Personality assessment protocol Version 2

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Abstract

This protocal characterized each focal fish (western mosquitofish, *Gambusia affinis*) for three standard indicators of personality using well-established experimental approaches: (1) boldness as latency to emerge from shelter and enter an unknown area, (2) activity in an open field tank and (3) sociability (i.e., shoaling tendencies), estimated as the time spent in the vicinity of a group of conspecifics. We tested each fish twice for its personality on two successive days, which allowed us to test for behavioral repeatability across both assessments.

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Guidelines

This protocol describes preparation of personality assessment, boldness, activity and shoaling tendency measurement methods of *Gambusia affinis* under laboratory condition.

Before start

We acclimated the fish to laboratory conditions for at least one month before we conducted behavioral experiments and maintained them in groups comprising both sexes at roughly even sex ratios, at densities of around 40 fish per tank, in several aerated and filtered 200-l aquaria under a 12:12 h light/dark regime. Aquaria were well equipped with plants, twigs and stones.

We fed the fish twice a day *ad libitum* with commercially available flake food, frozen blood worms (chironomid larvae), as well as *Artemia salina* nauplii and shrimps. In the stock tanks and all experimental tanks, water temperature was kept at 25 ± 1 °C. Water quality was maintained by exchanging half of the water every two weeks, while one tenth of the water was exchanged every day in case of the (smaller) isolation tanks. Aged tap water was used for water changes and throughout the entire experiment.

After acclimation period, we randomly selected adult focal fish from our stock tanks and isolated them, separated by sex, in 96-l tanks 24 hours prior to behavioral tests. To avoid aggressive interactions between individuals and to enable repeated testing of the same individuals, we kept each focal fish separately in 1.5-l transparent perforated plastic bottles, which allowed water exchange with the environment. Fish were returned to their isolation tanks between subsequent trials.

A web cam (KC-QB960AK, Keeper, Shenzhen, China) was fixed in a central position approximately 70 cm above the test tank during all behavioral observations, allowing us to remotely observe the focal fish from above. We introduced an air stone connected to an air pump into the test tanks between trials to guarantee well-oxygenated water, and we changed the water every day after a testing session (every 4 trials). The test arena consisted of a glass tank (80 × 30 × 30 cm) that was filled with aged tap water to a height of 15 cm. The tank was placed on a gray plastic sheet with a fine white grid (5 cm squares). All outer sides were covered with black plastic foil to minimize disturbance.

Protocol

Step 1.

To initiate a trial, we introduced the focal individual into a lateral shelter area (20 × 30 cm) of the testing tank, which was separated from the rest of the tank by an opaque trap door. The shelter area contained small stones and artificial plants for the fish to hide. We gave the focal fish 2 min for acclimatization before the trapdoor was remotely opened by a pulley system. We determined the latency the test subject needed to emerge from shelter, which is a common measure of boldness in fish, with bolder fish emerging faster. We terminated a trial after a maximum ceiling value of 5 min (i.e., if the focal fish did not leave the starting area) and gently moved the fish outside the container with the help of a small aquarium dip net.

Step 2.

Afterwards, we closed the trapdoor and let the fish explore the tank for 5 min before we started quantifying swimming activity. This habituation period was important as we were interested in individuals' activity levels rather than exploration of a novel environment. Even shorter periods of time for habituation were successfully employed in studies on individuals' activity levels in other poeciliid species. We counted numbers of squares crossed by the focal fish in the test arena (60 × 30 cm) within 5 min, assuming that more active fish would cross more grid squares.

Step 3.

Directly after the activity assessment, we removed a black cardboard divider that had blocked visual contact with a stimulus shoal that was situated in another tank (20 × 20 × 15 cm), adjacent to the small side of the test tank opposite of the starting area. Physical and chemical contact between fish residing in different tanks was not possible, leaving only visual cues as a potential stimulus. The tank contained three stimulus fish (SL, females: 28.46 ± 5.41 mm; males: 22.74 ± 3.33 mm), which were exchanged between trials. To avoid effects of sexual attraction and familiarity on shoaling behavior, we presented stimulus shoals of the same sex, and fish used to compose stimulus shoals were taken from a different stock tank than the one from which the focal fish stemmed. We waited until the focal individual habituated to the new situation and resumed swimming freely. During a 5 min observation period, we determined the time the focal individual spent within a visually marked association zone (10 cm in front of the stimulus tank) as a measure of sociability/shoaling. All tests were performed consecutively in the same arena to minimize handling stress.