Developing an Archerfish (*Toxotes jaculatrix*) Ethogram to Examine Self-Recognition and Mirror-Image Discrimination

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Consciousness, as defined by self-recognition, has historically differentiated humans from animals, leading several researchers to study if animal behaviors could be explained through human concepts such as self-awareness (1). By investigating the true extent of animal consciousness, society can better understand the boundaries for respectful coexistence and animal rights (2). Mirror self-recognition (MSR), the benchmark measurement for this type of consciousness, is most often evaluated through the traditional mark test, which requires a subject to perform a self-directed behavior toward a mark (3). However, this requirement limits the test's accuracy, as it has focused on species more physiologically able to engage with the mark, unlike fish (3). These results are also limited by the painful application of the mark (3). To address this apparent need for a self-recognition task that prompts a similar idiosyncratic response in forelimb lacking subjects, we designed our mirror exposure task to enable more flexibility by monitoring the archerfish's general reactions rather than a single behavior.

The project involved four archerfish in a 0.48 m x 0.53 m x 1.83 m, 454 L capacity tank that lasted eight days. We started each trial by exposing the reflective side of a 6.3" wide x 9.6" long handheld mirror on the tank's left or right side, based on random assignment. We observed the fish for 375 minutes in intervals ranging from thirty to sixty minutes, throughout three-minute windows marked by a digital timer, as consistent with earlier lab work. During the intervals, we noted any unique behaviors directed towards the mirror in the handwritten datasheet's *narrative section* to construct a prototypical archerfish ethogram (4). This ethogram will determine if any responses are evidence of mirror recognition, or if the pattern of behavior changes based on the mirror's presence, in an upcoming Master's Thesis (5). After each period, we counted the number of fish on each side of the tank, determined by a half-way line. Finally, we flipped the mirror back to its non-reflective side. We ranked the total behaviors as follows: 38% hovering, 38% repetitive swimming, 9% darting, 9% facing the mirror, 3% chasing, and 3% backward swimming. These eight-day observations rendered us evidence in distinguishing *repetitive swimming* as either *parallel* or *perpendicular* in our ethogram (Table 1). To discover if archerfish can self-recognize, we analyzed the fish's preference for the mirror condition and either side of the tank. Due to our small sample size ($n = 4$), we applied the Wilcoxon Signed Rank Test to identify interactions between the mirror/non-mirror condition, the left/right side of a tank, and fish location. Under the mirror conditions, the median number of fish on the left was 0.450, and on the right was 1.020. Under the non-mirror conditions, the median number of fish on the left was 1.60, and on the right was 1.850. Neither of the correlations was statistically significant. Therefore, we lack evidence, thus far, that archerfish can self-recognize.

Table 1. Behavioral Categories Used to Develop a Formal Ethogram for Archerfish Behaviors. These behavioral categories were developed to be used in future archerfish work (5).

Code:	Behaviors	Definition:
BS	Backward Swimming	Fish move backward away from the mirror.
H	Hovering	Fish mostly immobile/attempting to maintain a position when facing the mirror for an extended period (more than 2 seconds). Also noted as *stopping* and *facing the mirror*.
Pe	Perpendicular Movement	Fish swimming up/down in the y plane.
Pa	Parallel Movement	Movement toward the mirror in the forms of *head butting*, *pecking*, darting (*speeds away*), or pressing/hitting any body part against the glass
Ch	Chasing	One fish shortens the distance from another in a fast manner that may come off as territorial or aggressive.

This preliminary study exposed several limitations to our research. One such restriction included the small sample size (n = 4), which reduced opportunities to examine a complete MSR behavior spectrum. However, due to the small size of our lab's tank, we constricted our sample to minimize the risk of disease. Future studies will benefit both from monitoring more subjects and conducting more trials. Previous works recognized multiple marine species, such as cleaner wrasses and dolphins, that can express self-recognition (3, 6). Future research should focus on categorizing MSR behaviors in terms of normal social or aggressive aspects and developing a painless mark test for fish. Alternatively, self-recognition could be further tested based on other senses, such as smell, as seen in studies with dogs (7). The likelihood of a deeper psychological connection between humans and animals remains elusive as scientists continue to expand present knowledge of self-recognition in non-human species.

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