Although the focus of this article is not on explicit recommendations for instruction, a few suggestions may be helpful. Given how difficult it is to explain the evolution of troglodyte blindness, I do not recommend using the cavefish item as a pre-test or advanced organizer where it could be very discouraging to the students. For the same reasons, I do not encourage use of these items for summative assessment.

Questions about loss-of-function evolution could, however, be used in a wide variety of active learning settings, especially after students have a good understanding of the basics of evolution and can adequately explain the evolution of gain-of-function traits such as cheetah running speed. Generating possible evolutionary explanations of cavefish blindness could, for example, be a very demanding challenge used in the Explain and Explore phases of a 5e lesson (Atkin & Karplus, 1962) in which students are asked to work in small groups to propose creative explanations (perhaps without other resources, the internet, etc.). Teams could then present their proposals to other teams or the entire class to receive feedback, then prepare a report or present a poster on their conclusions. Teachers should look for reasoning that gives evidence of the common misconceptions given in Table 1. Likewise, loss-of-function examples could be used in the Extend phase of a 5e lesson in which the earlier stages involved understanding the basics of evolution.

To enhance interest and motivation, teachers might also present a brief Internet video showing blind *A. mexicanus* (e.g., https://www.youtube.com/watch?v=jOvcB30Yvrg) or invite students to find such videos on their own. Blind *A. mexicanus* specimens are also often available at local pet stores and are relatively inexpensive. Bringing some of the fish into the classroom for direct observation can be more stimulating for students than looking at pictures in a book or even watching videos. For the most fun, take students on a field trip to a large cave with guided tours if one is available close by. Involving students from other classes such as earth science can also make such field trips more feasible. (For students interested in *A. mexicanus* conservation or related topics, see Gross, 2012.)

○ Bonus Activity: Cavefish Blindness and the Nature of Science

Assuming you have addressed the nature of science in a variety of uncontroversial contexts (and that you fostered an atmosphere in which it is "safe" for students to discuss more controversial topics respectfully), the development of blindness in cavefish is an obvious context for further discussion of the nature of science. You might begin your class on the topic with questions such as: Why are most animals that live in caves blind? How do you know why, given that no one was in the cave when they lost their sight? The second question focuses on the difference between evolution and other biological subdisciplines, namely, that evolution is in part a historical science.

Other questions might be fruitful for discussion at the end of the cavefish lesson: What is the goal of science? Does our discussion of cavefish blindness meet that goal? ("Science is an attempt to *explain* natural phenomena" McComas et al., 1998.) What is the difference between observation and inference? What are some examples of each that we have talked about with cavefish? Why is it important in science to distinguish between observations and inferences? Another option is: "Suppose someone told you that they believe that God just created the cavefish blind in the first place? What would be a scientific response? Because science is empirically based on and/or derived from observations of the natural world" (Lederman, 2007, p. 833), a proper response would be that science is non-theistic (not atheistic), and therefore it takes no position on whether or not there is a supernatural power (God) or the actions thereof.

○ Acknowledgments

All photographs are from the public domain unless otherwise noted.

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c. Reviews and updates of recent advances in the life sciences in the form of an "Instant Update" that bring readers up-to-date in a specific area
d. Imaginative views of the future of biology education and suggestions for coping with changes in schools, classrooms and students
e. Other timely and relevant and interesting content like discussions of the role of the Next Generation Science Standards in biology teaching, considerations of the history of biology with implications for the classroom, considerations of the continuum of biology instruction from K-12 to post-secondary teaching environments, contributions that consider the likely/ideal future of science and biology instruction.

Research on Learning (up to 4000 words) includes reports of original research on innovative teaching strategies, learning methods, or curriculum comparisons. Studies should be based on sound research questions, hypotheses, discussion of an appropriate design and procedures, data and analysis, discussion on study limitations, and recommendations for improved learning.

Inquiry and Investigations (up to 3000 words) is the section of *ABT* that features discussion of innovative and engaging laboratory and field-based strategies. Strategies in this section should be original, focused at a particular grade/age level of student, with all necessary instructions, materials list, worksheets and assessment tools, practical, related to either a particular program such as AP and/or linked to standards like NGSS. The most appropriate contributions in this category are laboratory experiences that engage students in inquiry.

Tips, Tricks and Techniques (up to 1500 words but may be much shorter) replaces the How-To-Do-It and Quick Fix articles. This section features a range of suggestions useful for teachers including laboratory, field and classroom activities, motivational strategies to assist students in learning specific concept, modifications of traditional activities, new ways to prepare some aspect of laboratory instruction, etc.

Submission Guidelines

All manuscripts must be submitted online at http://mc.manuscriptcentral.com/ucpress-abt

- Authors will be asked to register the first time they enter the site. After receiving a password, authors can proceed to upload their manuscripts through a step-by-step process. Assistance is always available in the "Author Help" link found in the menu on the left side of the page. Additional assistance is available from the Managing Editor (managingeditor@nabt.org).
- Manuscripts must be submitted as Word or WordPerfect files.
- Format manuscripts for 8.5 × 11-inch paper, 12-point font, double-spaced throughout, including tables, figure legends, and references.
- Please place figures (including photos) and tables where they are first cited in the text along with appropriate labels. Make sure to include figure and table citations in the text as it is not always obvious where they should be placed. At the time of initial submission, figures, tables and images should be low resolution so that the final file size remains manageable.
- If your article is accepted, we will require that figures be submitted as individual figure files in higher resolution form. See below for file format and resolution requirements.
- **NOTE:** Authors should be aware that color is rarely used within the journal so all artwork, figures, tables, etc. must be legible in black and white. If color is important to understanding your figures, please consider alternative ways of conveying the information.
- Authors are encouraged to submit multimedia files. Acceptable file formats include MP3, AVI, MOV, WMV, and FLV.

Editorial Procedures

- Communications will be directed to only the first author of multiple-authored articles.
- At least three individuals who have expertise in the respective content area will review each article.
- Although the editors attempt to make decisions on articles as soon as possible after receipt, this process can take six to eight months with the actual date of publication to follow. Authors will be emailed editorial decisions as soon as they are available.
- Accepted manuscripts will be forwarded to the Copy Editor for editing. This process may involve making changes in style and content. However, the author is ultimately responsible for scientific and technical accuracy. Page proofs will be sent to authors for final review before publication at which time, only minor changes can be made.

continued

Writing & Style Guidelines

The *Chicago Manual of Style, 14th Edition* is to be used in regards to questions of punctuation, abbreviation, and style. List all references in alphabetical order on a separate page at the end of the manuscript. References must be complete and in *ABT* style. Please review a past issue for examples. Use first person and a friendly tone whenever appropriate. Use concise words to emphasize your point rather than capitalization, underlining, italics, or boldface. Use the SI (metric) system for all weights and measures.

NOTE: All authors must be current members of NABT or a charge of $100 per page must be paid before publication.

Several times a year the *ABT* has issues that focus on a specific area of biology education. Future focus issues are published in most issues. The editors highly encourage potential authors to consider writing their manuscripts to align with the future focus topics.

Thank you for your interest in *The American Biology Teacher.* We look forward to seeing your manuscripts soon.

William McComas, Editor-in-Chief, ABTEditor@nabt.org
Mark Penrose, Managing Editor, managingeditor@nabt.org

Preparing Figure Artwork

General Requirements

- When your article is accepted, we will require that figures be submitted as individual figure files in higher resolution format. See below for file format and resolution requirements.
- **NOTE:** Authors should be aware that color is rarely used within the journal so all artwork, figures, tables, etc. must be legible in black and white. If color is important to understanding your figures, please consider alternative ways of conveying the information.

Halftone (photographic) figures

Digital files must meet the following guidelines:

- Minimum resolution of 300 DPI, though 600 DPI is preferred.
- Acceptable file formats are TIFF and JPEG.
- Set to one-column (3.5″ wide) or two-column size (7″ wide).
- If figure originates from a web site, please include the URL in the figure caption. Please note that screen captures of figures from a website are normally too low in resolution for use.

Line art figures

- Minimum resolution of 600 DPI, though 1200 DPI is preferred.
- Acceptable file formats are TIFF, BMP, and EPS.
- Set to one-column (3.5″ wide) or two-column size (7″ wide).

If you have any questions, contact Mark Penrose at managingeditor@nabt.org.

Submitting *ABT* Cover Images

Submissions of cover photographs from NABT members are strongly encouraged. Covers are selected based on the quality of the image, originality, overall composition, and overall interest to life science educators. *ABT* has high standards for cover image requirements and it is important for potential photographers to understand that the size of the cover image generally precludes images taken with cell phones, point-and-shoot camera and even some older model digital SLR cameras.

Please follow the requirements listed below.

1. E-mail possible cover images for review to Assistant Editor, Kathleen Westrich at kmwestrich@yahoo.com.
2. Choose images with a vertical subject orientation and a good story to tell.
3. Avoid cropping the subject too tightly. It is best to provide an area of background around the subject.
4. Include a brief description of the image, details of the shot (i.e., circumstances, time of day, location, type of camera, camera settings, etc.), and biographical information in your e-mail message.
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6. Please ensure that the image meets the minimum standards for publication listed below and has not been editors or enhanced in any way. The digital file must meet the minimum resolution of 300 pixels per inch (PPI)—preferred is 400 PPI— and at a size of 8.5 x 11.25″. We accept TIFF or JPEG images only.
7. For exceptional images, the editors will also accept sharp, clear, color 35 mm slides. Submit only the original; duplicates will not be accepted. Be sure to clearly label your slides with your name and contact information in ink. Contact Assistant Editor Kathy Westrich beforehand to discuss the possibility of submitting a 35mm slide or other non-digital format for consideration as an *ABT* cover.