COMPARING THE ROLE OF THE LATERAL LINE DURING RHEOTAXIS BETWEEN A SEDENTARY AND MOBILE SPECIES

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A Thesis

Submitted to the Graduate College of Bowling Green State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2014

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Rheotaxis is a robust, multisensory behavior with many potential benefits for fish and other aquatic animals. Visual (optic flow) cues appear to be sufficient for rheotaxis, but other sensory cues can clearly compensate for the loss of vision. The role of various non-visual sensory systems, in particularly the flow-sensing lateral line, is poorly understood—largely due to widely methods employed to study rheotaxis. Here, we examine how sedentary behavior affects the relative importance of lateral line cues by comparing sedentary armored catfish (*Corydoras julii*) with mobile blind cavefish (*Astyanax mexicanus*). Armored catfish were observed to be sedentary and moved in a saltatory fashion, whereas blind cavefish were significantly more mobile and tended to move continuously. No effect of lateral line disruption was observed in mobile blind cavefish, suggesting it is not necessary for rheotaxis at any of the speeds tested. By contrast, rheotaxis was significantly affected by lateral line disruption in sedentary armored catfish. Taken together, these results suggest that sedentary behavior might promote reliance on lateral line cues.
ACKNOWLEDGEMENTS

The author would like to thank: Sheryl Coombs for help with all aspects of the design, execution, analysis and final write up of this thesis, Jake Graving for assistance in conducting behavioral and anatomical experiments, Dan Wiegmann and Robert Huber for help with the design of the experiment as well as participation in the thesis committee, Derek Paley and Sachit Butail for contributions to tracking software, Matt Kulpa for feedback, and Steve Queen for help in designing and constructing the flow tank. Finally, the author would like to thank Christina Bak-Coleman for her unending support.
INTRODUCTION

Fish placed in moving water often exhibit an unconditioned orienting response, typically orienting either upstream (positive rheotaxis) or downstream (negative rheotaxis) (Lyon, 1904; Arnold, 1974). Rheotaxis is of strong ecological significance to fish as it facilitates interception of downstream-drifting prey and odors (Kleerekoper, 1978; Gardiner and Atema, 2007), directional guidance of migratory behaviors (Thorpe et al., 1981; Tytler et al., 1978), and energetic cost savings for fish attempting to avoid being swept downstream (Montgomery et al., 1995). Rheotaxis is a taxonomically widespread behavior that occurs throughout the life cycle of fish under widely-varying behavioral, flow, and sensory conditions (Arnold, 1974). For example, rheotaxis has been observed in diurnal, stream-dwelling giant danio (*Devario aequipinnatus*) (Bak-Coleman et al., 2013; McClure et al., 2006) as well blind cavefish (*Astyanax mexicanus*) that inhabit lightless, subterranean cave pools (Baker and Montgomery, 1999b). Moreover, sedentary fish living on the substrate (e.g. flatfish) (Arnold, 1969) rheotact, as do fish swimming at different elevations in the water column. The diversity of ecological and sensory conditions under which rheotaxis occurs is indicative of the robustness of this behavior and its reliance on multiple sensory cues.

When visual cues are available, displacement downstream causes apparent movement of objects across the retina, known as optic flow. Optic flow appears to be a very important cue that can be sufficient, if not dominant, as fish exhibit rheotactic responses to a fictive optic flow stimulus in the absence of any true water movement or body-movement caused by downstream displacement (Lyon, 1904). When visual cues are absent or degraded (e.g. nighttime, turbid water, deep water), fish can successfully rheotact using non-visual sensory modalities such as the
vestibular system, touch, and the flow-sensing lateral line (Lyon, 1904, Montgomery et al., 1997, Baker and Montgomery, 1999a, 1999b).

While each of these non-visual sensory modalities could theoretically be used for rheotaxis, their actual contribution to this behavior is poorly understood for several reasons. One reason is that there is presently no good way to block the vestibular system without disrupting the ability of fish to maintain an upright posture, making it difficult, if not impossible, to assess the unique contribution of the vestibular system. A second reason is that there is conflicting evidence for and against a role of the lateral line in rheotaxis. Early studies, in which transection of the posterior lateral line nerve failed to alter rheotactic performance, suggested that the lateral line was unnecessary for rheotaxis (Dijkgraaf, 1933; Hahn, 1960).

This early, nineteenth century view, begun by Lyon’s (1904) classic studies on the importance of optic flow cues and championed in later years by Dijkgraaf (1933; 1963) was upheld for nearly a century until challenged by a series of experiments in the late 1990’s on three different species (Montgomery et al., 1997; Baker and Montgomery, 1999a,b). These studies provided evidence that the lateral line system and, in particular one submodality (superficial neuromasts on the skin surface), were indeed important for rheotaxis, but only for slow flows less than ~ 1 body length s⁻¹ (Montgomery et al, 1997; Baker and Montgomery, 1999a,b). This ‘revised’ view of lateral line involvement curried favor for another decade until more recent studies provided evidence against a major involvement of the lateral line at any flow speed (Van Trump and McHenry, 2013; Bak-Coleman et al., 2013). In general, all studies to date raise questions about the precise circumstances under which the lateral line may or may not play a role in rheotaxis.
There are at least two important theoretical considerations that are often overlooked in rheotactic studies and that may help to explain the discrepancies in the literature. One is the role that behavior, in particular, sedentary vs. mobile behavior, plays in determining the utility of various sensory modalities during rheotaxis. Sedentary benthic fish tend to be more negatively buoyant and may additionally have morphological and behavioral adaptation for coupling themselves to the substrate (Hart and Fanelli, 1999; Blake, 2006) thereby avoiding being swept downstream. Sedentary behaviors tend to minimize vestibular and optic flow cues, while maximizing lateral line cues (water flowing across the skin surface). By contrast, more neutrally-buoyant fish that are higher in the water column tend to be coupled to the surrounding water and thus, are more readily swept downstream, presumably generating useful vestibular and optic flow cues while reducing the strength of lateral line cues. In this regard it is interesting to note that two of the species found to exhibit a lateral-line dependent effect (the torrentfish and the Antarctic fish, *Pagothenia borchgrevinki*) exhibited sedentary, benthic behaviors. Moreover, effects were limited to slow flows (≤1 bodylength(BL) s^{-1}) – presumably those that were insufficient to displace the fish downstream (Baker and Montgomery, 1999a; Montgomery et al., 1997).

A second overlooked factor is the spatial nature of the flow itself – i.e. the extent to which flow is spatially uniform (flow speed and direction everywhere the same) or non-uniform. Indeed, Dijkgraaf (1933, 1963) observed that lateral line deprivation reduced the ability of fish to orient towards a narrow jet stream, but not to more globally uniform flows. From a biophysical point of view, the lateral line system is designed to respond to spatial non-uniformities in flow (Kalmijn, 1989). If the fish and surrounding water move together at the same speed and direction, as might be expected in a globally uniform flow, non-uniformities would not arise and
there would be no stimulus to the lateral line. In the case of a narrow jet stream, however, a velocity gradient exists between the center of the jet stream and the surrounding water, thus making it possible for the fish and surrounding water to be moving at different speeds, thereby stimulating the lateral line. Furthermore, non-uniform flow could impact the utility of vestibular cues by displacing the fish in directions other than downstream, effectively creating sensory noise that could interfere with the fish’s ability to determine flow direction. Taken together, spatially non-uniform flows are likely to increase reliance on the lateral line, while at the same time decreasing reliance on vestibular cues. Although difficult to say for certain, differences between studies in the way that flow was generated could very well have resulted in substantial differences in the spatial nature of the flow field (see discussion for further detail).

In addition to differences in the spatial nature of the flow field and species-specific behaviors, differences between studies in other test conditions may have contributed to the inconsistency of results. For example, several studies reporting an effect of lateral line deprivation tested groups of fish (5 – 20) simultaneously in a small, rectangular space (e.g. Montgomery et al., 1997; Suli et al., 2012). Under these circumstances, the packing density of multiple fish in a confined area might arguably lead to an orientation bias in the streamwise direction. Any disruption of this orientation preference in lateral line deprived fish could thus be due to an indirect impairment in their ability to maintain distance and orientation with respect to each other (rather than the current), as has been observed in lateral line-deprived schools of fish (Partridge and Pitcher, 1980; Fauchere et al., 2010). As a second example, the intended effects of various lateral line blocking techniques have recently come into question. Among the class of ototoxic drugs (aminoglycosides) used in rheotactic studies, the efficacy of gentamycin to selectively block canal but not superficial neuromasts has been come into question (Van Trump
et al., 2010). Likewise, streptomycin has been shown to be effective in some but not all species (Brown et al., 2011). Finally, high concentrations of cobalt chloride (as were used by Baker and Montgomery (1999b) on blind cavefish) may have overall toxic effects on behavior in general in addition to local effects on the lateral line system alone (Janssen, 2000).

In this study, we make direct comparisons of rheotactic performance and lateral line deprivation effects in two species: a sedentary, benthic armored catfish (*Corydoras julii*) and a mobile epibenthic species, the blind cavefish (*Astyanax mexicanus*). The blind cavefish was chosen for several reasons, including its propensity to swim near the substrate and its heavy reliance on non-visual senses, in particular the lateral line (reviewed in Montgomery et al., 2001). Given that this species has previously been shown to exhibit lateral-line dependent rheotaxis (Baker and Montgomery, 1999b), the retest of the same species in this study provides an opportunity to control for possible confounding factors of past studies, such as tank shape and simultaneous testing of multiple fish. The results of this study show that lateral line deprivation of solitary fish leads to a decrement in rheotactic performance in benthic armored catfish, but not epibenthic blind cavefish and that, this effect is associated with a measurable difference in the mobility of these two species.
MATERIALS AND METHODS

Experimental Overview

We compared the rheotactic performance of a benthic siluriform armored catfish (Corydoras julii) to that of the epibenthic characid blind cavefish under identical test conditions. Fish of both species were evenly divided into a lateral line-deprived and sham treatment groups. To rule out possible streamwise biases and other effects caused by simultaneous testing of multiple fish, both species were tested individually in an enclosed area of equal width, length and height (25 cm). A repeated measures design was used in each treatment group to determine each fish’s response to an ascending series of flow speeds from 0-10 cm s\(^{-1}\) in a spatially uniform flow field. Both species were also tested in the dark under IR light to eliminate visual cues and to maximize the potential for reliance on non-visual senses. Their behavior was video recorded and automatically tracked to document differences in mobility and to determine the effects of lateral line deprivation on rheotactic performance, both in terms of the degree to which they oriented upstream at different flow speeds and the minimum (threshold) flow speed required to elicit a rheotactic response.

Experimental Animals

All fish were obtained from commercial aquarium suppliers, and housed in 76-liter tanks (up to 8 fish per tank) at ambient temperature (21-23°C) on a 12:12, light/dark cycle. Fish were fed daily with commercially-available tetramin flakes and pellets. In total, 16 blind cavefish (42-50 mm in total length) and 13 armored catfish (34-44 mm in total length) were used. All protocols for the maintenance, care and experimental use of the animals in this study were approved by the Bowling Green State University Institutional Animal Care and Use Committee.
Experimental Set-Up

Fish were tested in the working area (25 x 25 x 25 cm) of a flow tank designed to produce spatially uniform flows (Vogel and Labarbera, 1978). The working area was constructed out of translucent Plexiglas with flow-through tank dividers (Penn Plax, Hauppauge, NY, USA) on the upstream and downstream ends. The main body of the flow tank was a rectangular channel (154 x 28 x 35 cm). Water was cycled via a polyvinyl chloride (PVC) tube (20.3 cm in diameter) that was attached to both ends. Unidirectional flow was produced using a chem-stirrer (IKA Laborteknik RW 20DZM, Staufen, Germany) attached to a 12.7 cm aluminum impellor blade. Experiments were conducted in the dark (vision disabled) under upwelling infrared (IR) light that was produced by a matrix of IR emitting diodes located directly below the working arena. A white plastic sheet on the bottom of the tank served as a diffuser for the upwelling light. Fish behavior was recorded with a video camera (with IR light detecting capabilities) located directly above the working arena.

Turbulence created by the impellers was reduced with three collimators: one coarse and one fine collimator at the upstream end and one coarse collimator at the downstream end. Coarse and fine collimators were constructed of large (1 cm x 3 cm) and small (0.5cm x 3 cm) soda straws, respectively. The fine mesh tank dividers at the upstream and downstream ends of the working area also helped to reduce turbulence. A video camera (Sony Handicam) was placed approximately 1 meter above the working area of the tank.

The flow speed associated with a range of different impeller speeds (60-600 RPM) was measured by videotaping traces of methylene blue dye delivered simultaneously via an array of 3 equally-spaced, 20 gauge syringe needles at 3 different elevations (6.25, 12.5, and 18.75 cm).
above the tank floor. Distanced moved from frame to frame by each dye streak was calculated and averaged across the working area. For each impeller speed, the flow speed was calculated as the average speed of movement across all dye traces for any given elevation. A regression line was fit to the flow speed versus motor speed data points across all elevations ($R^2 = 0.98$) and the motor speed required for each of the flow speed conditions was computed from the regression line. Dye streaks and Particle Imaging Velocimetry, as described elsewhere (Elder et al. 2013), confirmed that flow speed was spatially uniform in both horizontal and vertical planes with the exception of boundary layer (BL) effects near tank surfaces. BL velocity profiles were not measured but BL thickness was estimated from equations to describe BL development along a flat plate (equations 8.2 and 8.3 in Vogel 1996)(Table 1). For these estimates, the characteristic length is given as the distance from the upstream collimator to the upstream (25 cm) and downstream (50 cm) edge of the working arena.

*Disabling the Lateral Line System*

The lateral line was disabled (LL- group) by immersing fish in a 5.5 L treatment tank containing 0.5 g/L streptomycin sulfate for 3 hours prior to day 1 of testing (Baker and Montgomery, 1999b). The LL+ group of fish was also immersed in a sham treatment tank without streptomycin for the same period of time. Lateral line blockage was verified using DASPEI after testing. DASPEI is a vital dye that is taken up by the transduction channels of lateral line hair cells (Meyers et al., 2003; Van Trump et al., 2010). Absence of dye uptake is an indication that the transduction channels, and thus the functional viability of hair cells, have been effectively blocked by the streptomycin treatment. Fish were immersed in a 0.008% DASPEI solution for 10 minutes. Fish were then anesthetized in a 0.01% buffered solution of MS-222 and
observed under an epi-fluorescent stereomicroscope to determine the extent of dye uptake by
neuromasts of the lateral line.

*Experimental Procedures*

Fish were transferred from the sham or streptomycin treatment tank using a plastic lined
net to avoid damage to the lateral-line. Fish were given a 30 minute period (in the dark under IR
light) to acclimate before testing (videotaping) began. A series of 8 (0, 1, 2, 3, 4, 7, 8, 10 cm s⁻¹)
flow speeds were then presented to the fish in ascending order to reduce carry over effects
resulting from prior exposure to a higher flow. For the no-flow control, the impeller motor was
turned on with the gear disengaged to control for effects of motor noise. For each flow-speed, 2
minutes were allotted for the flow speed to ramp up to the desired speed before behavior was
recorded for 3 minutes. The flow speed was then accelerated to the next speed and the process
was repeated until all speeds had been tested.

*Data Collection and Analysis*

Fish behavior in the flow tank was recorded at a rate of 5 frames s⁻¹, using a Sony
Handicam mounted above the center of the working area. The video was captured using video
capture software (Winnov, Version 3.3, Santa Clara, CA, USA) in a nearby room. Video was
then broken down into images and analyzed using custom written tracking software (Butail and
Paley, 2012) as well as the circular statistics toolbox (Berens 2009). The tracking software
utilized MATLAB’s image processing toolbox to first fit an ellipse around the body of each fish
and subsequently determine the centroid, major axis, and minor axis of each ellipse in a
Cartesian coordinate system relative to the video frame. This information was then used to
calculate the orientation of the fish relative to the upstream direction as well as the fish’s location
with respect to the walls of the tank. Because the elevation of the fish in the tank was not measured, there were potential depth-of-field errors in the estimated stream-wise and crosswise position of fish. Maximum possible errors were measured to be less than 4% of the stream/crosswise length of the test arena. Due to the benthic behavior of both species, however, errors due to large vertical excursions in the tank were likely minimal. All orientations and locations were verified by overlaying them on the original video and manually reviewing them in a frame-by-frame fashion. Finally, data were occasionally lost when the identifying characteristics of the fish were lost for several frames, e.g., when a fish swam in a vertical direction or when it rolled sideways. Thus the number of video frames per each flow speed trial ranged from a minimum of 777 to a maximum of 900 with an average of 898.

**Rheotactic Metrics**

Rheotactic performance was measured with two metrics: the rheotactic index (RI), and rheotactic threshold (RT). The rheotactic index is an overall measure of rheotactic performance that is criterion free. It is derived from the cumulative frequency distribution (CFD) of observed fish headings (Bak-Coleman et al., 2013). CFDs determined from observed fish headings are compared to the CFD based on a theoretical distribution in which the headings are uniformly distributed. RI is defined as the signed area between the theoretical and observed CFD, normalized so that it ranges between positive and negative one. A value of ±1 indicates that fish are oriented directly upstream (+1) or downstream (-1) in 100% of the samples, whereas a value of 0 indicates no upstream or downstream preference. RI near the maximum positive (+1) or negative (-1) values require there be a low angular dispersion of fish headings (i.e. a high vector strength) and a small angular deviation from either the upstream (positive RI) or downstream (negative RI) direction. If either the angular dispersion or deviation from the
upstream/downstream direction is less than ideal, the absolute value of the resultant RI will be less than its maximum value. For a complete description and evaluation of this metric, see Bak-Coleman et al. (2013).

The rheotactic threshold is based on the relationship between RI and flow speed and was defined as the lowest flow speed that causes the RI to rise two standard deviations above the mean RI measured for the no flow condition. No-flow RI values were first compared across treatment groups within each species to ascertain that there were no orientation biases. In the absence of any orientation biases (RI ~ 0), these values were then pooled for each species across treatment groups to determine the mean no-flow RI and the criterion for the rheotactic threshold (2 standard deviations above the mean). If the threshold value fell between two measured flow speeds, linear interpolation was used to estimate the threshold flow speed.

**Data Analysis**

All statistical analyses were performed using R (R Core Team, 2013). Generalized linear mixed models (GLMM) were used for the bulk of the analysis, as they enable both fixed (flow speed, lateral line condition, species) and random (individual) factors to be examined under the repeated measures design of this study. Full-factorial models looked for the effects of flow speed, lateral line condition, and species on various dependent variables. Post-hoc tests used sequential Dunn-Sidak to control for experiment-wise error arising from multiple comparisons. To test whether or not RI varied significantly between LL+ and LL- treatment groups in the no flow condition, an unpaired t-test was performed for each species. The no-flow RI data were then pooled to calculate the arithmetic mean and associated standard deviation for each species. A two-tailed unpaired t-test was used to determine the effect of lateral line deprivation on
rheotactic threshold. Rheotactic thresholds in armored catfish appeared to have unequal variance, so a one-tailed unpaired Welch’s t-test was employed. Upon finding a clear effect of lateral line deprivation in armored catfish, a stepwise descriptive discriminant analysis was performed to gain insight into how mobility differed between LL+ and LL- groups.
RESULTS

DASPEI Verification of Lateral Line Treatments

The utility of DASPEI as a means of verifying that the lateral line disruption was determined to be highly species-specific. Blind cavefish represented a “best-case” scenario showing strong DASPEI labeling of both canal and superficial neuromasts in sham-treated fish (Fig. 1), but only a handful of faintly labeled neuromasts (< 1%) in streptomycin-treated fish (Fig. 1). By contrast, DASPEI labeling of armored catfish was difficult to interpret for two reasons. One, DASPEI was sometimes taken up by numerous, small structures, presumed to be chemosensory organs on the skin surface, making it difficult to discern small superficial neuromasts. Two, the more heavily pigmented skin of armored catfish (compared to the pigment-free skin of blind cavefish) made it more difficult to identify canal neuromasts, which are typically easier to identify than superficial neuromasts because of their smaller number, larger size and more stereotyped locations. Despite these difficulties, canal neuromasts were readily identifiable on lateral line enabled catfish, but not on lateral line disabled catfish. To improve observer reliability, agreement between two observers that canal neuromasts were present was required for inclusion of sham-treated fish into the lateral line intact (LL+) group. Likewise, agreement that no canal neuromasts were present was required for inclusion of streptomycin-treated fish into the lateral line disrupted (LL-) group. Only one LL+ fish (a blind cavefish) was excluded based on these criteria.

Behavioral Postures and Movements of Blind Cavefish and Armored Catfish

Casual observations of both blind cavefish and armored catfish indicate that both species maintained positions close to the substrate, but that their movements and postures relative to the
substrate were quite different (Fig. 2A,B). As previously documented, blind cavefish were observed to swim in a burst and coast fashion (Windsor et al., 2008; Tan et al., 2011), with the ventral surface of their body or head making light contact with the substrate, while keeping their paired pectoral fins largely off of the substrate (Fig. 2A). By contrast, armored catfish were more likely to remain stationary on the substrate, with their head propped upwards by their paired pectoral and pelvic fins (Fig. 2B). Armored catfish moved in a saltatory fashion, remaining motionless on the substrate for long periods of time (Fig. 2D), whereas blind cavefish swam continuously (Fig. 2C). GLMM of mobility indicated a significant interaction between flow speed and species ($F_{7, 167} = 2.387, P = .024$). While mobility remained somewhat constant across flow speeds in blind cavefish, in armored catfish it was very low at low flow speeds and increased at speeds above 3 cm s$^{-1}$ (Fig 3). Overall, blind cave fish were confirmed to be much more mobile than armored catfish ($F_{1,25} = 30.479, P = < .0001$). Finally, an effect of flow speed was observed suggesting that mobility is flow speed dependent, even after accounting for species and treatment effects ($F_{1,167} = 2.725, P = .011$).

**Rheotactic Performance**

We begin this section with an overall description of rheotactic performance in each species as a function of flow speed and lateral line treatment. This is followed by a summary of (1) the main effects, as determined from the GLMM analysis and (2) the factors that best explain treatment effects in armored catfish, as determined from the discriminant analysis. Frequency distributions (density plots) of fish headings re: upstream clearly show that rheotactic performance of both blind cavefish increases with flow speed (Fig. 4). Whereas lateral line deprivation appeared to have little effect on the degree to which blind cavefish orient upstream, subtle effects can be discerned for armored catfish at flow speeds < 3cm s$^{-1}$ (Fig. 4A). For
example, the distribution of headings in the LL+ fish at the 1 cm s$^{-1}$ condition are more leptokurtic and centered around 0° whereas the distribution for LL- fish are more platykurtic and negatively skewed towards about +45° (Fig. 4B). In the 2 cm s$^{-1}$ condition, LL- fish were slightly off from upstream and slightly more platykurtic.

As to be expected from orientation density plots (Fig. 4), the derived metric of rheotactic performance, RI, likewise increases with flow speed for each treatment group in both species (Fig. 5). Furthermore, the increase in RI is much steeper and the overall magnitude of this index is much higher in armored catfish compared to blind cavefish. The RI of fish in no flow did not differ significantly between LL+ and LL- individuals for either blind cavefish (unpaired t test: $t_{14} = -0.893, P = 0.1931$) or armored catfish (unpaired t test: $t_{10} = -0.617, P = 0.551$) (Fig. 5). Therefore, RIs in no flow were pooled across treatment groups in each species to yield an average RI that was very close to zero ($X \pm SE = 0.0018 \pm 0.011, N = 16$ for blind cavefish and $X \pm SE = -0.016 \pm 0.0548, N=12$ for armored catfish), suggesting no underlying directional bias. The criterion RI for determining RT was two standard deviations above the no-flow pooled mean ($=0.09$ for blind cavefish and 0.37 for armored catfish). Comparison of RTs between LL+ and LL- groups in blind cavefish revealed no significant effect of lateral line deprivation (two-tailed unpaired t test $t_{14} = 2.00, P = .06$). In fact, RT was slightly, though not quite significantly higher (poorer) in LL+ fish ($X \pm SE = 0.90 \pm 0.137, N = 8$) than LL- fish ($X \pm SE = 0.540 \pm 0.120, N = 8$) (Fig. 6). In contrast, RT was significantly lower (better) in LL+ armored catfish ($X \pm SE = 0.852 \pm 0.189, N=6$) than LL- fish ($X \pm SE = 1.86 \pm 0.285, N = 6$) (two-tailed Welch’s unpaired t test: $t_{8.69} = -2.506, P = 0.034$) (Fig. 6).

A GLMM analysis of RI indicated a significant interaction between flow, species, and lateral line condition ($F_{32.41}=3.966, p < .0001$). This interaction was characterized by a significant
decrease in performance in lateral line disrupted (LL-) armored catfish at the 1 cm s\(^{-1}\) \((t_{38} = -3.727, P = .001)\) and 2 cm s\(^{-1}\) condition \((t_{35} = -2.948, P = .001)\) (Fig. 5). At no other flow speeds was an effect of lateral line disruption observed (Table 2). Additionally, a significant main effect of flow was observed \((F_{8, 50} = 213.083, p < .0001)\) and characterized by increased rheotaxis as a function of flow speed (Fig 5). Finally, armored catfish exhibited significantly higher overall rheotactic performance than blind cavefish, regardless of flow speed \((F_{1,23} = 43.236, p < .0001)\) (Fig 5).

To examine the lateral line effects in armored catfish in greater detail, we performed a linear discriminant analysis (LDA) on the 1 cm s\(^{-1}\) dataset. This flow speed was chosen because it was between the rheotactic threshold speeds for LL+ and LL- groups, and would thus provide information regarding behavioral differences at a flow speed where the effect resides. The model generated by the LDA was able to successfully discriminate 12 out of 12 fish (100%). The LDA (Table 1, Fig. 7) indicated that the strongest discriminating factors were mean vector strength (Batshelet 1981) and stream-wise positional variability. Higher vector strength, a measure of orientation consistency, and lower stream-wise positional variability were more associated with lateral line enabled fish. Lateral line enabled fish were also associated with lower levels of mobility, as well as mean angles closer to the upstream direction. Taken together, lateral line enabled fish were characterized by decreased movement and increased rheotactic performance. In fact, RI is a nearly a linear function of mobility in lateral line enabled fish (Fig. 8).

**Space Utilization**

Spatial density plots of the combined frequency with which different fish spend time in different regions of the flow tank indicate that blind cavefish (Fig. 9A) and armored catfish (Fig.
9B) utilized space differently, especially at the lower flow speeds. For example, blind cavefish spent more time near all four the walls of the arena than armored catfish. The wall-hugging behavior of blind cavefish is also evidenced by the quadramodal distribution of fish headings in the no-flow and low flow speed conditions (Fig. 4A). The peaks at $0^\circ$, $\pm 90^\circ$ and $\pm 180^\circ$ correspond to the orientation of the fish as it follows each wall, while keeping the long axis of its body nearly parallel to the wall surface (Sharma et al., 2009). GLMM indicated a significant decrease in wall proximity (distance in cm to nearest wall) as a function of flow speed for blind cave-fish ($F_{7, 98}=2.341, P = .030$). As flow speed increases, the peaks at $\pm 90^\circ$ appear to shift to $\pm 60^\circ$ and eventually become a single broad peak centered at $0^\circ$, which arises from upstream rheotactic behavior. Though not quite significant, GLMM indicated wall-proximity of blind cavefish was affected by an interaction between flow and lateral line condition ($F_{7, 98}=1.945, P = .070$). This is likely due to the wall-hugging tendencies of LL+ individuals at flow speeds less than $\sim 4$ cm s$^{-1}$, which appear to be slightly stronger than those of LL- individuals (Fig. 9). That is, LL+ fish exhibit strong wall-following around the entire perimeter of the arena for flow speeds up to 4 cm s$^{-1}$, whereas LL- individuals stop wall-following after around 1 cm s$^{-1}$, spending a higher percentage of their time near the upstream barrier. Finally, although not quite significant, GLMM indicated that ($F_{1, 14}=3.313, P = .090$), LL+ individuals may be closer to walls overall.

In addition to observed wall-following behaviors in blind cavefish, the spatial density plots show that both species move further upstream as flow speed increases (Fig. 9). In particular, armored catfish (especially LL+ fish) appear to have a distinct preference for the downstream end of the tank at low flow speeds.
DISCUSSION

Our study is the first to compare rheotactic behavior of a sedentary and non-sedentary species under identical test conditions. This study provides evidence that immobility and, by implication, the degree to which fish are coupled to the substrate, are important factors in determining the sensory basis of rheotaxis. This study shows that lateral line deprivation caused a decrease in the rheotactic performance of visually-deprived armored catfish, especially at low flow speeds. In contrast, lateral line deprivation caused no effect in blind cavefish at any flow speed, suggesting that these fish rely more heavily on other non-visual sensory modalities, namely tactile and vestibular cues.

Context-Dependency of Multisensory Cues

These findings can be interpreted in terms of the sensory cues available under different flow and behavioral conditions. As both vestibular and optic flow cues are generated during downstream displacement, fish that exhibit substrate coupling will not have access to these cues at flow speeds insufficient to cause displacement. Moreover, substrate coupling should increase the signal to the lateral line as it maximizes flow across the skin surface, which, in turn, can provide fish with information about current speed and direction (Chagnaud and Coombs, 2014). Conversely, fish that are not tightly coupled to the substrate will be more readily displaced downstream, thus generating useful optic flow and vestibular cues at the cost of a reduced stimulation of the lateral line. Thus, sedentary behavior may promote reliance on the lateral line by increasing the stimulus to the lateral line while reducing sensory redundancy provided by other modalities. Furthermore, the benthic positioning of many sedentary fish ensures that they
will be exposed to non-uniform flows in the vertical plane – i.e. to velocity gradient in the boundary layer between the substrate and the surrounding water.

For fish that are mobile, other factors may also reduce the utility of the lateral line. For example, the octavolateralis efferent system is known to inhibit the lateral line during active swimming, presumably to reduce the effects of self-generated noise (Flock and Russel, 1976). For blind cavefish, which swim in a burst and coast fashion, the information to the lateral line is thus intermittent, being theoretically suppressed during the burst but not coast phase of the swim cycle. Evidence for this effect is the decreased obstacle avoidance ability of blind cavefish during the burst phase of the swim cycle (Windsor et al., 2008). In the context of rheotaxis, the absence of continuous lateral line sensory feedback could cause heading estimation errors to accumulate at a faster rate. During frequent periods of immobility in armored catfish, the octavolateralis efferent system is theoretically not activated and thus, these fish presumably have access to continuous lateral line feedback. Moreover, when sedentary, these fish do not require information about their own body movements in order to determine the speed and direction of the surrounding current. That is, the net movement between the fish and the surrounding water (the stimulus to the lateral line) is always caused by the movement of the surrounding water alone. In contrast, the net motion for mobile fish like blind cavefish involves relative movements between the water and the fish. Thus, these fish need to factor out body motion (self-generated or exogenously imposed) in order to determine the speed and direction of the surrounding current.

The results of our discriminant analysis (Table 1, Fig. 7) on armored catfish are largely consistent with the overall idea that substrate coupling and immobility enhance lateral line-dependent rheotaxis. That is, measures relating to positional stability indicated increased positional stability in LL+ fish when compared to LL- fish. From a theoretical perspective,
maintaining a stable position and coupling to the substrate maximizes flow across the skin surface, thereby enhancing the stimulus to the lateral line. By contrast, such a behavioral tendency would eliminate vestibular cues at low flow speeds, as the flow is insufficient to displace the fish downstream.

The increased positional stability in lateral line intact catfish indicate that these fish may be adopting a motionless strategy to maximize the stimulus to the lateral line. Further indications of this strategy can be seen by the decrease in mobility of LL+ fish when switched from the no-flow to the 1 cm s\(^{-1}\) flow condition (Fig. 3). By contrast, LL- fish may be allowing themselves to be displaced downstream in order to gain access to vestibular cues. Alternatively, they may be unable to maintain position without access to lateral line cues. Future work to test these possibilities by examining differences in LL+ and LL- swimming kinematics would be of great value to understanding how animals might alter their behavior based on the availability of sensory information.

As predicted, the armored catfish results of this study are consistent with lateral line deprivation effects reported in other species with similar benthic/sedentary behaviors (Montgomery et al., 1997; Baker and Montgomery, 1999a). In addition, Arnold (1969) provides some evidence that benthic flatfish (*Pleuronectes platessa*) also rely on lateral line information, as rheotactic responses were observed in the dark at flow speeds that did not displace the fish downstream. Similarly, epaulette sharks (*Hemiscyllium ocellatum*) exhibit rheotaxis when they are located on the benthos, but not when they are higher in the water column (Peach, 2001). Finally, our blind cavefish results are consistent with the absence of lateral line effects observed in other species with non-sedentary behaviors, such as the surface-dwelling giant danio (Bak-Coleman et al., 2013). In giant danio, lateral line deprivation did not cause a significant reduction
in the rheotactic performance of visually-deprived giant danio; however, it did alter the spatiotemporal dynamics of rheotactic behavior. Thus, while these fish can obviously compensate for the loss of lateral line information, they rely on different sensorimotor strategies to do so.

Although the lateral-line dependent effect observed in this study is similar to that observed in other benthic or sedentary species (Montgomery et al., 1997), the size of the effect in armored catfish (~ 1 cm/s shift in threshold) was relatively small compared to that reported for other species. That is, lateral line deprivation caused the rheotactic threshold of bald notothen to increase from 2 to 8 cm s\(^{-1}\), a shift of 6 cm s\(^{-1}\) (Montgomery et al., 1997; Baker and Montgomery, 1999b). The deprivation effect was somewhat smaller in torrentfish (a shift of ~2 cm s\(^{-1}\)) (Montgomery et al., 1997), but nevertheless approximately twice that observed for armored catfish. There are at least two possible factors that could contribute to effect size differences. One is study-specific differences in how rheotactic performance was measured (see discussion of Methodological differences below) and the other is species-specific differences in the ability of fish to hold station (stay coupled to the substrate) (Webb, 1989; Blake, 2006). The ability of benthic fish to withstand downstream displacement depends on a number of different factors, including the mass and specific gravity of the fish, whether or not a gas-filled swimbladder is present, and other morphological and behavioral adaptations (e. g. body shape, fin position, oral suckers etc.) for remaining coupled to the substrate (Blake, 2006). It is presently unclear how or whether armored catfish, torrentfish and bald notothen differ in their station holding abilities, as comparable measures of these abilities (e. g. slip speed and critical swim speed, Webb, 1989; Pavlov, 2000) are unavailable. However, it is noteworthy that the species with the largest threshold shift, the bald notothen, was considerably larger (14 – 21 cm in length) than the other
two species (3 – 5 cm in length). Based on total mass alone, the bald notothen might be predicted to better withstand downstream displacement than the other species and thus, to rely more heavily on lateral line information over a wider range of flow speeds.

Methodological Differences Impacting Observed Lateral Line Effects

Inconsistent with the predicted lateral line effects along the sedentary/non-sedentary dichotomy are three studies on non-sedentary fish, all of which showed decrements in rheotactic performance after blocking the lateral line in either larval zebrafish (Suli et al., 2012; Olszewski et al., 2012) or blind cavefish (Baker and Montgomery, 1999b). However, these studies differed from the present study in several important ways, including how flow was generated, the number of fish tested simultaneously, the shape of the flow tank, how the lateral line was blocked and developmental stage of the fish (larval vs. adult).

In terms of flow generation, the present study utilized an impeller to circulate water in a closed-system circuit of nearly constant diameter throughout to minimize spatial heterogeneities in the flow (Vogel and LaBarbera, 1978). Under such spatially uniform flow conditions, fish displaced downstream are more likely to move at the same speed as the surrounding water, thus minimizing lateral line cues, while maximizing vestibular, tactile and optic flow cues (if visual conditions permit). Under spatially non-uniform conditions (e. g. turbulence, velocity gradients), it is possible for the fish and the surrounding water to be moving at different speeds and directions, thus enhancing the stimulus to the lateral line. Furthermore, the displacement of the fish in directions other than downstream could generate vestibular noise that may interfere with the utility of vestibular cues. Unlike the present study, Suli et al. (2012) and Baker and Montgomery (1999a) utilized pumps to generate flow and these likely involved small-diameter
inlet and outlet connections to the main flow chamber. Unpublished PIV data from our lab suggests that such an arrangement (even with collimators present) would likely create a high-velocity stream down the center of the tank, low-flow in surrounding regions and steep velocity gradients in between. Furthermore, the third study (Olsiezewki et al., 2012), which employed a circular tank with a drain in the center, produced measureable velocity gradients, with velocity increasing in a radial direction towards the center of the tank. Thus, it is possible that the presence of velocity gradients may have facilitated lateral-line dependence in these cases. As fish in a natural setting experience a wide range of flow heterogeneity, study of lateral line functioning under such situations would be an important direction to pursue.

Tank-shape and the simultaneous testing of groups of fish may also have affected the probability of lateral line effects in previous studies (Baker and Montgomery, 1999a; Suli et al., 2012). These studies tested either 5 (Baker and Montgomery, 1999b) or 20 (Suli et al., 2012) fish at a time in a tank that was much longer in the stream-wise than cross-stream direction. These conditions could have led to packing density effects that caused fish to align more frequently along the streamwise axis of the tank. Under these circumstances, lateral line effects could be interpreted as a disruption in the abilities of fish to maintain their orientation and distance with respect to other fish in a confined area. Indeed, the lateral line has been shown to play a role in maintaining inter-fish distances (Partridge and Pitcher, 1980) and orientations (Faucher et al., 2010) in schooling fish. Furthermore, lateral line deprivation results in increased collisions among schooling fishes (Partridge and Pitcher, 1980). In this regard, it is worth noting that Olszewski et al. (2012) tested solitary fish in a circular arena and thus, in this case, tank shape/group effects (but not velocity gradients) can be ruled out as a potential explanation for observed lateral line effects.
Another key difference between studies is the way in which the lateral line system was blocked. Blocking techniques used in rheotaxis studies fall into two general categories: (1) pharmacological techniques in which fish are immersed in a solution of either cobalt chloride or aminoglycoside antibiotics (typically streptomycin or gentamicin) to block the transduction channels of lateral line hair cells and (2) physical ablation techniques in which the skin surface of the fish is scraped. Both cobalt chloride and streptomycin have been used in numerous studies to block the entire lateral line system (i.e. both canal and superficial neuromasts) (Karlsen and Sand, 1985; Blaxter and Fuiman, 1989; present study), whereas gentamycin and skin scrapes have been used to block either canal or superficial neuromasts, respectively, while leaving the remaining submodality intact (Song et al., 1995; Montgomery et al., 1997; Baker and Montgomery, 1999a,b). Recent studies have questioned the validity of gentamycin as a selective blocker of canal neuromasts, since both canal and superficial neuromasts of gentamicin-treated fish failed to take up DASPEI, indicating that hair cell transduction channels were compromised in both submodalities (Van Trump et al., 2010). For this reason it is difficult to reconcile results from different treatment groups in the blind cavefish study by Baker and Montgomery (1999b). That is, gentamicin treatments caused no decline in rheotactic performance (also confirmed by Van Trump and McHenry, 2012), whereas cobalt chloride and skin scrape treatments did. One possible explanation of what appears to be an irreconcilable set of results is that both cobalt chloride and skin scrape treatments caused more than just sensory deprivation effects – i.e. more global effects at the behavioral level, perhaps due to cobalt chloride toxicity (Janssen, 2000) and the repeated handling and anesthesia required for the skin scrapes (Montgomery and Baker, 1999b).
As a final point of difference between studies, both zebrafish studies were performed on larval fish (Suli et al., 2012; Olszewski et al., 2012) and it is unclear how the relative importance of various sensory modalities changes throughout the life history of fishes. In adults of a closely related species, giant danio, no effect of lateral line deprivation was observed (Bak-Coleman et al., 2013). In this regard, it is interesting to point out that the specific gravity of larval zebrafish decreases at about the same time as the swimbladder inflates (Robertson et al., 2007). In response to predation by suction predators, this change in specific gravity was estimated to decrease the suction-flow stimulus to the lateral line by as much as 80%, indicating that adult zebrafish may be less dependent on their lateral line than larval fish (Stewart and McHenry, 2011). Similarly, the stimulus to the lateral line during rheotaxis may also be decreased following a developmental change in specific gravity, creating lateral line dependence in larval, but not adult fish. Future studies of how the sensory basis of rheotaxis changes during development, especially during critical transition phases like the inflation of the swimbladder, are needed.

**Mechanisms Underlying Lateral Line Facilitated Rheotaxis**

Previous work has suggested the superficial, but not canal neuromasts of the lateral line are responsible for rheotaxis in slow flows (Montgomery et al., 1997; Baker and Montgomery, 1999a; Baker and Montgomery, 1999b). As streptomycin does not selectively block superficial neuromasts, the present study is unable to evaluate the claim that the superficial but not canal neuromasts facilitate rheotactic behavior in armored catfish. However, superficial neuromasts are known to respond primarily to low-frequency flows, perhaps all the way down to DC flow (Chaugnaud and Coombs, 2014). Thus, it is reasonable to assume that flow detection in this low
frequency range is enhanced in sedentary fishes, if only because these fishes are not generating self-generated flow noise that could interfere with the detection of slow ambient currents.

*Space Utilization During Rheotactic Behaviors*

As noted previously, both blind cavefish and armored catfish spend more time upstream than downstream at the higher flow speeds, when both species are presumably relying on vestibular and/or tactile cues instead of lateral line cues. At low flow speeds, when lateral line information is most likely to be available, LL+ armored catfish show a distinct preference to stay near the downstream end of the tank. In this respect, it is well known that boundary layer thickness increases as a function of increasing distance from the leading (upstream) edge of a flat plate in flow and also with decreasing flow speeds (Vogel, 1996). Thus, it is to be expected that boundary layer thickness along the substrate of the flow tank will vary as a function of flow speed and streamwise location in the tank (Table 3). As such, it is possible that boundary layer thickness plays a role in the spatial preference of armored catfish – perhaps to maximize the stimulus to the lateral line in the vertical plane, where the velocity gradient of the boundary layer resides.

In contrast to armored catfish, which exhibit downstream spatial preferences, in slow flows, blind cavefish exhibit distinct wall following behavior – not only for slow flows (< ~ 4 cm s\(^{-1}\), but also in no flow (Fig. 9A). This behavior is evidenced by characteristics modes at 0°, ±90°, and ±180° in the frequency distribution of orientations (Fig. 4A) as well as a tendency to take up positions near all four boundaries of the working area (Fig. 9A). Wall-following is a well-documented behavior that occurs in visually deprived animals and is believed to be exploratory in nature (Sharma et al., 2009).
When fish are exposed to a novel environment in flow, they may be forced to make a behavioral choice between wall-following (thigmotaxis) and rheotaxis. The thigmotactic preferences appeared to be slightly more distinct in LL+ blind cavefish, where the modes were more pronounced and persisted into higher flow speeds than LL- fish. This is consistent with evidence that wall following behavior is mediated, at least in part, by the lateral line in blind cavefish (Patton et al., 2010, Windsor et al. 2011). This provides some behavioral evidence, consistent with the DASPEI anatomical results, that streptomycin did in fact disrupt the lateral line in blind cavefish.

Potential trade-offs between thigmotaxis and rheotaxis are not well understood, and it remains possible that rheotactic performance was suppressed at these low flow speeds in lateral line enabled individuals. While this may account for the slight (but statistically insignificant) increase in rheotactic performance in lateral line disabled individuals (Fig. 5, Fig. 6), the effect is subtle and unlikely to account for the large shift (~ 4 cm s\(^{-1}\)) in rheotactic thresholds previously noted for blind cavefish (Montgomery et al., 1997; Baker and Montgomery, 1999a). Furthermore, lateral line disabled fish in previous studies exhibited no evidence of rheotactic behavior below 16 cm s\(^{-1}\), whereas the present study clearly shows that lateral line disabled fish exhibit some level of rheotaxis at all flow speeds.

However, it is possible that lateral-line mediated wall-following behaviors could have masqueraded as lateral-line dependent rheotactic behaviors in previous studies of blind cavefish (Baker and Montgomery, 1999b). That is, wall-following behavior in combination with the rectangular shape of the tank may have resulted in a higher incidence of wall following along the streamwise compared to the cross-stream walls of the tank. In flow, the incidence of wall-following behaviors in the upstream direction would be expected to be even further inflated,
since fish are likely to spend more time swimming upstream than downstream. The current study on blind cavefish reduces the possibility of streamwise bias since a square arena was used. Nevertheless, the strong proclivity of blind cavefish to follow walls could very well have suppressed rheotactic tendencies in this study. Future studies in which wall-following tendencies are reduced by giving fish ample time to acclimate to a novel environment are needed to rule out this possibility.

Conclusion

To conclude, this paper presents strong evidence that rheotaxis in slow flows (<3 cm s⁻¹) is dependent upon the lateral line under conditions unlikely to provide useable vestibular, optic flow or tactile slippage cues – i.e. in a species (armored catfish) that is sedentary and coupled to the substrate. In contrast, rheotaxis was found to be independent of the lateral line in a species (blind cavefish) that likely minimizes useable lateral line cues by exhibiting high levels of mobility near the substrate. These results help to resolve some of the discrepancy that has persisted in the literature. Future work under flow conditions that maximize flow speed heterogeneities in the horizontal plane may reveal increased importance of the lateral line even in non-sedentary species of fish. Finally, while this study establishes that the lateral line is utilized by benthic fish that rest motionless on the substrate, the mechanisms by which fish use lateral line information in rheotaxis is still poorly understood (Coombs and Montgomery, In Press).
REFERENCES


### APPENDIX A: TABLE 1

Table 1: Descriptive Discriminant Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficients</th>
<th>Mean</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standardized</td>
<td>Correlation</td>
<td>LL+</td>
<td>LL-</td>
</tr>
<tr>
<td>Position&lt;sub&gt;str&lt;/sub&gt;</td>
<td>2.72</td>
<td>-0.13</td>
<td>7.99</td>
<td>9.21</td>
</tr>
<tr>
<td>Position&lt;sub&gt;crw&lt;/sub&gt;</td>
<td>1.36</td>
<td>0.46</td>
<td>13.65</td>
<td>9.32</td>
</tr>
<tr>
<td>STD&lt;sub&gt;str&lt;/sub&gt;</td>
<td>-5.25</td>
<td>-0.48</td>
<td>2.42</td>
<td>4.86</td>
</tr>
<tr>
<td>STD&lt;sub&gt;crw&lt;/sub&gt;</td>
<td>-1.18</td>
<td>-0.08</td>
<td>3.42</td>
<td>3.73</td>
</tr>
<tr>
<td>VS</td>
<td>-5.46</td>
<td>0.38</td>
<td>0.57</td>
<td>0.38</td>
</tr>
<tr>
<td>Orientation</td>
<td>-2.60</td>
<td>-0.71</td>
<td>8.74</td>
<td>46.29</td>
</tr>
<tr>
<td>Mobility</td>
<td>-2.39</td>
<td>-0.48</td>
<td>0.58</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Position<sub>str</sub>, average streamwise position (cm); Position<sub>crw</sub>, average cross-stream position (cm); STD<sub>str</sub> = standard deviation of streamwise position (cm); STD<sub>crw</sub> = standard deviation of cross-stream position (cm); VS = Vector strength of orientation distribution. Orientation, mean angle(°) re: upstream; Mobility (cm s<sup>-1</sup>). Magnitude of standardized coefficients indicates relative importance in the discriminant function. Correlation coefficients indicate correlation between individual scores for a given variable and the corresponding score on canonical 1.
### APPENDIX B: TABLE 2

**Table 2:** Post-hoc comparison of rheotaxis between LL+ and LL- fish

<table>
<thead>
<tr>
<th>Flow (cm s(^{-1}))</th>
<th>Species</th>
<th>Contrast Estimate (RI(<em>{LL^+}) - RI(</em>{LL^-})) ± 95% C.I.</th>
<th>S.E.</th>
<th>t(df)</th>
<th>Adj. Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>AC</td>
<td>-0.056 ± 0.183</td>
<td>0.090</td>
<td>-0.625(31)</td>
<td>0.537</td>
</tr>
<tr>
<td></td>
<td>BCF</td>
<td>-0.021 ± 0.159</td>
<td>0.078</td>
<td>-0.274(32)</td>
<td>0.786</td>
</tr>
<tr>
<td>1</td>
<td>AC</td>
<td><strong>0.269 ± 0.146</strong></td>
<td><strong>0.072</strong></td>
<td><strong>3.727(38)</strong></td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td></td>
<td>BCF</td>
<td>-0.049 ± 0.0127</td>
<td>0.063</td>
<td>-0.781(38)</td>
<td>0.439</td>
</tr>
<tr>
<td>2</td>
<td>AC</td>
<td><strong>0.245 ± 0.169</strong></td>
<td><strong>0.083</strong></td>
<td><strong>2.948(35)</strong></td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td></td>
<td>BCF</td>
<td>-0.100 ± 0.147</td>
<td>0.072</td>
<td>-1.384(35)</td>
<td>0.175</td>
</tr>
<tr>
<td>3</td>
<td>AC</td>
<td>-0.001 ± 0.138</td>
<td>0.068</td>
<td>-0.010(41)</td>
<td>0.992</td>
</tr>
<tr>
<td></td>
<td>BCF</td>
<td>-0.019 ± 0.121</td>
<td>0.060</td>
<td>-0.315(41)</td>
<td>0.754</td>
</tr>
<tr>
<td>4</td>
<td>AC</td>
<td>0.012 ± 0.113</td>
<td>0.056</td>
<td>0.206(41)</td>
<td>0.838</td>
</tr>
<tr>
<td></td>
<td>BCF</td>
<td>0.016 ± 0.100</td>
<td>0.050</td>
<td>0.325(41)</td>
<td>0.747</td>
</tr>
<tr>
<td>6</td>
<td>AC</td>
<td>0.074 ± 0.104</td>
<td>0.052</td>
<td>1.424(38)</td>
<td>0.862</td>
</tr>
<tr>
<td></td>
<td>BCF</td>
<td>-0.008 ± 0.090</td>
<td>0.044</td>
<td>-0.176(35)</td>
<td>0.862</td>
</tr>
<tr>
<td>0</td>
<td>AC</td>
<td>0.069 ± 0.104</td>
<td>0.050</td>
<td>1.374(22)</td>
<td>0.183</td>
</tr>
<tr>
<td></td>
<td>BCF</td>
<td>-0.019 ± 0.092</td>
<td>0.044</td>
<td>-0.431(21)</td>
<td>0.671</td>
</tr>
<tr>
<td>10</td>
<td>AC</td>
<td>0.033 ± 0.107</td>
<td>0.052</td>
<td>0.638(20)</td>
<td>0.530</td>
</tr>
<tr>
<td></td>
<td>BCF</td>
<td>-0.008 ± 0.094</td>
<td>0.045</td>
<td>-0.172(20)</td>
<td>0.865</td>
</tr>
</tbody>
</table>

Pairwise comparisons of rheotactic performance (RI) between LL+ and LL- individuals for both armored catfish (AC) and blind cavefish (BCF). Dunn-śidak corrections were used to control for experimentwise error. Bold rows indicate significance. Contrast estimates represent the actual difference between the two groups (i.e. RI\(_{LL^+}\) - RI\(_{LL^-}\)).
APPENDIX C: TABLE 3

Table 3: Calculation of boundary layer

<table>
<thead>
<tr>
<th>Flow Speed (cm s(^{-1}))</th>
<th>Characteristic Length (cm)</th>
<th>Local 'Re'</th>
<th>BL (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>2500</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>5000</td>
<td>1.8</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>10000</td>
<td>1.25</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>20000</td>
<td>0.9</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>25000</td>
<td>0.8</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>5000</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
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<td>10000</td>
<td>2.5</td>
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<tr>
<td>8</td>
<td>50</td>
<td>40000</td>
<td>1.25</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>50000</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Fig. 1 DASPEI Verification: DASPEI-labeled neuromasts in blind cavefish (BCF) and armored catfish (AC). Labeling of superficial and canal neuromasts in blind cavefish was strong for sham-treated (LL+) (left panel) individuals, but undetectable for streptomycin-treated (LL-)(right panel) individuals. In armored catfish, numerous small structures, believed to be chemosensory in nature, were labeled, making it difficult to distinguish these small structures from superficial neuromasts. Nevertheless, DASPEI labeling of canal neuromasts above the eye and along the trunk (outlined area) was observed in sham-treated, but not streptomycin treated individuals. 1 = Nares, 2 = Eye socket (BCF), Eye (AC)
**Fig. 2 Comparison of Body Posture:** Typical postures (A,B) and time waveforms of changes in orientation and position (C,D) of blind cavefish (A,C) and armored catfish (B,D) near the substrate at flow speeds of 1 cm s\(^{-1}\). Armored catfish move infrequently, tending to rest motionless on the substrate, while propped up on their pectoral and/or pelvic fins. Blind cavefish make transient contacts with the substrate while swimming non-stop, either parallel to the substrate or in a head-down posture.
Fig. 3 Comparison of Mobility: Mean + SEM as a function of flow for sham-treated, lateral line enabled (Solid) and streptomycin-treated, lateral line deprived (dashed) individuals of blind cavefish (red) and armored catfish (blue).
APPENDIX G: FIGURE 4

Fig. 4 Orientation Frequency Distributions: Distribution of fish orientations for sham (LL+) and streptomycin-treated (LL-) blind cavefish (BCF) and armored catfish (AC) at low (A) and high (B) flow speeds.
**APPENDIX H: FIGURE 5**

![Graph showing Rheotaxis and Flowspeed](image)

**Fig. 5 Rheotaxis and Flowspeed:** Mean RI and SEM as a function of flow speed for lateral line enabled (solid lines) and disabled (dashed lines) blind cavefish (squares) and armored catfish (circles). Dotted and dashed horizontal lines indicate threshold criteria (mean RI in the absence of flow + 2 standard deviations) for determining rheotactic thresholds in armored catfish and blind cavefish, respectively. While no effect of lateral line deprivation was observed in blind cavefish, a significant effect was observed in armored catfish at 1 and 2 cm/s flow speeds.
**APPENDIX I: FIGURE 6**

**Fig. 6 Rheotactic Threshold:** Rheotactic threshold for sham-treated, lateral line enabled (LL+) and streptomycin-treated, lateral line deprived (LL-) individuals of blind cavefish (BCF) and armored catfish (AC). Rheotactic thresholds were significantly elevated in LL- compared to LL+ individuals of AC but not BCF.
APPENDIX J: FIGURE 7

**Fig. 7 Discriminant Analysis:** Canonical plot resulting from 1 cm s\(^{-1}\) discriminant analysis. The discriminant analysis was successfully able to discriminate 100% of fish as either being lateral line enabled (blue diamonds) or disabled (red squares). All fish are plotted here, though nearly identical canonical values for pairs of fish obscured some of the markers.
**APPENDIX K: FIGURE 8**

**Fig. 8 Rheotactic Index and Mobility:** The relationship between rheotactic index and mobility in lateral line enabled AC (blue diamonds) and BCF (red squares). For AC, there is a strong relationship between rheotaxis and mobility at both 1 \( r = 0.93 \) and 2 \( r = 0.97 \) cm s\(^{-1}\). A similar relationship exists for blind cavefish in both 1 \( r = 0.73 \) and 2 \( r = 0.67 \) cm s\(^{-1}\), though the relationship isn’t quite as strong and the slope is much less steep.
APPENDIX L: FIGURE 9

Fig. 9 Spatial Density Plots: Spatial density of plots for blind cavefish (A) and armored catfish (B). Each plot represents the combined positional data across all individuals within a treatment group (LL+, lateral line enabled or LL-, lateral line disabled) and for any given flow speed.
APPENDIX M: IACUC APPROVAL

Dr. Sheryl Coombs
Biological Sciences
Bowling Green State University

Re: IACUC Protocol 13-009

Title:
Information Processing by the Lateral Line System of Fish

Dear Dr. Coombs:

On October 31, 2013 the above referenced protocol received final approval after review of the requested modifications by Designated Member Review. The modifications have been incorporated into the official copy of your protocol (see modifications below).

This approval expires on October 30, 2014, by which time renewal must be requested if you wish to continue work on the protocol. The Office of Research Compliance will send notification reminding you of the need for renewal in advance of that date.

Please have all members of your research team read the approved version of the protocol. Please also remember to keep a copy of the approved protocol in the animal facility room(s) in which your animals are housed and in any associated procedure rooms (contact the UAF staff for assistance in this regard).

Please consult with the staff of the Animal Facility about your requirements to get started on this project. Good luck with your project.

Sincerely,

[Signature]

Hillary Harris, Ph.D.
IACUC Administrator