# CAN FRESHWATER DARTERS (*PERCINA*) PASS THE MIRROR TEST?


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In 1970, the first application of the mirror test (also called the "mark test") was performed on Chimpanzees. In the mirror test, the subject is given access to a mirror and allowed to interact with its reflection. The mirror is then removed and a mark is placed on the subject's body somewhere it cannot see – on the Chimpanzees, marks were placed on the forehead and the ear. The subject is once again given access to the mirror. If the organism interacts with the mark in some way (typically by touch), we can extrapolate that they identified it as unusual. Therefore, they "pass" the test and are considered to be self-aware. The mirror test was hugely influential and became a touchstone of studies on animal intelligence and behavior. It inspired similar studies on many more primates and eventually non-ape species: Bottlenose Dolphins, Asian Elephants, and Eurasian Magpies have all "passed" the mirror test. Though the mirror test was never without criticism, researchers continue to return to it as they explore concepts of self-awareness and intelligence.

In 2019, Kohda and colleagues (Kohda et al. 2019) published a study suggesting that the Cleaner Wrasse *Labroides dimidiatus*—a small saltwater fish—is capable of passing the mirror test. In their test, they created marks by injecting fish with Visible Implant Elastomer (VIE), a type of tag injected just underneath the skin to create a small colored mark. According to the authors, the fish saw the VIE mark in the mirror and attempted to scrape it off by rubbing against substrate. This paper made waves, especially among those not familiar with fish cognition. Fishes are frequently underestimated, but they are much more intelligent than people often give them credit for. They possess a sophisticated cognition system and substantial memory skills. They have complex social interactions, including the ability to cooperate with others. A fish's senses are sophisticated and, in many cases, stronger than our own. When comparing the diverse skillset of fishes to that of primates, there are fewer differences than one might expect. It is not unreasonable to suggest the cognitive ability of fishes might in some way be comparable to mammals. Still, this is the first time that a fish has ever passed the mirror test, which is no small feat. This development led us to wonder: are other fishes capable of this, too? We set out to perform the mirror test on two species of freshwater fish.

The following experiments were conducted by 19 undergraduate students enrolled in introductory biology for majors at the

Photos by the authors.

Scott Nelson is the lead author of this study and an undergraduate student researcher and lab assistant at Emporia State University with an interest in ecology and zoology. Scott is a dual major in biology and art; when they're not in the lab, they can be found in the printmaking studio. Dr. Erika Martin is an Assistant Professor of Biology and Biology Education at Emporia State University.

The rest of the authors are introductory biology students, primarily freshman biology majors, plus an additional undergraduate researcher. These experiments were integrated into the classroom; through this project, students gained hands-on experience in scientific research.

**Figure 1. Collecting fishes from the Neosho River.**

**Figure 2. Ozark Logperch (top) and Slenderhead Darter (bottom) with orange VIE marks.**

university level plus two undergraduate research assistants under the advisement of a professor.

Darters (Percidae) are small, sometimes brightly colored freshwater fishes native to North America. We selected Percidae for their assumed ability to see color. Many species of Percidae use color as a mechanism for mate choice; therefore, they would theoretically be a good fit for a mirror test with colorful VIE marks. We used two species of darters in the genus *Percina,* Ozark Logperch *P. fulvitaenia* and Slenderhead Darter *P. phoxocephala*, to see if these freshwater fishes could pass the mirror test.

Five Ozark Logperch and seven Slenderhead Darter were collected from the Neosho River (38.426203, -96.171907) and Cottonwood River (38.385914, -96.181818) near Emporia, Kansas, USA (Figure 1). Fishes were housed in individual microcosm aquaria. The room the microcosms were housed in was kept on a consistent 9-hour photoperiod. Fishes were fed a diet of bloodworms and brine shrimp on alternating days. All fish housing, handling, and care was performed in compliance with Public Health Service policy and the Guide for the Care and Use of Laboratory Animals.

First, we sought to quantify whether unusual fish behavior was due to mirror recognition or simply the introduction of an unusual object. Each microcosm was haphazardly assigned either a real mirror or a fake mirror made of black plastic. Fishes were injected laterally with orange VIE tags prior to the beginning of the experiment (Figure 2). Additionally, we placed a single large rock in each microcosm to enhance the tank environment and provide a small amount of shelter. Fishes were observed four separate times over 14 days (Figure 3). Each fish was assigned to one or two undergraduate students to observe, and students named their fish according to its species; "S" names for Slenderhead Darter and "L" names for Ozark Logperch (e.g., Skittles and Lovely). During observations, fishes were monitored by their assigned student(s) for five minutes. A single real or fake mirror was gently placed in the microcosm as close to the individual fish as possible without disturbing it. Fish were then monitored for another five minutes. The observer recorded the fish's activity. This process was repeated for each of the four observations. In all of these observations, a fish only interacted with the mirror once; a Slenderhead Darter in the real mirror group swam in circles toward the mirror, then went to an opposite corner of the microcosm and rubbed and pushed against the back wall.

After the initial experiment, we conducted a secondary test in which we redesigned our experiment slightly to encourage mirror interaction. The large rock was removed to discourage hiding and increase visibility, and small gravel substrate was added to enhance the environment. A Plexiglas divider was placed in each microcosm to cut tank size in half. Two mirrors were placed on opposing ends of the new smaller enclosed area. The fake mirror variable was eliminated. Fishes were injected a second time in a similar lateral location with green VIE tags prior to the beginning of the second experiment. Fish were once again observed in five-minute intervals four separate times over 14 days. Our efforts to increase mirror interaction seemed to be effective; four Slenderhead Darters and one Ozark Logperch exhibited unusual behavior interactions when the mirror was present during at least one observational period. Most fishes exhibited some interest in the mirror but were not observed actively interacting with their reflection.

We also considered the possibility of observer effects on behavior, where fishes behave differently with human observers present. With this in mind, we conducted a third and final test. No changes were made to microcosm environments, but two cameras (HoverCam Solo8 Spark, MFR #HCS8S) were set up on microcosms 3 and 4 (housing a Slenderhead Darter and an Ozark Logperch, respectively). Mirrors remained in the microcosm for the entire 9-hr recording period. Two student observers later independently watched both videos and recorded the behaviors and activity of the fish. These observations supported the results from the previous two experiments: neither fish species exhibited behavior indicative of being self-aware. However, the fishes were much more active in video recordings without human presence than in the previous experiments when humans were present. The video method appeared to be more effective in documenting fish behavior. Nonetheless, no behaviors suggesting self-awareness were observed.

In all the experiments, we were unable to find evidence of self-awareness in either of these species of *Percina*. Though some isolated incidences of odd behavior occurred, they were infrequent and could have been attributed to other things, such as irritation from injection or handling. Perhaps these fishes are incapable of self-awareness, or perhaps the mirror test is an inappropriate test for these species.

Darters have the anatomy and physiology to see color. It is predicted that they use this sense for mate selection, as the males become bright and colorful during spawning. Interestingly, when this prediction has been tested, results have varied. Some studies have indicated that certain darter species do use color for mate choice (Splendid Darter *E. barrenense* and Banded Darter *E. zonale*; Williams and Mendelson 2010), whereas other species do not

**Figure 3. Students working in the lab and practicing recording observations.**

(Roanoke Darter *P. roanoka*; Ciccotto, Gumm, and Mendelson 2013). The visual capacity of the Ozark Logperch and Slenderhead Darter has not yet been studied. If the two species used in our study do not use color as a visual cue, then use of colorful VIE markers likely is not an appropriate evaluation of self-awareness for these species.

The mirror test is inherently biased towards highly visual species that see in a way similar to humans. It is possible that the application of a visual test is biased against organisms that rely more on non-visual stimuli. A test using non-visual cues, such as pheromones or lateral line sensitivity, might be more appropriate. A recent study by Horowitz (2017) has found success using an olfactory self-awareness test on domestic dogs. In this study, each dog was presented with a sample of their own urine, either unmodified or augmented with an additional odor. The dogs showed far more investigative interest in the modified urine sample. A second experiment found that the dogs were more interested in the modified urine sample than the additional odor on its own. These results suggest that the dogs recognized their own odors and showed investigative interest in the scent modifications. This kind of "olfactory mirror test" has great potential for application in other species that utilize chemical cues. Further research on Percidae pheromone reactivity is necessary before this kind of test can be applied, but it may be a better fit than the visual test we performed.

The mirror test's efficacy as a test of self-awareness has been subject to much scrutiny. Some studies have suggested that the capacity to "pass" the mirror test is not an intrinsic ability or inability at all; it is instead a skill that can be learned. Perhaps the ability to pass the mirror test (and perhaps the nature of self-awareness itself) reflects a complex continuum rather than a pass/fail dichotomy. As more discoveries are made about animal cognition, our concept of self-awareness is further challenged, and we must continually remodel our approach to evaluating it. Perhaps future studies, rather than investigate whether a species possesses self-awareness, should focus on whether a species can *learn* self-awareness. Is it possible to teach these darters to recognize their reflection? Would that be a better indicator of self-awareness and intelligence?

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