CONSPECIFIC SOUND LOCALIZATION IN *OTOCINCLUS AFFINIS*

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1 INTRODUCTION

Fishes in the superorder Otophysi are mostly freshwater teleosts that include catfishes, knifefishes, minnows, carps, characins, suckers and loaches [1]. One unifying characteristic of this group is the Weberian apparatus, a series of bones that connect their swim bladder with their ears [2]. In the catfish family Loricariidae, the Weberian apparatus is minimized, since the swim bladder is directly adjacent to each side of their ears [3]. Loricariid catfishes feature a highly derived swim bladder morphology characterized by complete division of the bladder into two separate spheres that are each surrounded by a megaphone-like bony capsule.

Several structural features of this swim bladder capsule apparatus have been hypothesized to have acoustic functionality, but little is understood about either the morphological range of these structures in the family Loricariidae, or about their acoustical functionality. We hypothesize that having a bi-lobed swim bladder may provide loricariid catfishes with an improved ability to localize sounds. In order to start testing this hypothesis, we first had to develop a protocol for attracting *Otocinclus affinis*, a small loricariid catfish, to a sound source. We did this by using operant conditioning, in which a food reward was paired with a conspecific sound stimulus [4].

Like many catfishes [5], we have found that *O. affinis* produces sounds by stridulating their pectoral spines to make broad-band clicks (unpublished data). Although these sounds are produced when the fish are handled, the normal behavioral context of these sounds are currently unknown. Following operant conditioning of *O. affinis* to a conspecific click sound, we then used a multiple speaker array to examine the ability of *O. affinis* to localize to a sound source in a large, round tank.

2 METHODS

2.1 ANIMALS AND TRAINING EXPERIMENTS

Two hundred *O. affinis,* with a total length between 1.5 and 2 cm, were obtained from Segrest Farms (Gibsonton, FL, USA) and kept in a 122 x 30 cm (200-L) tank with water temperature at approximately 26.5 °C and a 12 h light/12 h dark automatic lighting regime. Two groups of 42-50 fish each were placed in a similar tank in the same room and trained to associate a conspecific sound stimulus with food (algae pellets) by giving them the food and sound stimuli simultaneously three times a week for 6-8 weeks, until at least 70% of the fish were attracted to the sound stimulus. The naïve group (N=42), kept in a separate control tank, only received food during this period.

Four types of experiments were then performed- training, test-1, test-2, and naïve. During training trials (N=3), food (algae pellets) and a conspecific sound stimulus were presented simultaneously. During the test-1 trials (N=3), only the conspecific sound-stimulus was presented to trained fish. During the test-2 trials (N=3), only a 4-sec, 500-Hz sound-stimulus was presented to trained fish. During naïve trials (N=3), only the conspecific sound-stimulus was presented to unconditioned fish. For each trial, behavior was videotaped 15-min before, during, and after the sound stimulus using a Sony HandyCam with a wide-angle lens in night vision mode. The aquarium was illuminated by an infrared light to prevent disturbance of the fish.

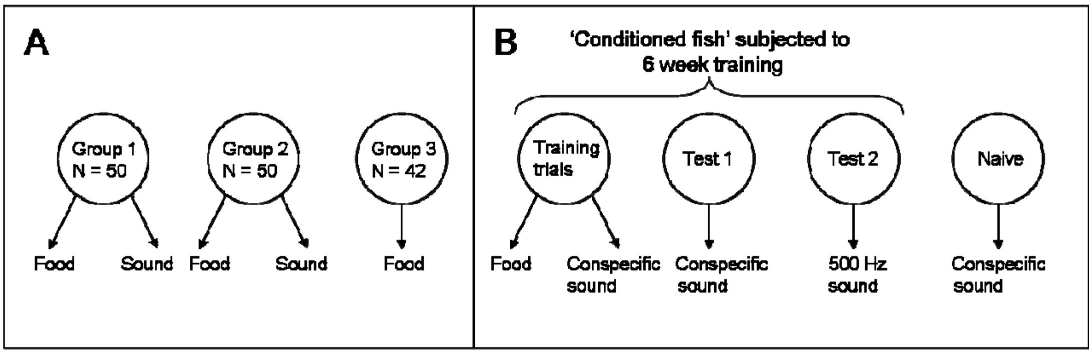

Figure 1: Schematic of experimental design.

Caption text: A) Groups 1 & 2 were experimental groups that were conditioned to a conspecific sound stimulus for six weeks, while Group 3 was a unconditioned control. B) Conditioned fish (Groups 1 & 2) were used for Training, Test 1, and Test 2 trials, with unconditioned controls (Group 3) were used in Naïve trials.

The sound stimuli used in these experiments were a 4-sec recording of a single *O. affinis* click (Fig. 1) or a 500-Hz tone, played repeatedly in a loop, with a 0.07s delay between recordings, continuously for 15 min. Sound was played through a computer to a 5.3 amp/200 watt monoblock amplifier (AudioSource, Portland, OR, USA) to a University Sound UW-30 underwater speaker (Electro-Voice, Burnsville, MN, USA). Sample video frames were analyzed at the end of every 3-min of each 15-min recording period. At each frame, fish were counted in each column of a grid (15 x 15 cm) on the back of the aquarium. The 500-Hz tone stimulus was generated by a

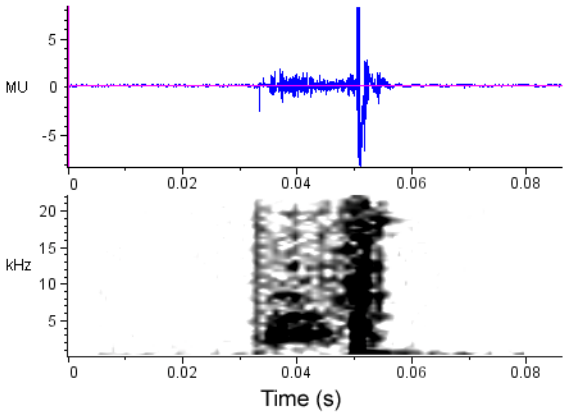

Figure 1: *O. affinis* click used for operant conditioning

Caption text: The waveform (above) and spectrogram (below) of the *O. affinis* broadband click used for operant conditioning.

2.2 LOCALIZATION EXPERIMENTS

Twenty-six trained *O. affinis* (used in previous test-2 conditioning experiments) were used to test the acuity of sound localization by randomizing the conspecific sound stimulus through four University Sound UW-30 underwater speakers mounted at 0, 90,180, and 270º along the edge of a round tank. Fish behavior was videotaped from above the tank. Fish, one at a time, were acclimated for 5-min in a small, clear tube located in the center of the tank. Then the tube was lifted to allow the fish to swim freely while the same sound stimulus used in the conditioning experiments was played for 5-min. Then the sound stimulus was turned off and fish behavior was recorded for an additional

5-min. The tank was divided into 4 quadrants, one associated with each underwater speaker. Immediately at the onset of the sound stimulus, it was recorded which quadrant each fish directly swam to and total time spent in each quadrant during the experiment. Analysis of variance (ANOVA) was used to test for differences among quadrants, followed by *post hoc* Tukey tests for pair-wise comparisons.

3 RESULTS

O. affinis were successfully conditioned to approach a speaker emitting the conspecific click stimulus. Over 75% of the fish approached the right side of the tank at some point during conspecific sound presentation, both with (Fig. 2A) and without pairing with food (Fig. 2B). A 500-Hz tone elicited a similar response in fish conditioned to respond to the conspecific sound (Fig. 2C). A conspecific sound by itself did not attract naïve fish to the speaker (Fig. 2D), suggesting that it was the conditioning to food, and not the conspecific sound itself, that attracted fish to the speaker.

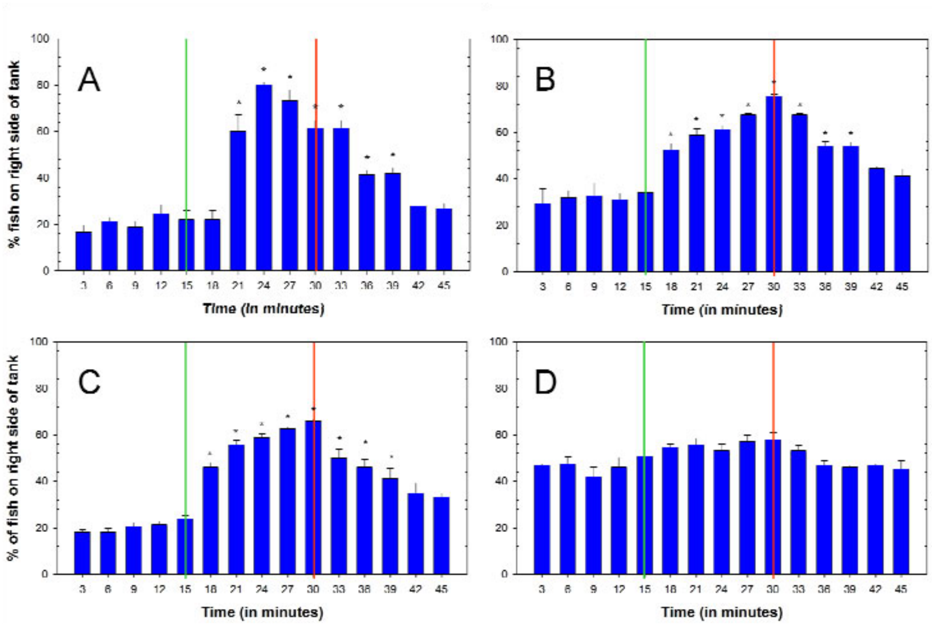

Figure 2: Percent of fish on the speaker side of the aquarium as a function of sound stimuli.

Caption text: Percentage of total *O. affinis* on the right side (speaker side) of the aquarium in A) training, B) test-1 (conspecific sound only), C) test-2 (500-Hz only), and D) naïve trials (conspecific sound only). Vertical lines represent the onset (at 15 min) and offset (at 30 min) of the sound stimulus. * significantly different from pre-stimulus controls, *post hoc* Tukey test, P < 0.05.

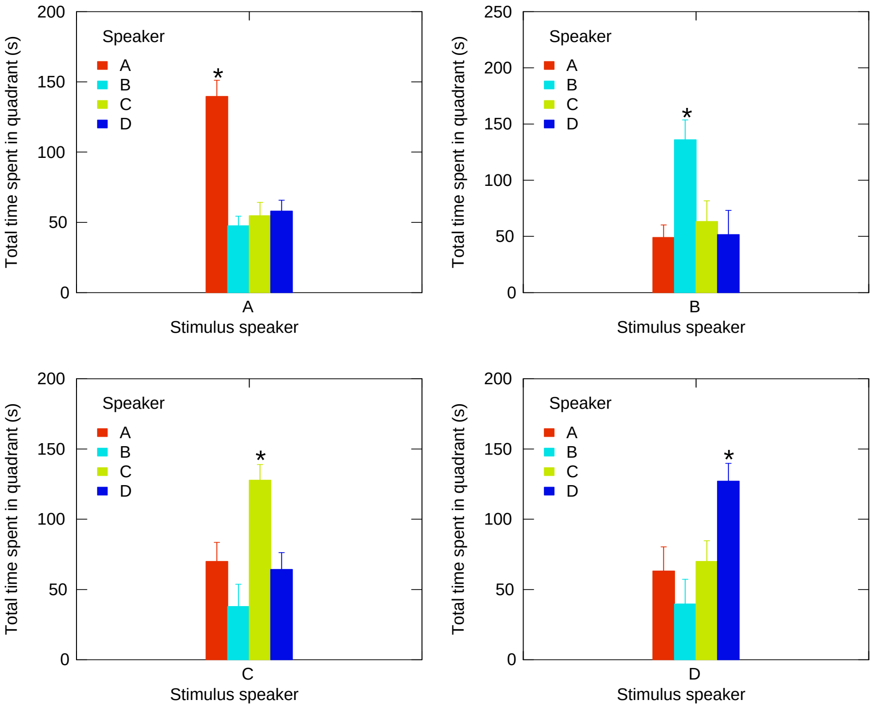

Figure 3: Time spent in quadrant as a function of emitting speaker

Caption text: Total time that *O. affinis* spent in each tank quadrant as a function of which speaker was emitting the conspecific sound stimulus. * significantly different, *post hoc* Tukey test, P < 0.05. At the beginning of each sound localization trial, 19 out of 26 fish (73%) swam to the quadrant of the correct sound-producing speaker first. Fish spent significantly more time near the sound-producing speaker than the other three speakers. This was true for all four speakers.

4 DISCUSSION

In summary, *O. affinis* is able to localize a conspecific sound source and are trainable via operant conditioning to approach a sound. Although there have been a number of studies examining sound localization in fishes (see review in Fay and Popper, 2005, [6]), the process of sound localization in fishes is incompletely understood. While most vertebrates use interaural intensity and time differences between opposite ears to localize sounds, the inner ears of fishes are too close together and the speed of sound in water too fast to make such cues beneficial for sound localization. The potential acoustical role of the unique peripheral auditory structures of loricariid catfishes is currently unknown.

Future experiments will use the described conditioning paradigm to further study sound localization abilities in *O. affinis.* In particular, the effects of swim bladder deflation on sound localization capabilities in *O. affinis* are currently being examined.

ACKNOWLEDGEMENTS

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