Go with the flow: The effect of flow rate on pitch with lateral line system inhibition

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Introduction

Aquatic environments often involve rapidly flowing, highly turbulent, or completely still water—often in combination. The animals living in these environments must successfully navigate to avoid obstacles, detect predators, and find prey—all key to their survival (Flammang & Lauder, 2013). Most aquatic animals do this through the use of specialized sensory systems: the lateral line and vision.

The lateral line system (LLS) allows animals to detect hydrodynamic stimuli such as disturbances at the surface or within bodies of water (Mogdans, 2019). These stimuli are detected through superficial and canal neuromasts–the LLS's receptor organs–distributed across the head and trunk (Webb, 2011). Neuromasts are small structures composed of sensory hair cells and supporting structures that send signals through afferent nerves to the central nervous system. These signals are then integrated and complex behavioral responses are initiated (Mogdans, 2019).

Fish with adequate vision have previously been suggested to primarily seek out prey visually, however studies have shown that LLS input can override vision during feeding behaviors (Janssen & Corocran, 1993). Interestingly, vision has also been found to influence fishes' decision to interact with turbulent environments moreso than the lateral line (Liao, 2006). These findings suggest that visual and LLS input not only supplement each other, but also compete to provide sensory information for decision making.

This limited knowledge of the relationship between vision and the LLS inspires the present inquiry. It is relatively unknown how disabling both vision and the LLS will affect fishes' swimming behaviors in turbulent environments. It is hypothesized that disabling both the visual and lateral line systems of the fish will affect pitch as a function of flow rate.

In order to assess the LLS, bluegill sunfish (*Lepomis macrochirus*) provide unique advantages as a model species. The littoral environments they reside in often contain many obstacles (Flammang & Lauder, 2013), requiring proficient navigation abilities and use of both the LLS and vision. Furthermore, as a diurnal species that relies heavily on vision for locating prey (Janssen & Corcoran, 1993), bluegills are a great model to assess behavior when vision is inhibited.

Methods

Study Design

This experiment allows for assessment of the possible effect of flow rate on the pitch of bluegills with an inhibited lateral line system. In this case, flow rate and the status of the lateral line were experimentally manipulated while pitch—the fish's upward or downward angle—was measured. In order to ensure that confounding variables were limited; other variables including the flapper speed, experimental tank, environment, and light condition remained constant across conditions. In the present experiment, the control condition (with inhibited vision, enabled LLS, three Hz flapper speed, and low flow rate) was compared to two experimental conditions in which the LLS was disabled and flow rate was varied.

Subjects

Three wild-caught bluegill sunfish (*Lepomis macrochirus*) were randomly selected for participation in this study. Animals were housed individually and fed a live worm and food pellet diet.

Experimental Apparatus

The experimental apparatus consisted of a large aquarium tank with adaptations specifically for assessing fishes' swimming behaviors in hydrodynamic environments. The water's flow speed was varied from low to high; as a function of body lengths/second. The water's turbulence could be manipulated via activation of flappers at the front of the tank. The flapper was set to the highest speed (three hertz) to generate the greatest amount of turbulence in the tank for each condition. Due to the fact that turbulence was vertically generated from the up and down-ward movement of the flappers, pitch was believed to be under the greatest influence of high turbulence. In addition, the tank room was completely dark to ensure that the bluegills' visual systems were inhibited for the duration of each trial. Figure 1A displays a representation of the experimental apparatus setup.

Procedure

The following experimental procedure was derived from Professor Margot Schwalbe's Spring 2022 "Neuroethology Lab - Flapper Project Handout" for the Neuroscience 301: Neuron to Brain Lab (Schwalbe, 2022) and was performed by Professor Schwalbe and members of the Schwalbe Lab. LLS inhibition was performed by treatment with 0.1 mM aqueous cobalt chloride for three hours prior to running the experiment.

For each fish and each trial, the subject was placed into the tank and oriented to face the flappers from several inches away and submerged to a depth approximately halfway from the top of the tank. Data collection via high-speed camera footage began at time point zero, after the fish had been oriented and immediately before the flapper was turned on. Once the flapper was turned on and began moving, the water's turbulence increased and the fishes' swimming behavior was recorded. For all trials, the tank room was completely dark for the entire duration of the experiment.

Data Collection

Footage from the camera capturing the lateral view was used to assess changes in pitch. The first few seconds following flapper activation were of interest in the present study and thus timepoints from the first 15 seconds of each recording were selected for further analysis. The software program ImageJ was used for analyzing the frames from these recordings. Using the angle tool, an angle was drawn from the end of the upper jaw to the flapper at the front of the tank with the vertex in the middle of the junction between caudal fin and peduncle. The line from the vertex to the tank front was kept at 180° across the image. Figure 1B displays this measurement technique on an example photo.

The angle formed from this was measured three times and the average angle was recorded. The angle was recorded as a positive value if the fish's head was pointing upwards towards the top of the tank and negative if the fish was pointing downwards. Angular data was collected for each of the conditions at the following time points: zero, five, and ten seconds.

Statistical Analysis

For all conditions and time points, uniformity of pitch angle was analyzed using Rayleigh's Test of Uniformity. For all tests, n = 3 and a significant result of $p < .05$ indicated a lack of uniformity of circular distribution for pitch angle, indicative of a preferred angle (i.e. approximately 0 degrees, into the flow of water).

For comparison of average pitch angles between conditions compiled across all time points (e.g. low flow/LL- vs low flow/LL+), a nonparametric Watson's U2two-sample test was performed, where n = 15 for each condition and a significant result of $p < .05$ indicated a significant difference in pitch angle between two independent conditions. Comparisons between conditions at specific timepoints using Watson's U2 were unable to be performed, as grouping variables in this way resulted in a total sample size less than required to perform the test.

Results

Pitch appeared to remain consistent across all time points regardless of status of the lateral line [inhibited (LL-) or enabled (LL+)] or flow rate (low/high) (see Figure 2). Rayleigh's test of uniformity showed a lack of uniform circular distribution of pitch with a mean of approximately 0° across all time points, for all conditions, $p < 0.05$.

Pitch was also unaffected by lateral line inhibition at a low flow rate (see Figure 3). Comparison at a low flow rate with lateral line inhibited (M = 357.9°, SD = 12.0°) or intact (M = 357.2°, SD = 12.0°) revealed no significant difference in pitch, Watson's U20.181, $p > 0.05$.

Pitch remains unaffected by flow rate when the lateral line is disabled (see Figure 4). Fish with inhibited lateral lines (LL-) showed no difference in pitch between low (M = 357.9°, SD = 12.0°) and high (M = 353.2°, SD = 7.4°) flow conditions, Watson's U20.171, $p > 0.05$. Additionally, there was no significant difference observed between high flow/LL- and control (low flow/LL+) conditions, Watson's U20.064, $p > 0.05$.

Conclusion

The present study assessed the effect of disabling vision and the LLS on bluegill's pitch when swimming in high turbulence environments. Previous studies suggest that the LLS and vision only slightly influence steady swimming in hydrodynamic environments while more strongly influencing behavioral preferences (Liao, 2006). The present findings support this idea that the LLS and vision may be primarily employed for deciding how to engage with stimuli, and not steady swimming maintenance. Despite the lack of support for the present hypothesis, these findings contribute towards a better understanding of the functioning of the LLS and provide avenues for further research. While LLS inhibition failed to affect pitch, greater statistical power and study design improvements in the future may yield these findings.

Understanding the way in which vision and the LLS function to regulate pitch is key to its applications for both species with and without a LLS, such as humans. For instance, a mechanically reproduced LLS could be used to enhance underwater vehicles, underwater-sensing technology, and robotics for human applications (Liu, 2016). However, the factories in which these devices would be built are likely to generate massive quantities of pollutants and toxins. Exposure to such toxins has been found to have detrimental effects on the development, regeneration, and functionality of the LLS (Coffin, 2017). Recent research has also shown that noise pollution generates continuous sounds underwater, interfering with the LLS's ability to detect relevant stimuli and thus affecting behavior (Weilgard, 2018). Consequently, this fundamental system must be better understood in order to help both humanity and marine ecosystems thrive.

Appendix A

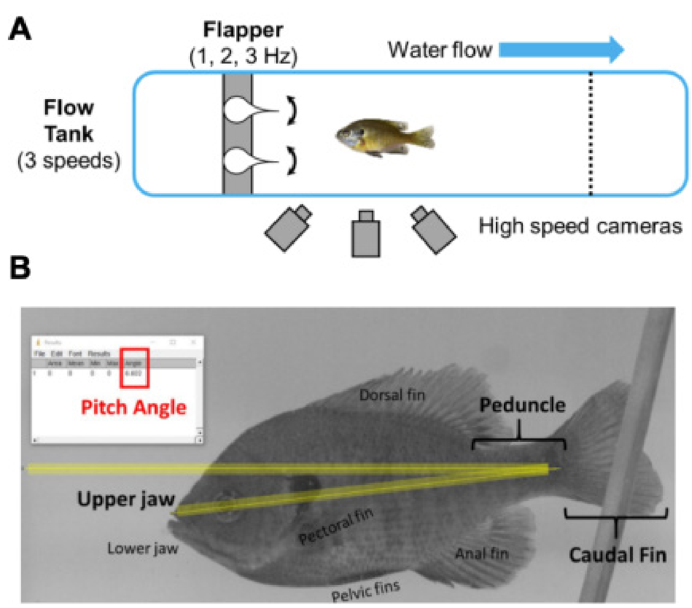

***Figure 1.* Diagrammatic representation of the experimental setup (A) and pitch measurement (B).** (A) Bluegill sunfish were placed into the aquatic tank and swimming behavior was observed and recorded via high-speed imaging. (B) Pitch was measured accordingly at distinct timepoints in each trial using the ImageJ software program and recorded. These images were provided by M. Schwalbe's Neuroethology Supplemental Material (2022).

Appendix B

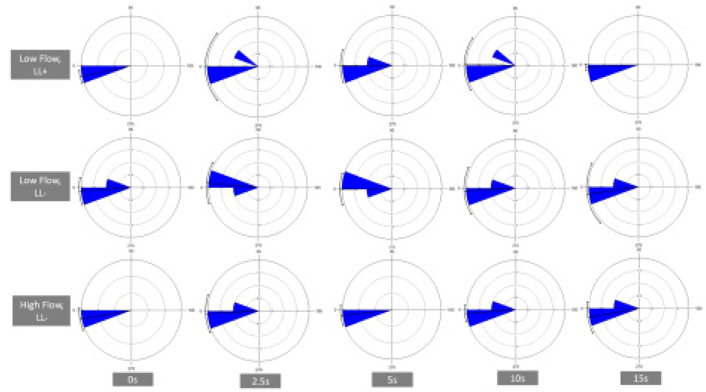

***Figure 2.* Pitch appears to remain consistent across time.** Pitch appears approximately the same across individual time points (0s, 2.5s, 5s, 10s, and 15s) regardless of lateral line inhibition (LL-) or manipulations of flow rate (low vs. high).

Appendix C

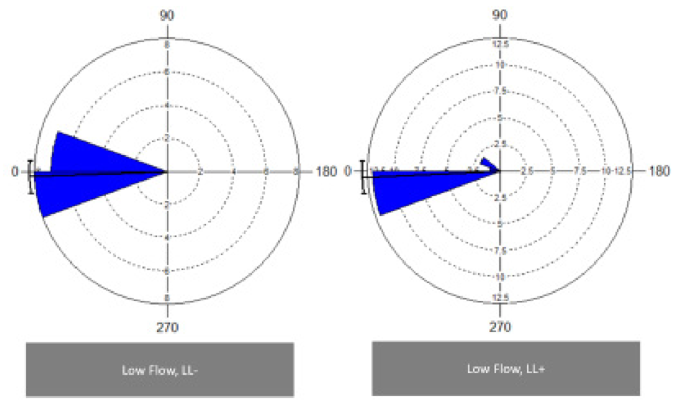

***Figure 3*. Pitch remains unaffected by lateral line inhibition.** At a low flow rate, pitch appears to be unaffected by inhibition of the lateral line system (LL-). No significant difference in pitch was found when comparing conditions despite lateral line status.

Appendix D

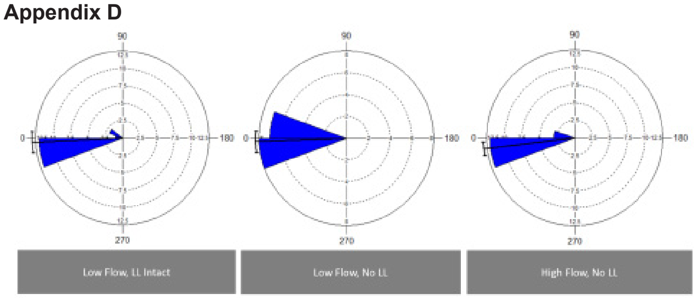

***Figure 4.* Pitch remains unaffected by flow rate.** Pitch showed no significant changes between low and high flow rate conditions. Fish with inhibited lateral lines (LL-) showed no difference in pitch between flow conditions. No significant difference was observed between high flow/LL and control conditions.

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