Undergraduates Learn Evolution Through Teaching Kindergartners About Blind Mexican Cavefish

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INTRODUCTION

The value of scientific outreach, both for investigators and the broader public, is largely self-evident. By moving science beyond the laboratory bench, investigators gain the opportunity to promote their work to a wider audience (1). This type of activity provides public access to sophisticated scientific principles they may otherwise not encounter (2). In this way, scientific outreach is a crucial mechanism through which scientists foster public support for science (3). The value of scientific outreach is also evidenced by several national funding agencies that formally require such activities to promote ‘broader impacts’ of sponsor-supported research (4-5).

Implementing an outreach activity can be difficult owing to (1) the time required to develop and create materials and (2) the personnel necessary to help deliver these activities (6). This paper seeks to address both potential obstacles. First, we describe an approach that fosters undergraduate-level student engagement in both the development and implementation of the outreach activity. Secondly, using our research animal model system (blind cavefish), we describe the implementation of undergraduate-designed activities that engaged kindergarten students in a series of learning exercises.

The overall goal of the outreach activity was to introduce kindergarten students to the blind Mexican cavefish (Astyanax mexicanus) as an example of microevolutionary change. This freshwater fish lives in limestone caves in the Sierra de El Abra of NE Mexico (7). Because this animal is closely related to a river-dwelling form with very different external morphologies and behaviors (8-9), cavefish nicely illustrate many fundamental biological principles of evolution. Our outreach activity focused on the evolved sensory changes between cave and surface fish, the process of microevolution, and the impact of environment on outward appearance (phenotype).

SCIENTIFIC TEACHING THEMES

Active learning

This teaching activity uses a cooperative learning approach. Undergraduates collaboratively worked together to brainstorm ideas for presenting evolutionary differences between cave...
and surface fish to kindergarten students. This brainstorming session was supplemented with direct discussions to increase the students’ background knowledge of the model system. The pre-outreach activities involved direct and creative use of props and crafts to creatively present morphological and behavioral differences to a young, naïve audience. Delivery of the outreach event involved working collaboratively, in teams of two people, to present the material effectively and enthusiastically, provide reinforcement for evolutionary topics throughout, and query outreach participants about the knowledge they gained after completion of the activities.

Assessment
Pre-outreach activities were assessed through informal peer-review (i.e., discussions among the team about each of the outreach topics to be presented). Students’ opinions and feedback about the outreach activity development and delivery were assessed through direct discussions following the event. These opinions can also be collected anonymously using a paper handout or web-based survey.

Inclusive teaching
We ensured inclusion for all undergraduate participants through open discussions as the outreach event was being developed. Through the process of brainstorming, all ideas were accepted and discussed, and each undergraduate student was encouraged to offer ideas. Additionally, all participants respectfully weighed the pros and cons of each idea before the group collectively moved towards construction of outreach materials. We ensured diversity in the implementation of our outreach program by developing outreach activities and props that could optimally foster participation of kindergartners, irrespective of differences in maturity-level, cognition or physical abilities.

How People Learn
To ensure engagement of students with different learning styles, a variety of activities were developed, allowing students to identify a preferred activity for the outreach event. These activities included showcasing morphological, behavioral, and ecological differences between the cave and surface forms of our study system. By presenting the outreach event in pairs, students were able to hone their respective topical strengths, while remaining engaged and involved in the broader evolutionary topics.

HOW FISH LOST THEIR EYES

Preparation of outreach activity and materials

Step One - Brainstorming
This outreach activity was organized and guided as a part of an ongoing outreach program developed by the lead author of this report: the lab head and an assistant professor in Biological Sciences at the University of Cincinnati. This activity was developed with two undergraduate research fellows and would be appropriate for course credit as part of an undergraduate research experience. To effectively deliver compelling and age-appropriate material, the lab head teamed with two undergraduate students to identify a suitable list of topics of interest for kindergarten-level learners. Our team started with a list of ~8 topics, which we refined to the following four activity-based themes: (1) Compare and contrast the ‘cave versus surface’ environmental differences; (2) Use images and crafts to understand how sensory morphologies affect behavior in cavefish; (3) Observe feeding and behavioral differences in cavefish; (4) Perform a “sensory deprivation” activity to model cavefish feeding behavior. Undergraduate students were involved in the conception and refinement of each stage of the development of these four themes. The principal learning objective for undergraduate students was to think creatively about the biology of our cavefish animal system, and to devise ways of presenting these principles in an age-appropriate manner. The two undergraduate students (A. Gangidine, R. Schafer) principally involved in the design, development and implementation of the outreach activity are co-authors on this manuscript.

Following the outreach, one undergraduate author reported that inclusion in the activity provided a very welcome break from the “being lectured to” approach they experienced in much of their undergraduate education. Specifically, one student really appreciated how the process of explaining material to a naïve audience motivated them to understand the material at a more comprehensive and sophisticated level. Both students reported how this created a healthy challenge of imparting their wisdom in a descriptive, creative yet meaningful way that could be interpreted by a kindergarten audience. Additionally, this student appreciated the fact that the outreach program focused on many aspects of cavefish, and cave biology in general. This student reported that their prior undergraduate research experiences required such a depth of knowledge on a focused topic, that it risked “losing the forest for the trees.” This outreach experience, in contrast, helped the student gain an appreciation for the multiple traits co-evolving in cavefish (e.g., both trait losses and gains), as well as the broader subjects of cave biology and evolution.

The other undergraduate author underscored the value of constructing the teaching materials. Specifically, the hours spent creating fish puppets that accurately depicted numerical differences in taste bud number and neuromasts granted the student a new appreciation for how different these organisms are despite their relatively recent evolutionary divergence. Additionally, this student reported that the pairing of morphological differences between cave and surface fish, followed by the use of Velcro dots to illustrate these differences (see below), greatly improved their appreciation for the interactions between expanded morphologies and keener senses. Finally, both undergraduate authors felt that the combined processes of critical thinking, open discussion, trial-and-error, and collaboration lent itself to both an excellent team-building experience, and greatly accelerated their understanding and appreciation for blind cavefish and the concept of evolution.

Step Two - Content Development

Once the brainstorming session identified suitable topics, the team then developed materials for each of the four teaching themes. The four teaching themes are summarized below, with a description of the content that was developed to support the learning goals of each activity.

1. Compare and contrast the ‘cave versus surface’ environmental differences.

Two activities were developed for this section.

- (A) Fish puppets: Cave and surface fish “puppets” were created by mounting several images of cave and surface fish
on a foam board and trimming the excess board (Figure 1). We used high quality, glossy paper to increase durability. Each fish puppet was glued to a wooden popsicle stick. We made enough puppets to provide a pair of cave and surface fish to each kindergartner learner. The entire kindergarten class for whom we initially developed our outreach included a total of 24 students. This class size was subdivided into two groups of 12 students each. Therefore, a group of 12 was the “typical” group size for the activities described below, with the exception of the final activity in which students were “blinded” (see below).

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• During the introduction to exercise #1, we compared and contrasted morphological differences between cave and surface fish. These “puppets” allowed quick evaluation of external differences in appearance between cavefish and surface fish during the introduction to exercise #1.

• Cave and surface environment poster: We developed a “compare and contrast” activity that featured habitat and fish characteristics. To represent the habitat, we printed a 20” X 30” poster showing four photographs of the cave environment on the left and four photographs of the surface environment on the right (Figure 2, page 4). To encourage engagement by the kindergartners, we developed five “contrast” points between the two environments using pictures and words mounted to foam poster board and trimmed to size. These points included ‘light’ (present in surface, absent in cave), ‘plants’ (present in surface, absent in cave), ‘predators’ (present in surface, absent in cave), ‘eyes’ (present in surface, absent in cave), and ‘bats’ (a more common feature in the cave). In addition, we showed the children mounted images of cave and surface fish and asked them which animal lived in the cave versus surface environment. The children volunteered to temporarily attach each “contrast” point to the poster board using clear Velcro attachments.

(2) Use of images and crafts to understand how sensory morphologies impact behavior in cavefish: Two activities were developed for this activity.

• Cavefish and surface fish “image booklets”: The first activities illustrated key morphological differences between cave and surface fish, as well as between the cave versus surface environment (Figure 3, page 4). The next activity examined the differences between surface fish and cavefish in detail. The image booklets consisted of a set of paired images showing: (a) a photograph of the fish head in profile + cranial skeletal image (Figure 3A,B,F,G); (b) a photograph of the fish head in profile + transparent overlay of taste buds (Figure 3C,H); (c) a photograph of the fish head in profile + transparent overlay of sensory neuromasts (Figure 3D,I); and (d) a light image of the fish head in profile + transparent overlay of iridescence (“silvery” pigmentation; Figure 3E,J). The use of transparent overlays on images a, c, and d, facilitated the students’ comprehension of morphological differences between the two fish, despite the fact that these morphologies are typically not visible to the naked eye.

• Interactive sensory puppets: The second activity used high-resolution images of the lateral head of cave and surface fish. These images were mounted on foam poster board and clear Velcro hook tags were attached to the positions on the head where taste buds would be found (Figure 4A-D, page 5). Surface fish have far fewer taste buds compared to cavefish (10); therefore, the domain of these sensory organs was limited to the lips. In contrast, cavefish have a dramatically expanded domain (as well as number) of taste buds, including virtually the entire dorsal and ventral regions of the head (11). This distribution was mirrored in the distribution of Velcro hooks. A second set of sensory puppets was also developed for neuromasts (Figure 4E-H). Neuromasts are mechanosensory hair cell organs found along the head and flank of many types of fish and amphibians. They play a role in detecting water movements, schooling behaviors, and in finding prey. Similar to taste buds, sensory neuromasts are greatly expanded in number and distribution in cavefish compared to surface fish (12-13). During the demonstration, the expansion of sensory organs in cavefish was illustrated using colored “pompoms” designed to model either enhanced taste sensation (pink and orange pompoms) or enhanced sensitivity to water movements due to neuromasts (blue pompoms). These pompoms (purchased at a local craft store) stick readily to the Velcro regions of the head indicating position and density of taste buds and neuromasts.

(3) Participate in a live demonstration to observe feeding and behavioral differences in cavefish: This teaching theme was designed to provide real-life evidence for behavioral and feeding differences between cave and surface fish (13-15). This demonstration required two tanks of fish, transported to the outreach activity in plastic tanks. These animals were transported in IACUC-approved animal containers. We also brought sufficient fresh system water (~3 gallons) and spare containers, as a precaution, to facilitate fresh water changes.
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Figure 2. A ‘cave versus surface’ poster illustrates differences between the cave and surface environment to outreach audience members. A large composite image composed of four panels of the cave (left column), and four photographs of the surface environment (right column) encouraged engagement from the audience through the use of “contrast” points. These points were added following discussions and queries between the audience and the outreach event leader. Shown is the poster before the activity (“pre-activity”; A) and after completion of the activity (“post-activity”; B).

Figure 3. Cavefish and surface fish “image booklets” illustrated key morphological differences between surface fish (A–E) and cavefish (F–J) that were not visible to the naked eye. The books included paired images of the fish head in profile (A, F), a cranial skeletal image (B, G), an overlay of taste buds (C, H), an overlay of sensory neuromasts (D, I), and an overlay of iridescent pigmentation (E, J).
### Taste Bud Activity

| Pre-Activity | Post-Activity |
|--------------|--------------|
| Surface fish | C            |
| Cavefish     | D            |

### Neuromast Activity

| Pre-Activity | Post-Activity |
|--------------|--------------|
| Surface fish | E            |
| Cavefish     | F            |

**Figure 4.** Interactive sensory “puppets” illustrated differences in sensory morphology between surface fish and cavefish. The presence of taste buds (A–D) and neuromasts (E–H) were indicated by the distribution of clear Velcro hooks on a set of sensory “puppets”. Sensory organ expansion in cavefish were illustrated using colored “pompoms” to model enhanced taste sensation (pink and red pompoms symbolized food; A–D) or enhanced sensitivity to water movements due to neuromasts (blue pompoms symbolized water; E–H). One can compare these puppets before (“pre-activity”; A, B, E, F) and after (“post-activity”; C, D, G, H) the activity to appreciate how regions of the head in cave and surface fish harbor variation in the position and densities of taste buds and neuromasts.

### Feeding Behavior Activity

**A** Surface fish

**B** Cavefish

**Figure 5.** A live feeding demonstration provided real-life evidence of behavioral differences between surface and cavefish. This demonstration involved two ~8-gallon tanks of fish, holding 6–8 commercially obtained surface fish (A) or cavefish (B). During this learning activity, live California black worms were fed to each tank of fish to illustrate contrasting feeding behavior between surface and cave morphs.
and animal isolation if an animal appeared distressed or unhealthy. Exposure to these animals presents a minimal threat to the health of children. However, for the safety of the children and the fish, only members of the outreach team handled the fish. Each ~8 gallon tank held 6–8 commercially obtained cavefish or surface fish. To facilitate this learning activity, we also brought a 50 mL conical plastic vial containing live California black worms and a plastic Pasteur pipet used to transfer the worms into the tanks for the feeding exercise (Figure 5). A ventilated cloth cover was placed over the fish tanks in order to avoid distraction for the children during prior exercises.

(4) Perform a “sensory deprivation” activity to model cavefish feeding behavior: This activity was designed to model cavefish reliance on other senses to find food, given the loss of a visual system. The kindergartner learners would be “deprived” of vision using oversized sunglasses that had been painted black to “blind” them. While wearing blackout sunglasses, the children would respond to “waves.” In this demonstration, the children moved in the direction of sound waves, rather than disturbances in ambient water, to find their “food.” Oversized plastic sunglasses were purchased at a local toy store (51), along with a toy duck call that was used to create a sound. Owing to other ambient noise in the classroom, we kept the sound localized to one place in the room (i.e., the sound source did not move). During this activity, only three children were “blinded” at a time to optimize their chances of success in finding the sound source, and to ensure we maintained an organized classroom. “Blinded” students were then spun around three times before asking them to find the source of the sound. During the activity, the rest of the children sat in a circle as spectators. Once the children successfully moved in the direction of the sound wave, they received a reward as a proxy for finding food in the darkness of the cave. For our activities, we provided each child two pink-colored gummy fish candies (these candies looked like albino cavefish!). To ensure inclusion for students with nut allergies, we strictly avoided candies processed in a facility that also handled nuts. Additionally, we checked with the classroom teachers and parents of the children in the class, to ensure handing out candy would not be problematic for anyone. As an alternative, several non-food options (e.g., small fish toys) can be substituted as a “reward” at the end of the activity.

Part Three - Implementation of Outreach Activity to Kindergartner Learners

At the introduction of the outreach activity, the lead instructor introduces themselves and the rest of the research team to the kindergartner children. At this point, the kindergarten teacher is asked to divide the class (~24 students total) into two equal-sized groups. The smaller groups then move sequentially through each of the following three activities, and the class members rejoin for the final activity.

Activity 1: Compare and contrast the ‘cave versus surface’ environmental differences

Number of kindergarten students: ~10 – 12; (Approximate time ~10 minutes). This kindergarten class was selected based on an established relationship between the lead author and Mariemont Public Elementary School, located in eastern Cincinnati. After consulting with two kindergarten teachers and the school principal, we determined that the final week of school (i.e., ~last week of May) was the optimal time to deliver this outreach activity. The teachers recommended delivering the outreach activity after second recess, which followed lunch, around 1:00pm in the afternoon.

Materials needed: (A) fish puppets, and (B) ‘Cave and Surface Environment’ poster

Learning environment: Children are seated on the floor facing the lead instructor who is holding the ‘Cave and Surface Environment’ poster. One undergraduate student leads the discussion while the other undergraduate student sits down with the kindergartner students to help facilitate discussion.

Scenario: As students enter into the learning environment, they are each handed one cavefish puppet and one surface fish puppet. The lead instructor is seated facing the children with the environment poster.

Lead Instructor: “By a show of hands, who thinks that these two fish look different from one another?”

(Children evaluate their puppets, many raise their hands)

Lead Instructor: “OK, who can tell me one way that these fish appear different from one another?”

(Instructor and undergraduate students call on children who brainstorm differences: e.g., cavefish do not have eyes or pigmentation)

Lead Instructor: “Excellent. Now, in order to understand why these fish are so different from one another, we have to explore their environments.” (Instructor points to the environment poster) “On this poster, we have two columns, on the left side are pictures from a cave and on the right side are pictures from a river. To start, show me which fish you think lives in the surface river.”

(Many children raise their surface fish puppet. Kindergarten students volunteer or are encouraged to affix surface fish to the poster)

Lead Instructor: “Excellent. Now, show me which fish lives in the cave?”

(Children raise their cavefish puppet. Kindergarten students affix cavefish to the poster)

Lead Instructor: “Why do you think this fish is the one that lives in the cave?”

(Children reference the fact that the cavefish do not have eyes)

Lead Instructor: “Now, when you look at these two environments – what is one big difference you see?”

(Children often first reference the fact that the surface river is lighted, while the cave has no light. Kindergarten students affix the sun picture and “no light” picture to the poster)

Lead Instructor: “Good. The surface has light, but the cave...
has no light. Do you think this has any effect on plants?”

(Children reference the fact that the surface environment has a lot of vegetation, while the cave has no vegetation. The lead instructor facilitates discussion around how absence of light does not permit plants to grow. Kindergarten students affix the plant picture and “no plants!” picture to the poster)

Lead Instructor: “Good. Now who knows what a predator is?”

(Children describe a predator – e.g. an animal that eats another animal)

Lead Instructor: “Which one of these animals do you think has a predator?”

(Children raise the surface fish puppet. Kindergarten students affix the hawk picture and “no predators!” picture to the poster. The lead instructor facilitates discussion around how hawks will prey on surface fish, but cavefish do not have any predators)

Lead Instructor: “Now, we have talked about a lot of things that are present in the surface environment. Can anyone think of something that is present in the cave, but not the river?”

(The undergraduates lead a discussion – children generally reference the fact that bats are present in the cave, but not the river environment. Kindergarten students affix the bat picture to the poster. Instructor and undergraduate students lead discussion around how bat droppings are one of the principal food sources for the cavefish in the wild)

Lead Instructor: “Now, look at your fish puppets. What is the most obvious difference between the cave and surface fish?”

(Children reference the fact that the cavefish do not have eyes. Kindergarten students affix the eye picture and “no eyes!” picture to the poster)

Lead Instructor: “Great job. Let’s review the differences between the cave and surface environments.” (Undergraders lead discussion by reviewing the differences between cave and surface, finishing with a reinforcement of the fact that cavefish have no eyes)

Lead Instructor: “As you have discovered, one of the clearest differences is that cavefish do not have eyes. In the next activity, you learn that although they do not have eyes, the cavefish are very strong in other senses.”

(Children transition to the next activity)

**Activity 2: Use images and crafts to understand how sensory morphologies impact behavior in cavefish**

**Number of kindergarten students:** ~10 – 12 (Approximate time ~10 minutes)

**Materials needed:** (A) cavefish and surface fish “image booklets” and (B) interactive sensory “puppets”

**Learning environment:** Children sit on the floor facing the undergraduate student, who will guide the discussion. In the first half of this activity, one set of kindergarten students hold the cavefish “image booklet”; while another set of kindergarten students hold the surface fish “image booklet”. In the second half of this activity, kindergarten students perform a demonstration of interactive sensory puppets for their respective morphotype by placing the Velcro-coated fish puppets in a shallow container filled with the colored pom-poms.

**Scenario:** Students transition into the learning environment, and take a seat in a semi-circle facing the two undergraduate student assistants.

Lead Instructor: “What did you learn in your first activity?”

(With prompting and guidance from the undergraduate students, the children describe differences between the cave and surface environments. The children also reference the fact that the cavefish do not have eyes)

Lead Instructor: “You learned that there are many differences between the way cavefish and surface fish appear, and that these differences are associated with living in the cave or the surface environment. You also learned that cavefish do not have eyes. Do you think the cavefish can still find food though?”

(Children exclaim, “yes!”)

Lead Instructor: “How can the cavefish find food if they don’t have any eyes?”

(Either through guidance from the undergraduate students, or independently, the children will usually suggest that they have other senses that have compensated for their lack of eyes)

Lead Instructor: “Very good, the cavefish have other senses that have become stronger after having lived in the caves for so long. The problem is that – unlike loss of eyes – you can’t see these sensory changes very well without a microscope. In this activity, we are going to show you some more differences between the cave and surface fish.”

(A set of kindergarten students hold either the cavefish or surface fish “image book”. Facing the rest of the children, they open their books to the same page, showing a high-resolution glossy image of the lateral fish head)

Lead Instructor: “Let’s explore whether these fish have different skull shapes.”

(The kindergarten students flip a page to a digital image of the cranial skeletons of each fish)

Lead Instructor: “Compare these two skull images. What are the differences you see between the cavefish and surface fish?”

(Either with guidance, or independently, the children will usually suggest that the orbit of the eye is misshapen in cavefish owing to the loss of an eye. The undergraduate students can also refer to evidence of bony fusions and fragmentations evident in the cavefish skull)
Lead Instructor: “Good, next let’s look at neuromasts. Neuromasts are sensory hair cells on the face and body of fish and some amphibians that allows them to sense water movements.”

(The kindergarten students flip a page to a glossy light image of the fish heads. They both turn a transparent page that shows the position of neuromasts on the lateral head)

Lead Instructor: “Normally you cannot see neuromasts without a microscope, so we are showing you where they are on the fish head with these purple colored dots.”

(Undergraduate students point to the positions of purple dots indicated by a spot of purple glitter glue)

Lead Instructor: “What are some differences that you see between cavefish and surface neuromasts?”

(The children describe how the cavefish have many more neuromasts than surface fish)

Lead Instructor: “Why would the cavefish have more neuromasts than surface fish?”

(With guidance and prompting, the undergraduate students help the children conclude that more neuromasts help the cavefish detect smaller water movements in the cave where they live; these smaller water movements are probably made by little crustaceans that the fish will prey upon)

Lead Instructor: “Very good. In a moment, we will illustrate how having more neuromasts actually makes the cavefish more sensitive to water movements. Next, we will look at taste buds. Taste buds help us find food that is safe or unsafe to eat.”

(The kindergarten students flip a page to a glossy light image of the fish heads. They both turn a transparent page that shows the position of taste buds on the lateral head)

Lead Instructor: “Just like neuromasts, you cannot see taste buds without a microscope, so we are showing you where they are on the fish head with these pink colored dots.”

(Undergraduate students point to the positions of pink dots indicated by spots of pink glitter glue)

Lead Instructor: “What are some differences that you see between cavefish and surface fish taste buds?”

(The children describe how the cavefish have many more taste buds than surface fish. While surface fish have taste buds only around their lips, cavefish have taste buds extending all along their dorsal skull and ventral jaws)

Lead Instructor: “Why would the cavefish have more taste buds than surface fish?”

(With guidance and prompting, the undergraduate students help the children conclude that more taste buds allow the cavefish to detect food very sensitively in the cave. At this time, the lead instructor can reinforce the fact that these two sensory adaptations are very important because there is very little food in the cave compared to the surface)

Lead Instructor: “Okay, the last thing we will look at is something called ‘iridescence’. Iridescence is the silvery or “shiny” appearance we often see in fish. Let’s compare between the cavefish and surface fish.”

(The kindergarten students flip a page to a glossy light image of the fish heads. They both turn a transparent page that shows the relative “shininess” of cave and surface fish using glitter flakes)

Lead Instructor: “What are some differences that you see between cavefish and surface fish?”

(The children describe how the cavefish have less “shininess” (iridescence) than surface fish)

Lead Instructor: “Why do you think the cavefish are less shiny than surface fish?”

(With guidance and prompting, the undergraduate students brainstorm different ideas. One suggestion is that the cavefish have a number of pigmentation changes (less brownish color, less iridescence, etc.), and these changes may be due to the fact that the cavefish can’t see one another. Therefore, the coloration appearance may not “matter” and the fish lose pigmentation over time)

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(With guidance and prompting, the undergraduate students brainstorm different ideas. One suggestion is that the cavefish have a number of pigmentation changes (less brownish color, less iridescence, etc.), and these changes may be due to the fact that the cavefish can’t see one another. Therefore, the coloration appearance may not “matter” and the fish lose pigmentation over time)
(Undergraduate students show the children the interactive taste bud puppets)

Lead Instructor: “On these puppets, we placed Velcro everywhere you normally see taste buds in cave and surface fish. Let’s have the cavefish and surface fish start sensing the ‘tastes’ that surround them (these tastes are represented by orange and red pompoms).”

(Kindergarten students are invited to place the puppets, face down, into a container filled with fuzzy orange and red pompoms)

Lead Instructor: “Which fish do you think will ‘detect’ more food?”

(The children usually provide diverse predictions. The kindergarten students reveal the results of the activity. Surface fish will have fewer orange/pink pompoms on their face compared to cavefish)

Lead Instructor: “Why do cavefish have more pompoms?”

(With prompting and guidance, the children conclude that cavefish have more orange/pink pompoms because they are more sensitive to food items in the water. Similar to neuromast expansion, owing to little amounts of food in the cave, cavefish must find any safe food to eat quickly in order to survive)

Lead Instructor: “Now that we have learned about these differences between cave and surface fish, let’s take a look at some live fish. As we move to the next station, think about how these fish behave differently, in their different environments.”

(Children transition to the next activity)

**Activity 3: Participate in a live demonstration to observe feeding and behavioral differences in cavefish**

**Number of kindergarten students:** 10 – 12

**Materials needed:** Two 8 gallon tanks, each holding ~6–8 cavefish or surface fish

**Learning environment:** Children stand around a table with a tank of cavefish and a tank of surface fish. The lead instructor facilitates discussion while each undergraduate student leads feeding either the cavefish or the surface fish. In the past, we have not allowed the kindergarten students to feed the fish. The reasons are: 1) it is important for the health of the fish that they do not receive too much food at one time. Too much flake food or black worms can ‘dirty’ the water and lead to an increase in nitrate levels which can cause discomfort for the fish; 2) in our experience, children of this age are too young to accurately control the amount of food expelled out of the plastic Pasteur pipets; and 3) having an undergraduate perform the feeding frees the kindergarten students to focus solely on the behavioral differences between cave and surface fish.

This species of fish is a very robust feeder, so starvation or restricted feeding is unnecessary. Cave and surface fish show robust feeding and foraging behaviors, albeit with observable differences in this behavior between morphotypes.

**Scenario:** Students transition into the learning environment, and take a seat around a table where the two tanks reside. The two undergraduate students are standing near the tanks, both of which are covered with a light cloth.

Lead Instructor: “In the first two activities, you learned about differences in the environment and sensory systems of cavefish and surface fish. In this activity, we will see how these differences affect how the fish behave in real life.”

(The undergraduate students lift up the cloth covering on both the surface fish and cavefish tanks. To reinforce the connection between environment and appearance from the first activity, each tank has a photograph of the cave environment or surface environment behind the cavefish and surface fish tanks, respectively)

(With prompting and guidance from the undergraduate students, the children discuss how the fish tend to stay near one another (“shoaling”), and they demonstrate directed swimming (“schooling”). Schooling can generally be stimulated by waving a hand next to the tank)

Lead Instructor: “Who can tell me which tank has cavefish?”

(The kindergarten students raise their hands and identify the appropriate tank)

Lead Instructor: “Very good, and why do you think these are cavefish in this tank?”

(The kindergarten students provide a number of reasons, with prompting and guidance from the undergraduate students, reinforcing what they learned based on photographs and puppets in the first two activities)

Lead Instructor: “Let’s start observing the cavefish behavior. Who can describe for me how these fish are swimming?”

(With prompting and guidance from the undergraduate students, the children discuss how – unlike the surface fish – cavefish demonstrate an absence of shoaling and schooling. At this time, the students may have several questions relating to the appearance and behaviors of the fish. Therefore, this represents an opportunity for unstructured observation and allows the students to think creatively and ask further questions)

Lead Instructor: “Let’s take a look at how well the cavefish can find food.”

(The undergraduate students use a plastic pipet to deliver several live black worms into the cavefish tank. Cavefish will quickly swim around – usually to the bottom of the tank first – and then towards the worms to feed)

Lead Instructor: “How did the cavefish do? Did they find the food as quickly as you would have guessed?”

(Kindergarten students are generally surprised by how quickly the cavefish are able to find food despite their lack of vision. With guidance from the undergraduate students, the children discuss how the cavefish likely found the food faster than predicted because their expanded senses compensated
for the absence of vision)

Lead Instructor: “Now, let’s look at the surface fish. Who can describe for me how these fish are swimming?”

(With prompting and guidance from the undergraduate students, the children discuss how the surface fish demonstrate an shoaling and schooling behaviors)

Lead Instructor: “Very good. Now, let’s feed the fish and observe their behavior.”

(The undergraduate students use a plastic pipet to deliver several live black worms into the tank. Surface fish will quickly swim around – sometimes to the top of the tank first – and then towards the worms to feed)

Lead Instructor: “Notice how fast the surface fish found the worms. What senses do you think these fish used to find their prey?”

(With prompting and guidance from the undergraduate students, the children discuss how the surface fish likely used their senses of smell, taste and vision)

Lead Instructor: “Very good. The surface fish relied on their vision to find the food. In contrast, how do you think the cavefish found the worms so well even though they do not have eyes?”

(The children may have a variety of answers. Some may predict the cavefish will find food slower because they do not have eyes. Some may explain that the cavefish found the worms so well because of their higher numbers of taste buds and neuromasts)

Lead Instructor: “Very good. Now we will transition to our final activity where you get the opportunity to live like a cavefish!”

Activity 4: Perform a “sensory deprivation” activity to model cavefish feeding behavior

Number of kindergarten students: 20 – 24

Materials needed: Oversized sunglasses with blackout lenses (or another type of effective blindfold); duck call or whistle; rewards for children (small toy or approved [non-allergenic] candy)

Learning environment: The entire classroom reunites for the final activity. Children are seated on the floor facing the undergraduate students with sufficient space for the children to move about the room. Each undergraduate student helps facilitate the activity by standing several feet to the right and left of the lead instructor with a duck call.

Scenario: Students transition into the learning environment, and take a seat in front of the lead instructor and undergraduate student assistants.

Lead Instructor: “Can anyone tell me something that we’ve learned about cavefish today?”

(With prompting and guidance from the undergraduate students, the children describe what they have learned. The undergraduate students can guide this activity as a means of reinforcing material learned in the first three activities. These include discussions of differences in the surface and cave environments [activity #1], expansions of the non-visual sensory systems [activity #2] and differences in swimming and feeding behavior between cave and surface fish [activity #3])

Lead Instructor: “Very good. Now it is time for you to behave like cavefish. The first thing we need to do is take away your vision. The way we will do this is by having you wear these sunglasses.”

(One of the undergraduate students blows through the duck call loudly)

Lead Instructor: “Remember, cavefish do not rely on their vision – they rely on their other senses. So, you need to behave like a cavefish and move in the direction of the sound waves (or duck call) in order to catch your food!”

(Along with organizational support from the teacher, children are selected in groups of three. The three students stand arms-length apart. The instructor and undergraduate students ‘spin’ the children around once. One of the undergraduate students then blows the duck call. The classroom teacher and undergraduate students support the children in finding the “food”)

Lead Instructor: “Very good! You were able to rely on other senses besides vision to find your food!”

(One of the undergraduate students is done. The activity continues until each child gets a turn)

Lead Instructor: “Wonderful job, everyone. You all did a great job today paying attention and learning about cavefish and why they look and act so different from surface fish. Thank you for allowing us to come into your classroom and share our love of these animals.”

(After an optional final question/answer session, the outreach activity ends)

SUGGESTIONS FOR DEVELOPING AND ADAPTING OUTREACH ACTIVITIES FOR OTHER ORGANISMAL SYSTEMS

(1) Know your audience. A successful outreach activity will present and communicate scientific principles to the audience in an understandable and accessible manner (16). For this reason, it is essential that careful consideration be given to the intended audience (17). Specifically, it is important to
consider the background knowledge, attention span, and educational level of audience members when developing teaching materials (18). Classroom teachers can be an excellent resource for helping determine the correct level of sophistication for an outreach event. The content and delivery of outreach materials should be frequently examined to ensure they align well with the target audience.

(2) Develop hands-on activities. Because outreach events are generally short and delivered to a given audience once, it is crucial that hands-on activities are developed (19). Although question-and-answer activities are essential for reinforcement of material, and helping the facilitators test comprehension, they should not be solely relied upon. Rather, creative and engaging hands-on activities that allow the students to interact with the material will have the strongest, and most lasting, impact (20).

(3) Be creative. There are many ways to communicate an interesting scientific principle. This is especially important during the brainstorming phase, when diverse ideas should be encouraged and explored. The creative use of images, crafts and activities brings the excitement of science to the public in an effective and memorable way (21).

(4) Focus on your main message(s). Depending on the length of an outreach activity, it will be important to select the proper amount of content (22). Once determined, it is key to select three or four main points that the audience should understand at the conclusion of the outreach event. Keep these key points in mind throughout the presentation, and use reinforcement and referencing to ensure the audience focuses their attention on these points.

(5) Use reinforcement. The content of an outreach event should be developed so that the underlying material can be reinforced throughout the presentation. This method of reinforcement will be very helpful for ensuring that the key points of the presentation are received (and understood) by the audience (23). Further, since it aids learning, reinforcement will ensure that the students get the most out of an outreach activity, even though it is a time-limited event. In future outreach events, we are developing small items (cavefish-themed keychains, stickers, or small toys) that will provide a fun take-away item to help the children remember the outreach activity.

**Active Learning - Undergraduate Students**

Undergraduate students participating in the development of this outreach activity exercised several scientific process skills via the following active learning approaches. The undergraduate students exercised a spectrum of Bloom’s Cognitive Levels (24), including ‘Foundational’ (i.e., the development of factual knowledge and comprehension of cavefish biology); ‘Application and Analysis’ (i.e., assimilating information and developing physical props); and ‘Synthesis/Evaluation/Creation’ (i.e., the development of a set of outreach activities that collectively advance kindergarten-level learners’ knowledge of cavefish biology).

(1) Brainstorming: To effectively brainstorm outreach activities showcasing the study system, undergraduate students had to first review the prior research. This review provided the opportunity to (a) develop ideas for which morphologies and behaviors to showcase, and (b) create a solid foundation of knowledge to help answer questions for the kindergarten students throughout the activity (25).

(2) Collaborative Work: Collaborative work included brainstorming, designing vignettes showcasing cavefish morphologies and behaviors, and the development of physical props. Collectively, this work required extensive collaboration between the lead instructor and the undergraduate students (26). In this sense, the creation of the overall activity was analogous to designing and conducting a series of “experiments.” These “experiments” eventually developed into the activities delivered to the kindergarten students.

(3) Physical Model: The outreach activity required the development of diverse sets of props to showcase different aspects of cavefish biology. In addition to requiring brainstorming and collaborative work, the undergraduate students developed skills in displaying and modeling information.

**Active Learning - Kindergarten Students**

Kindergarten students participating in this outreach activity similarly exercised scientific process skills via the following pedagogical approaches. The kindergarten students exercised Bloom’s Cognitive Levels (24) of ‘Foundational’ (i.e., learning about fundamental differences between cave and surface fish); ‘Application and Analysis’ (i.e., understanding the connection between increased numbers of neuromasts and better food-finding); and ‘Synthesis/Evaluation/Creation’ (i.e., synthesizing information from the first three demonstrations and modeling cavefish behavior during the final exercise).

(1) Brainstorming: Through the use of guided discussions, Kindergartners were encouraged at every activity to brainstorm possible explanations of several aspects of cavefish biology (27). By design, each activity required the kindergarten students to gather information, make observations, and predict outcomes (e.g., “which fish will find food faster?”). The final behavioral task allowed the students to model cavefish behavior, which required that they interpreted prior information correctly in order to perform the tasks.

(2) Strip Sequence: The first activity, showcasing environmental differences between cave and surface fish, represented a modified strip sequence activity. Specifically, it required the students to focus their attention on key differences between the cave and surface fish. Throughout all of the activities, Kindergartners were asked to explain their answers, providing the opportunity to communicate their findings.

**Assessment - Undergraduate Students**

The undergraduate students were informally evaluated, during the outreach development, through discussions with the lead instructor. Through the use of rehearsals before the outreach event, the undergraduate students were also able to self-evaluate their knowledge of the material. As a component of this rehearsal, the lead instructor would “quiz” the undergraduate students prior to the activity focusing on questions that may arise from the kindergarten students.

**Assessment - Kindergarten Students**

Kindergarten students were informally queried throughout the exercises. This included a series of short answer questions, which were targeted both to individuals as well as the entire group. The environmental activity required the students to place items in the proper context (i.e., a modified “strip sequence”). All students were encouraged to participate in the broader discussion, respond to reflection prompts, and solve
problems (e.g., “how does the cavefish find food?”) throughout the exercise.

Inclusive Teaching - Undergraduate Students
A key goal of this teaching activity was to enable any undergraduate student—irrespective of gender, race, knowledge level, or college year—to participate in the development of an outreach event (28). The inaugural presentation of this outreach activity included a first-year undergraduate female and a fourth-year undergraduate male. The lead instructor encouraged participation and input from every team member throughout the development and implementation of the outreach event.

Inclusive Teaching - Kindergarten Students
Kindergarten students were the audience for this outreach activity, which was designed to advance scientific engagement beyond the classroom. The development of teaching materials was carefully constructed to match knowledge and attention levels of this age group. Therefore, the outreach event was designed to be inclusive of kindergarten level learners. Owing to the biology of our animal system, this activity focused on multiple sense modalities including sight (versus absence of sight), touch (versus enhanced touch sensitivity), and taste (versus taste enhancement). To promote diversity in science, during implementation of the outreach activity all students were encouraged to participate and received accolades for taking part. Moreover, the entire outreach event was inclusive of several other lab participants, providing diverse role models to kindergarten learners in terms of gender, race and age levels.

DISCUSSION
The outreach teaching tools presented here were designed to enable other investigators to move their science beyond the lab bench, and to a broader public audience. This teaching tool is both practical, since it provides a step-by-step guide for generating an outreach event, and efficient. The efficiency of this tool is that it fosters engagement of the public in science, as well as the inclusion of undergraduate students in development and implementation of the event. In this way, outreach can provide an expanded outlet for the science being conducted in our labs, but also represents a powerful learning tool for target audiences and undergraduate students alike (29). By focusing on both aspects of learning, investigators can make substantial impact both within their labs and in contemporary society.

SUPPORTING MATERIALS
• S1.Blind cavefish-Image of oversized ‘black-out’ glasses

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