RESEARCH ARTICLE

The spatiotemporal dynamics of rheotactic behavior depends on flow speed and available sensory information

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SUMMARY

Rheotaxis is a robust, multisensory behavior with many potential benefits for fish and other aquatic organisms. Visual (optic flow) cues appear to be sufficient for rheotaxis, but other sensory cues can clearly compensate for the loss of vision. Nevertheless, the nature of multisensory interactions and the relative contributions of different senses under varying conditions are poorly understood – largely because there is so little description of the actual behavior. Here, we examined the effects of different flow speeds and different sensory conditions on the spatiotemporal dynamics of rheotaxis. Although the overall ability of giant danio (*Devario aequipinnatus*) to head upstream is largely unaffected by either unimodal or bimodal deprivation of visual and/or lateral line senses, the spatiotemporal form of the behavior is altered in subtle ways. When deprived of vision, fish move further upstream, but the angular accuracy of the upstream heading is reduced. In addition, visually deprived fish exhibit left/right sweeping movements near the upstream barrier at low flow speeds. Sweeping movements are abolished when these fish are additionally deprived of lateral line information. These results indicate that fish adopt different sensorimotor strategies to compensate for the loss of one or more senses and that the nature of multisensory interactions is a complex function of flow speed.

Key words: current, flow, lateral line, optic flow, orientation, rheotaxis.

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INTRODUCTION

Fish and other aquatic organisms exhibit unconditioned orienting responses (rheotaxis) to water currents (Lyon, 1904; Arnold, 1974), which are typically in either an upstream (positive rheotaxis) or downstream (negative rheotaxis) direction. Rheotaxis confers many potential benefits, including directional guidance for migratory behaviors (Thorpe et al., 1981; Tytler et al., 1978), improved interception of downstream-drifting prey and odors (Kleerekoper, 1978; Gardiner and Atema, 2007), and energetic cost savings for fish attempting to maintain streamwise position in currents strong enough to displace them downstream (Montgomery et al., 1995).

Although not systematically studied across taxa, rheotaxis appears to be a widespread behavior, occurring across diverse species living in freshwater or marine microhabitats that vary widely in flow characteristics. For example, rheotaxis has been observed in both benthic (e.g. trout, torrentfish) and mid-water (e.g. dace) species that inhabit freshwater streams and rivers (Montgomery et al., 1997; Pavlov and Tjurjukov, 2005), as well as benthic (e.g. Lake Michigan mottled sculpin, sole) and mid-water (e.g. bluegill) species that inhabit freshwater or marine bodies of water with little to no current (e.g. small ponds) or currents with variable directions and speeds (e.g. lakes and oceans) (Hanson and Jacobson, 1984; Champalbert et al., 1994; Kanter and Coombs, 2003).

Rheotaxis is also a very robust behavior, taking place under a variety of sensory conditions. Optic flow cues appear to be sufficient for many species (Lyon, 1904), but not necessary, as rheotaxis persists in the absence of visual cues in species like the blind cavefish (Montgomery et al., 1997; Baker and Montgomery, 1999b). Non-

visual cues implicated in rheotaxis include tactile (Lyon, 1904; Baker and Montgomery, 1999a; Baker and Montgomery, 1999b), vestibular (Pavlov and Tjurjukov, 1993) and lateral line (Montgomery et al., 1997; Baker and Montgomery, 1999a; Baker and Montgomery, 1999b) cues. In the absence of lateral line cues, substantial reduction in rheotactic performance (to chance levels) has been observed in blind cavefish and several other species, but only at flow speeds less than ~5 cm s⁻¹ [~1 fish body length (BL) s⁻¹] (Montgomery et al., 1997). For flow speeds greater than this, no change in performance is observed. Additionally, odor cues have been shown to lower the minimum flow speed (rheotactic threshold) required to elicit rheotactic behavior (Baker and Montgomery, 1999b). Finally, larval zebrafish exhibit rheotactic behavior to extremely slow flows (<0.2 cm s⁻¹) and this behavior is significantly impaired in the dark – but not in the light – when the lateral line is pharmacologically blocked (Suli et al., 2012). Thus, it is clear that multisensory contributions to rheotaxis are complex and likely to depend on many factors, including flow speed, available sensory information, time of year, life stage/body size and behavioral context.

Unfortunately, our ability to assess the complex nature of multisensory contributions to rheotaxis is presently limited because there are so few descriptive data of the actual behavior across species. The lack of adequate descriptions is quite surprising given the widespread and fundamental nature of this behavior. Furthermore, quantitative characterizations of rheotactic behavior have largely relied on a single metric of group performance. Typically reported is the instantaneous proportion of fish (usually in a group of four or five fish) that are heading within some angular criterion (e.g.

±45 deg) of upstream (Pavlov and Tjurjukov, 1993; Montgomery et al., 1997; Baker and Montgomery, 1999a; Baker and Montgomery, 1999b). Unfortunately, the various angular criteria applied in these prior studies appear to be arbitrarily selected and they vary widely (from ±20 to ±90 deg of upstream). Furthermore, the step-wise increments of the group metric (e.g. steps of 0.2 for a group of five) limit its utility in providing fine-scale information about performance differences. Furthermore, many of these studies have used this metric without careful consideration of factors that may bias the fish's orientation, such as social preferences, tank shape, packing density, and sensory information from conspecifics.

Vector strength, which is a scalar measure of the coherence in the angular distribution of orientations (Batschelet, 1981), is another metric that has been used to quantify the rheotactic performance of solitary fish (Kanter and Coombs, 2003; Coombs and Grossman, 2006). However, vector magnitude by itself carries no information on direction, and thus the vector strength associated with a fish heading predominantly upstream could be equal to the vector strength associated with a fish heading directly downstream. Thus, additional metrics of vector direction must be used in combination with vector strength, such as vector orientation.

Finally, no single measure of rheotaxis captures the subtle changes in the spatiotemporal dynamics of rheotactic behavior resulting from reliance on different sensory cues. For example, unlike fish relying on visual (optic flow) cues, fish relying on non-visual cues do not have constant access to a stationary external frame of reference (e.g. surrounding river banks), unless they are a benthic fish and in constant contact with the river bed. Thus, when deprived of visual cues, mid-water fish may need to alter their behavior so that they are periodically in contact with the substrate to provide a (tactile) external frame of reference, as suggested previously (Lyon, 1904; Baker and Montgomery, 1999a). The effect of these fundamental differences in sensory input on behavioral output remains largely undocumented, except in the context of obstacle entrainment behaviors (Sutterlin and Waddy, 1975; Liao et al., 2003; Montgomery et al., 2003; Przybilla et al., 2010), which have a rheotactic component.

The primary goal of the present study was to provide a detailed descriptive account of how the rheotactic behavior of solitary fish changes over time and space under various flow and sensory conditions. In particular, we wanted to investigate the flow-speed dependence of rheotactic behavior when all senses are enabled, when either vision (V) or lateral line (LL) senses are disabled (V+/LL− and V−/LL+), and when both are disabled (V−/LL−) and only tactile, chemical and vestibular senses remain. Additionally, we evaluate a new metric, the rheotactic index (RI), which does not rely on *a priori* angular criteria and whose magnitude represents rheotactic performance and whose sign (positive or negative) represents swimming direction (upstream or downstream).

The results of this study show that rheotactic behavior of giant danio, *Devario aequipinnatus* (McClelland 1839), is very robust and persists even when both visual and lateral line senses have been disabled. Although the overall strength of rheotactic behavior is largely unaffected by either unimodal or bimodal deprivation of visual or lateral line information, the spatiotemporal form of the behavior is altered in subtle ways, suggesting that sensorimotor strategies are likewise altered to compensate for sensory loss.

MATERIALS AND METHODS
Overview of the experimental design
The behavior of solitary giant danio was video recorded and analyzed to examine the relative roles of vision and the lateral line on the rheotactic behavior at flow speeds above $1\,BL\,s^{-1}$, where vision is likely to play a dominant role, and below $1\,BL\,s^{-1}$, where the lateral line is believed to play a more substantial role (Montgomery et al., 1997). We used a mixed design with two within-subject factors (flow speed and visual condition) and one between-subject factor (lateral line condition). Flow speed had two levels (low and high speed, 3 and $7\,cm\,s^{-1}$), as did each sensory condition [vision enabled (V+) or disabled (V−) and lateral line enabled (LL+) or disabled (LL−)]. In addition, tests were run in a no-flow control condition to ensure that in the absence of flow, behavior was not biased in either the upstream or downstream direction. A repeated-measure, counterbalanced design was used to manipulate flow and visual conditions within each of the independent lateral line treatment groups (for further details, see 'Experimental procedures', below).

Experimental animals
Giant danio (4.8–5.8 cm in standard length) were obtained from commercial aquarium suppliers. Total lengths were not measured, but based on estimates from similar-sized fish, these were likely to be in the 6–7.3 cm range. These species are pelagic, stream-dwelling fish native to Asia and accustomed to living in small streams and rivers with fast water currents (McClure et al., 2006). All fish were housed communally in 20 gallon (~76 l) tanks at room temperature (21–23°C) on a 12 h:12 h, light/dark cycle. Fish were fed *ad libitum* commercial flake food (Tetramin Tropical) 6 days a week; on test days, fish were fed after all test sessions were completed. All protocols for the maintenance, care and experimental use of the animals in this study were approved by the Bowling Green State University's Institutional Animal Care and Use Committee.

Experimental set-up
Fish were tested in the working area (25×25×25 cm) of a flow tank similar to that described elsewhere (Vogel and LaBarbera, 1978). The working area was constructed out of translucent, non-reflective Plexiglas with non-reflective, flow-through tank dividers (Penn Plax, Hauppauge, NY, USA) on the upstream and downstream ends. The main body of the tank was a rectangular channel (154×28×35 cm) constructed of Plexiglas and filled to a depth of 25 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 Labortechnik RW 20DZM, Staufen, Germany) attached to a 12.7 cm aluminum impellor blade. For experiments conducted in the light (vision enabled), the experimental arena was lit with upwelling light from 15 W fluorescent bulbs as well as down-welling light from overhead fluorescent lighting. For experiments conducted in the dark (vision disabled), upwelling light was produced by a matrix of infrared (IR)-emitting diodes located directly below the working arena. A white plastic sheet on the bottom of the tank served as a diffuser for upwelling light sources. 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 course and one fine collimator at the upstream end and one course collimator at the downstream end. Coarse collimators were constructed of either large (1 cm) or small (0.5 cm) diameter soda straws, each 3 cm long. The fine mesh tank dividers at the upstream and downstream ends of the tank also helped to reduce turbulence.

Flow speed calibration
The flow speed associated with a range of different impeller speeds (60–600 r.p.m.) was measured by videotaping traces of Methylene

Blue dye delivered simultaneously *via* an array of three equally spaced, 20 gauge syringe needles at three different elevations (6.25, 12.5 and 18.75 cm) above the tank floor. Distance 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 fitted 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 equation.

Experimental procedures

Each fish underwent 2 days of tests: 1 day in the light under visible light (vision enabled) and the other in the dark under IR light (vision disabled). Fish were acclimated to the flow tank for a period of 10 min before testing at the no-flow condition began. Once the flow was turned on, fish were given another 10 min period to acclimate to each new flow speed before testing (videotaping) began. This time period falls well within the range of flow acclimation periods typically used in previous studies of rheotaxis (Webb, 1998; Pavlov et al., 1995; Baker and Montgomery, 1999a; Baker and Montgomery, 1999b) and provides some period of acclimation while minimizing the potential confounding effects of fatigue due to long acclimation periods in continuous flow. Each test session consisted of three, 3 min trials during which behavior was video recorded. Trial 1 was always the no-flow (control) condition, whereas trials 2 and 3 were either high (7 cm s^{-1}) or low (3 cm s^{-1}) flow conditions. The flow speed for each subsequent trial was adjusted at the end of each trial and this adjustment was followed by a 10 min interval to ensure that the desired flow speed was reached before the next test trial began. To prevent order effects, the order of light/dark test days was counterbalanced across fish within each treatment group and the order of high/low flow test conditions was counterbalanced both within and across fish.

Disabling the lateral line system

The lateral line was disabled (LL– group) by immersing fish in a 19 l treatment tank containing 0.5 g l^{-1} streptomycin sulfate for 3 h prior to day 1 of testing (Baker and Montgomery, 1999a). 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 day 2 of testing. Fish were immersed in a 0.008% DASPEI solution for 10 min. Fish were then anesthetized in a 0.01% buffered solution of MS-222 and observed under an epi-fluorescence stereomicroscope to determine whether fluorescent DASPEI dye had been taken up by the transduction channels of the lateral line hair cells (Meyers et al., 2003; Van Trump et al., 2010). The absence of fluorescent 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.

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 1.3 m 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 streamwise and crosswise position of fish. These errors were measured to be less than 4% of the streamwise/crosswise length of the test arena. 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 fish became temporarily untrackable for several frames, e.g. when fish swam in a vertical direction or when they rolled sideways. Thus, the sample size (no. of video frames per trial) ranged from a minimum of 657 to a maximum of 900 with an average of 890.

Rheotactic metrics

Six different metrics of rheotactic performance, all based on hundreds of samples of fish headings (see above), were computed: RI, vector strength (Batschelet, 1981), and the proportion of headings within ±10, 25, 45 or 90 deg of upstream. We refer to the proportion of headings within the strictest criterion (±10 deg) as rheotactic accuracy (RA). The RI was derived from the cumulative frequency distribution (CFD) of observed fish headings (red function in Fig. 1E). CFDs determined from observed fish headings were compared with the CFD based on a theoretical (uniform) distribution drawn from completely random headings (dashed blue function in Fig. 1E). RI is defined as the signed area between the observed and theoretical CFD, normalized so that it takes values between ±1. A CFD above the blue dashed line has a RI between 0 and +1, meaning that fish headings are biased in the upstream direction, whereas a CFD below the blue dashed line has a RI between 0 and −1, reflecting an overall downstream bias (Fig. 1E). A value of ±1 indicates that fish headed 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 requires that there be both a low angular dispersion of fish headings and a small angular deviation of the mean heading from either the upstream (positive RI) or downstream (negative RI) direction. If either the angular dispersion or deviation from the upstream/downstream direction is high, the absolute value of the resultant RI will be less than its maximum value.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 19 (SPSS Inc., Chicago, IL, USA). Spearman's rank correlation was used to compare various measures of rheotactic performance. A repeated measures MANOVA was used to analyze the effects of two within-group factors (flow speed magnitude, high or low; and visual condition, enabled or disabled) and one between-group factor (lateral line, enabled or disabled) on five dependent variables: RI, RA, standard deviation of streamwise and crosswise position (s.d.$_{str}$ and s.d.$_{cross}$), and mean streamwise position ($\bar{x}_{str}$) Before analysis, RI, RA and $\bar{x}_{str}$ were arcsine transformed to meet the assumptions of a normally distributed data set. Out of a total of 320 data points (5 independent variables×8 replicates×2 flow conditions×4 sensory conditions), seven outliers were identified. The outliers were retained but their values were replaced with the next highest values from other fish in the same condition. Across all eight conditions and five dependent variables (40 distributions total), only two were found to be significantly non-normal as assessed by Shapiro–Wilk's test (P<0.05). Given the large number of distributions tested, and
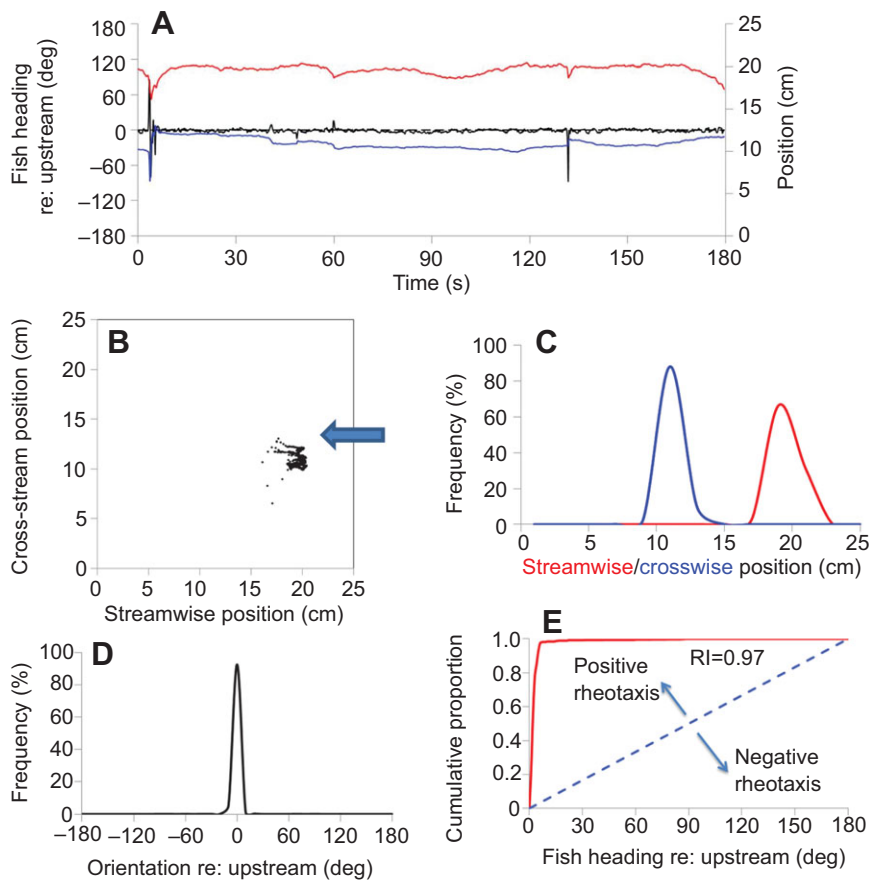
Fig. 1. Rheotactic performance of one out of four giant danio individuals that exhibited nearly perfect rheotaxis under high flow speed and V+ (vision-enabled) conditions. This fish was able to maintain an upstream orientation and nearly fixed spatial position within the tank for the entire test period of 3 min. (A) Temporal changes in the fish's heading (black function, left-hand *y*-axis) and either streamwise (red function, right-hand *y*-axis) or crosswise (blue function, right-hand *y*-axis) position in the test arena. (B) Scatter plot of crosswise *versus* streamwise position in the tank and (C) frequency distributions of crosswise (blue) and streamwise (red) positions. (D) Frequency distribution of the fish's angular headings re: upstream and (E) cumulative frequency distribution (CFD) drawn from the observed distribution in D (red function) and from a random (theoretical) distribution of headings (dashed blue function). The rheotactic index (RI) is the signed area encompassed between the observed (red) and theoretical (blue) CFD curves. A CFD above the blue dashed theoretical curve produces RI between 0 and +1, meaning that fish headings are biased in the upstream direction, whereas a CFD below the theoretical curve produces RI between 0 and −1, reflecting an overall downstream bias.

the robustness of MANOVA to deviations from normality, these distributions were included and no further transformations were made.

Because there were *a priori* expectations from the literature (Montgomery et al., 1997) that lateral line deprivation might have an effect under a limited set of conditions only – namely, at low but not high flow speeds – a discriminant analysis was performed to determine whether any of the four possible flow speed/visual combinations produced a significant discrimination of LL+ and LL– based on all five dependent variables. To compensate for increased Type 1 errors due to multiple comparisons, Dunn–Sidak corrections were used to set the significance level α at 0.012.

RESULTS

DASPEI verification of lateral line blocking technique
Sham-treated fish showed strong DASPEI labeling of both canal and superficial neuromasts, whereas those treated with streptomycin showed little if any labeling of either neuromast type. Some faint labeling of a few neuromasts (<1%) could be observed in a few cases, but this was generally restricted to a region surrounding the centrally located sensory epithelium and was presumed to represent slow endocytotic uptake by surrounding support cells rather than rapid uptake *via* transduction channels of sensory hair cells (Meyers et al., 2003; Van Trump et al., 2010).

Summary of main effects
A brief summary of the main effects is presented here, followed by a more detailed description of the results and the nature of the relationships between the independent and dependent variables. Multivariate tests indicated significant main effects for within-

subjects factors of flow speed ($F_{5,10}$=24.908, P<0.001, partial η^2=0.926) and visual condition ($F_{5,10}$=14.576, P<0.001, partial η^2=0.879) and no significant interaction effects (Table 1). Subsequent univariate tests indicated that switching from a low to a high flow speed had the effect of increasing rheotactic strength (RI and RA), while causing an upstream shift in the mean streamwise position ($\bar{x}_{str}$). Visual deprivation also resulted in an upstream shift in mean streamwise position as well as diminished RA and increased streamwise and crosswise variability (s.d.$_{str}$, s.d.$_{cross}$).

The only combination of visual/flow speed conditions in which a significant differentiation between LL+ and LL– fish could be found was the V–/low flow condition, where 74% of the variance was accounted for by the discriminant function (Table 2). As the strongest predictors of lateral line condition, RI and positional variability (both streamwise and crosswise) were higher in lateral

Table 1. Multivariate tests results from mixed MANOVA

Effect	d.f.	F	P	Partial η²	Observed power
Between-subjects					
Lateral line	5,10	1.969	0.169	0.496	0.431
Within-subjects					
Flow	5,10	24.908	**<0.001**	0.926	1.000
Vision	5,10	14.576	**<0.001**	0.879	1.000
Interactions					
Flow×LL	5,10	2.365	0.116	0.542	0.509
V×LL	5,10	0.388	0.846	0.162	0.110
Flow×V×LL	5,10	2.211	0.134	0.525	0.479

V, vision; LL, lateral line.
Bold values indicate significance at P<0.05.

Table 2. Results of discriminant analyses separating LL+ and LL– fish under different visual (V+/V–) and flow speed (low/high) conditions

						Standardized discriminant coefficients				
Condition	Wilk's λ	χ^2	d.f.	P	Canonical correlation	RI	RA	$\bar{x}_{str}$	s.d.$_{str}$	s.d.$_{cross}$
V–/low	0.260	15.49	5	**0.008**	0.860	1.25	–0.60	–0.51	1.07	1.22
V–/high	0.597	5.93	5	0.313	0.64	–2.0	1.52	1.42	–0.30	0.90
V+/low	0.488	8.25	5	0.143	0.72	0.55	–0.90	1.93	–1.87	1.13
V+/high	0.472	8.63	5	0.125	0.73	0.26	–0.38	0.99	1.24	–1.62

Bold values are significant at $P<0.012$ (Dunn–Sidak corrected for overall $\alpha=0.05$). Positive coefficients indicate traits that have higher values in LL– fish compared with LL+ fish. The absolute value of the coefficient is proportional to the predictive strength of each trait.

line-deprived fish than in lateral line-enabled fish. This counter-intuitive finding is likely related to a unique sweeping behavior observed in LL+ fish at the low flow speed (described later). Lateral line-deprived fish also showed weak tendencies for lower RA and streamwise positions that were further downstream.

The spatiotemporal dynamics of rheotactic behavior under ideal and no-flow (control) conditions

Near-maximum rheotactic performance (RI>0.95) was observed in 4 out of 16 individuals at the high flow speed only; all of these individuals had access to visual cues, but only half of them had access to lateral line cues. Under the V+/high flow speed set of conditions, the available sensory information is presumably better or more salient than that available under other sensory/flow speed combinations. Best performance in these conditions was characterized by very little temporal variation in upstream heading, streamwise (red functions) position, or crosswise (blue functions) position in the working arena, as illustrated for a single individual in Fig. 1A. As a consequence, fish positions for this individual were restricted to a narrow region of space (Fig. 1B,C). Fish headings were likewise restricted to a narrow region of upstream directions centered on 0 deg (Fig. 1D), resulting in a RI (0.97) very near its maximum value (1.0) for positive rheotaxis (Fig. 1E).

During the no-flow condition, fish headings and positions in the tank varied widely over time, as illustrated for one individual in Fig. 2A. The average swim speed of fish in the no-flow condition was $4.22\,cm\,s^{-1}$ [95% CI (3.8, 4.6)]. The V–/LL+ fish in this example
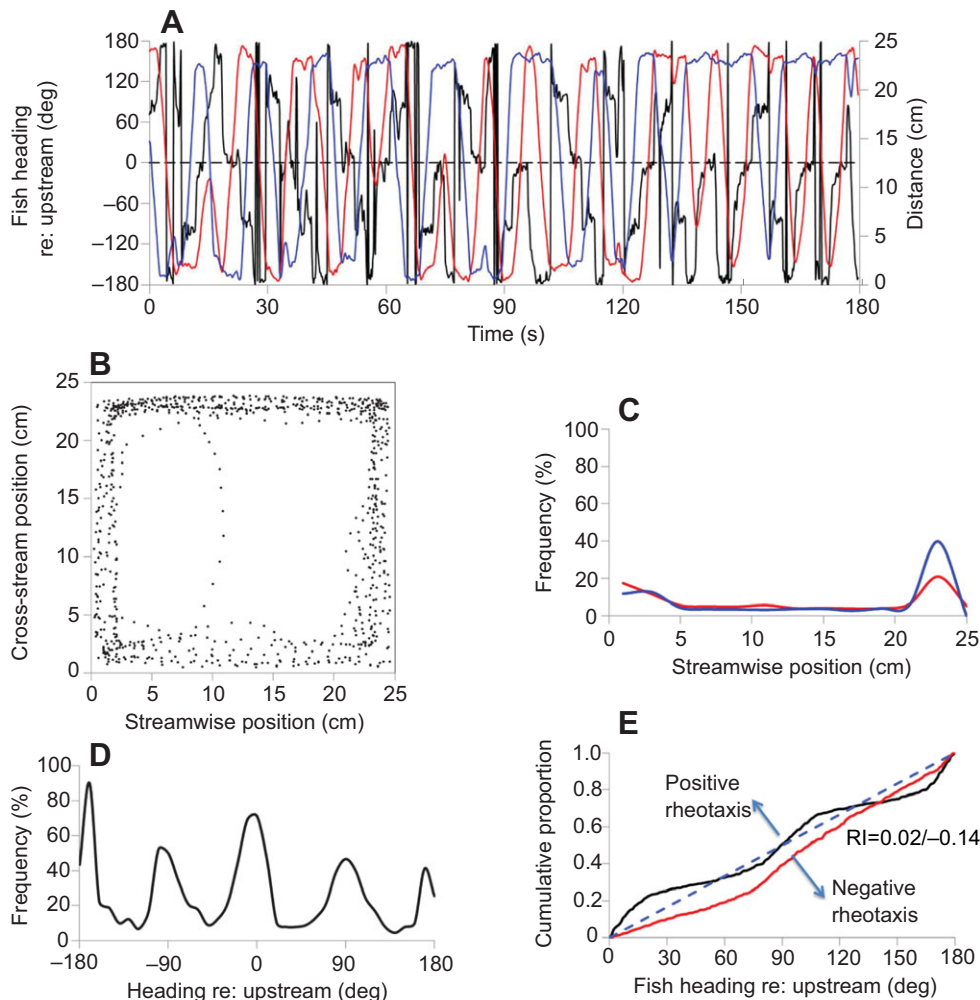

Fig. 2. Behavioral performance of one individual in the absence of flow illustrating the tendency of visually deprived individuals to follow the walls of the test arena. (See Fig. 1 for further details.) In E, the CFDs of two sensory conditions in the same LL+ individual are compared: vision enabled (V+, red) and disabled (V–, black).
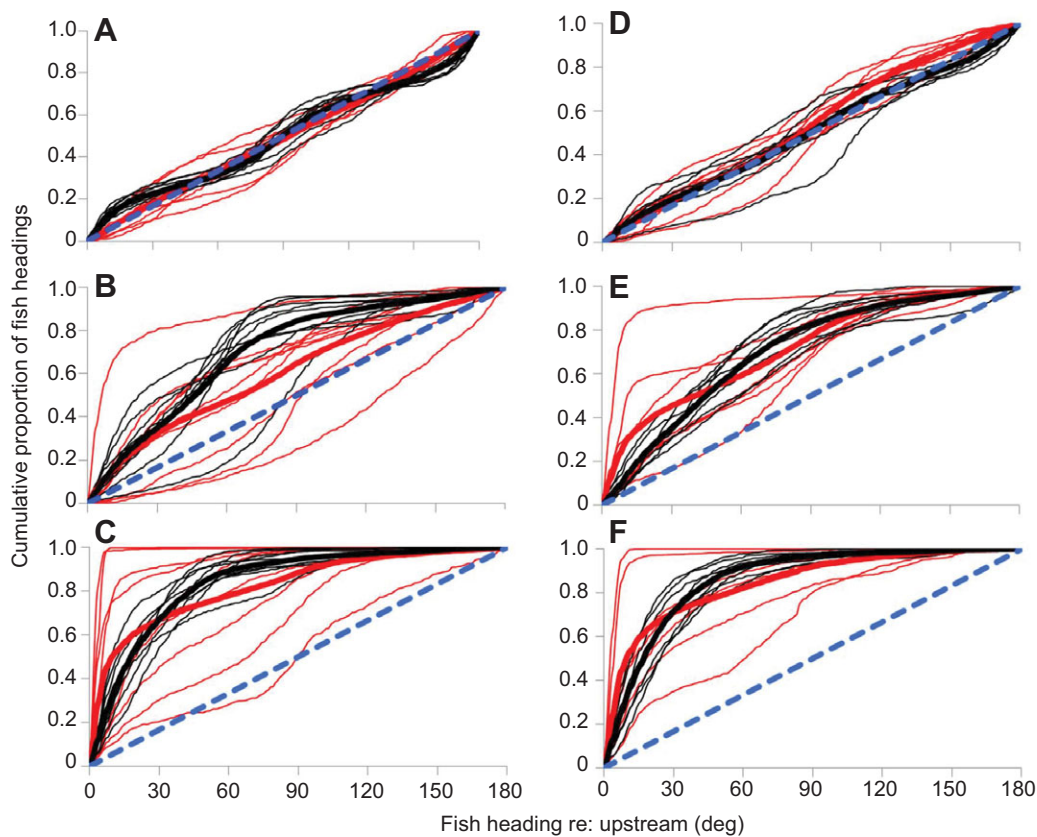
Fig. 3. CFDs of fish headings for the lateral line-enabled (LL+; A–C) and lateral line-deprived (LL–; D–F) treatment groups, each tested under vision-enabled (V+, red functions) and vision-disabled (V–, black functions) conditions. Each treatment group was also tested at three flow speeds: the no-flow control condition (A,D), the low (3 cm s⁻¹) flow speed condition (B,E) and the high (7 cm s⁻¹) flow speed condition (C,F).

illustrates the general tendency observed for fish in the dark to follow the walls of the working arena (Fig. 2B), resulting in preferential locations along the upstream/downstream or left/right barriers of the test arena (Fig. 2C). As a result, the distribution of fish headings (Fig. 2D) has four modes centered on 0 deg (towards the upstream barrier), ±90 deg (towards the left or right wall) and 180 deg (towards the downstream barrier). This resulted in a CFD (Fig. 2E, black line) that wandered slightly above and below the CFD (blue dashed) curve that is based on a theoretical uniform (random) distribution of fish headings. In contrast, the red function, which illustrates a CFD function from the same fish in a V+/no-flow condition, shows a slight negative bias. Nevertheless, both red and black CFD functions yield a RI near zero, indicating neither upstream nor downstream preference.

CFD functions across individuals were consistent in showing (1) little to no preference for either an upstream or downstream direction in the no-flow condition (Fig. 3A,D), (2) an upstream bias (positive rheotaxis) in the majority of individuals in flow (Fig. 3B,C,E,F) and (3) a higher incidence (4 out of 16) of near-perfect performance by individuals in the V+/high flow speed condition (Fig. 3C,F) compared with other conditions (0 out of 16 in all other conditions). In addition, CFDs at the high flow speed appear to be considerably more variable in the V+ condition (red lines) than in the V– (black lines) condition.

Evaluating rheotactic metrics

In order to confirm the utility of the RI and how it varied with other rheotactic metrics, we performed multiple Spearman's rank

Table 3. Spearman's rank correlations between various rheotactic metrics

Measure	RI	MA	r	±10 deg	±25 deg	±45 deg	±90 deg
Metrics							
RI	1.000	−0.824***	0.595***	0.690***	0.751***	0.777***	0.772***
MA	–	1.000	−0.514***	−0.684**	−0.740**	−0.792***	−0.775***
r		–	1.000	0.551***	0.646***	0.685***	0.841***
Window criteria							
±10 deg			–	1.000	0.956***	0.907***	0.672***
±25 deg				–	1.000***	0.979***	0.742***
±45 deg					–	1.000	0.785***
±90 deg						–	1.000

MA, absolute value of mean angle (re: upstream); r, vector strength; window criteria, the number of samples within the specified angle of upstream.
Spearman's rank correlations were performed on arcsine transformed data to determine the degree of similarity between various measures of rheotaxis. There is strong, significant agreement between all measures of rheotaxis. Relationships between mean angle and other measures are negative because of upstream being defined as 0 deg.
*P<0.05, **P<0.01, ***P<0.001 (two-tailed test).

correlations among six different metrics computed from the same pooled data set (N=96 for each metric) (Table 3). Although RI was significantly and positively correlated with vector strength (ρ=0.595, P<0.001), the absolute value of RI was consistently less than the vector strength. This trend reflects the fact that vector strength can be high even when there is a substantial deviation between the mean observed direction and the upstream direction. Thus, the negative correlation between RI and mean direction (ρ=−0.824, P<0.001) is much stronger than between vector strength and mean direction (ρ=−0.514, P<0.001).

RI was also strongly correlated with measures of rheotaxis based on the proportion of video frames in which the fish's heading fell within ±10, 25, 45 or 90 deg of upstream (Table 3, Fig. 4). As is to be expected, the proportion of upstream headings generally increased as the angular criterion changed from very conservative (e.g. ±10 deg) to very liberal (e.g. ±90 deg) (Fig. 4). Under conditions that result in a RI near zero (headings uniformly distributed), the expected proportion of headings within ±10 deg and ±90 deg of upstream is 0.056 (20/360 deg) and 0.5 (180/360 deg), respectively. Thus, the magnitude of this metric can shift by nearly half its dynamic range (i.e. by 0.44) when switching from a conservative to a liberal criterion.

Effect of flow speed and sensory condition on overall rheotactic performance (RI and RA)

As Table 4 indicates, flow speed had a significant effect on both RI (based on CFDs of fish headings) and RA (measured by the percentage of headings within ±10 deg of upstream), both of which were higher in the high flow speed compared with the low flow speed condition (Figs 3 and 5). Although sensory condition (visual or lateral line deprivation) had no effect on rheotactic performance, as measured by RI (Table 4), subtle effects on the shapes of frequency distributions of fish headings were observed. Three trends were noted. For the first trend, fish headings in the V+ condition (Fig. 6A,B) were more narrowly distributed around the upstream direction than those in the V− condition (Fig. 6C,D). These effects were quantified by RA (Fig. 5C,D), which is more sensitive to clustering around the mean direction. This observation is reflected by the univariate analysis, which showed a significant effect of visual deprivation on RA but not RI (Table 4). No significant effect of lateral line deprivation on either RI or RA was observed (Table 4).

The second trend, observed in the V−/LL+ condition, was a trimodal frequency distribution with three distinct modes (0 deg and

Table 4. Results of follow-up univariate ANOVA for effects of flow speed and visual deprivation

Effect	d.f.	F	P	Partial η²	Direction
Low → high flow					
RI	1,14	66.797	**<0.001**	0.827	Increase
RA	1,14	17.366	**0.001**	0.554	Increase
$\bar{x}_{str}$	1,14	22.134	**<0.001**	0.613	Increase
s.d._str	1,14	8.446	**0.012**	0.376	Decrease
s.d._cross	1,14	0.717	0.411	0.049	–
V+ → V−					
RI	1,14	0.706	0.415	0.048	–
RA	1,14	4.662	**0.049**	0.250	Decrease
$\bar{x}_{str}$	1,14	78.042	**<0.001**	0.848	Increase
s.d._str	1,14	11.894	**0.004**	0.409	Increase
s.d._cross	1,14	26.735	**<0.001**	0.656	Increase

RI, rheotactic index; RA, rheotactic accuracy; $\bar{x}_{str}$, mean streamwise position; s.d._str, streamwise standard deviation; s.d._cross, crosswise standard deviation; V+, vision enabled; V−, vision disabled.
Bold values indicate significance at P<0.05.

±60 deg) at the low (Fig. 6C, Fig. 7C) but not high (Fig. 6C) flow speed. The trimodal distribution arises from a common behavior in which fish perform repeated, cross-stream sweeping motions near the upstream barrier (Fig. 7). During each sweep, which lasts on the order of a few seconds, fish maintain a nearly fixed angle of attack (±60 deg) with respect to upstream (Fig. 7A–C). In one individual, this behavior was so prominent that it resulted in a bimodal instead of trimodal distribution of fish headings (Fig. 8A,C). This trend was nearly abolished in fish with blocked lateral lines (Fig. 9A,B). Sweep behaviors of V− fish at the low flow speed (Fig. 8A) were quite distinct from behaviors observed at the high flow speed (Fig. 8B). Cross-stream excursions as well as deviations from the upstream direction were much wider at the low flow speed than at the high flow speed. Moreover, streamwise excursions were wider at the high flow speed than at the low flow speed (Fig. 8C,D). Behavior at the high flow speed involved short periods of downstream drift followed by upstream recovery (Fig. 8B) with little deviation in the upstream heading, resulting in a unimodal distribution of fish headings.

The third trend, as previously noted, was associated with the wall-following behaviors of visually deprived fish in the absence of flow (Fig. 2 and Fig. 7D–F). Wall following resulted in quadramodal frequency distributions with modes at 0 deg (directly upstream), ±180 deg (directly downstream) and ±90 deg (in the cross-stream direction towards either the left or right sides of the tank) (Fig. 2D, Fig. 7F). Unlike sweeping behaviors, in which fish maintained an angle of approximately ±60 deg with respect to the upstream barrier (Fig. 7A–C), wall-following behaviors were characterized by fish maintaining a nearly parallel orientation to the wall surface while traveling along the boundaries of the tank (Fig. 7D–F). However, wall-following behaviors were similar to sweeping behaviors in that both were greatly suppressed in lateral line-deprived fish (Fig. 9).

Effect of flow speed and sensory condition on streamwise position

Univariate analysis indicated a significant effect of visual condition on streamwise position, with fish in the dark (V−) tending to maintain a more upstream position compared with fish in the light (V+) (Fig. 10, Fig. 11A,B). In addition, inter-individual variability in streamwise position appeared to be much higher in the light (preferred positions varied along the entire upstream/downstream axis) than in the dark (nearly all individuals being within 5–10 cm of the upstream barrier; e.g. compare the red and black functions
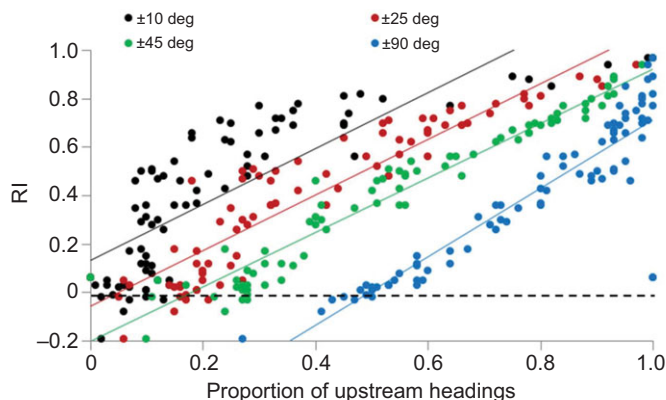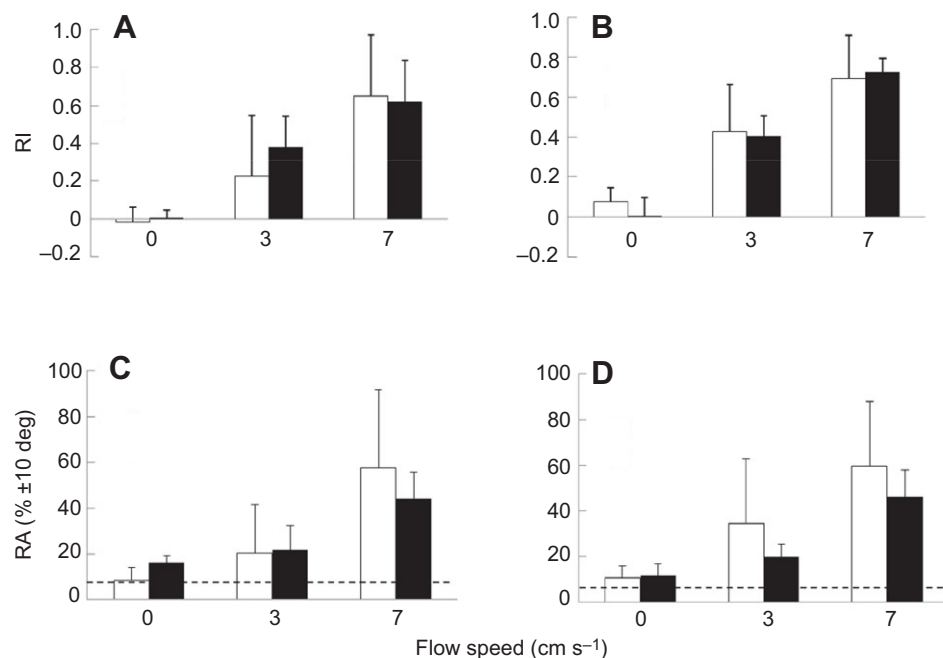

Fig. 4. (A) The relationship between RI and the proportion of headings within ±10, 25, 45 or 90 deg of upstream (data from all conditions combined).

Fig. 5. RI (A,B) and percentage headings within ±10 deg of upstream (rheotactic accuracy, RA; C,D) as a function of flow speed for the lateral line-enabled (LL+; A,C) and lateral line-deprived (LL–; B,D) treatment groups, each tested under vision-enabled (V+, white bars) and vision-disabled (V–, black bars) conditions. Dashed lines in C and D indicate chance levels.

in Fig. 10E). Although fish did exhibit weak positional preferences in the no-flow control (Fig. 10A,B), these were largely emergent properties of wall-following behaviors during which fish spent more time at upstream and downstream ends of the tank (as well as left and right walls) than at the center (Fig. 7D–F). Fish also tended to shift slightly upstream at the high compared with the low flow speed (Fig. 11A,B, Table 4).

The relationship between RI and positional variability

To determine whether positional stability (i.e. low variability of position in space) was necessary for strong rheotactic performance, RI was plotted as a function of positional (streamwise, x; or cross-stream, y) variability (standard deviations from the mean x or y position) for four different data sets representing all possible pairwise comparisons of sensory conditions (i.e. V+/LL+, V+/LL–, V–/LL+ and V–/LL–), separated by flow speed (Fig. 12). At the high flow speed, the RI was uncorrelated with both streamwise and cross-stream variability in all sensory conditions (Fig. 12, Table 5). At the low flow speed, RI was again uncorrelated with both streamwise and cross-stream variability in all sensory conditions except the V–/LL–

condition, in which RI was negatively correlated with both streamwise and crosswise variability (Fig. 12D). To summarize, RI was negatively correlated with positional variability under a restricted set of conditions, i.e. when the flow speed was low and both vision and the lateral line were disabled. Under these circumstances, positional stability may be necessary for strong rheotactic performance.

DISCUSSION

This study demonstrates that loss of vision or lateral line alone or in combination does not compromise the ability of giant danio to head in a general upstream direction. At the same time, however, sensory deprivation does cause subtle alterations in the spatiotemporal form of rheotactic behavior. When deprived of vision, the angular accuracy of the upstream heading is reduced (Fig. 6, Table 4) and fish move further upstream (Figs 9, 10). At low flow speeds, visually deprived fish exhibit left/right sweeping movements near the upstream barrier, which are abolished when fish are additionally deprived of lateral line information. Whether these alterations reduce rheotactic benefits (e.g. energetic cost savings) is unknown, but they illustrate the complexity and context dependency of multisensory interactions.
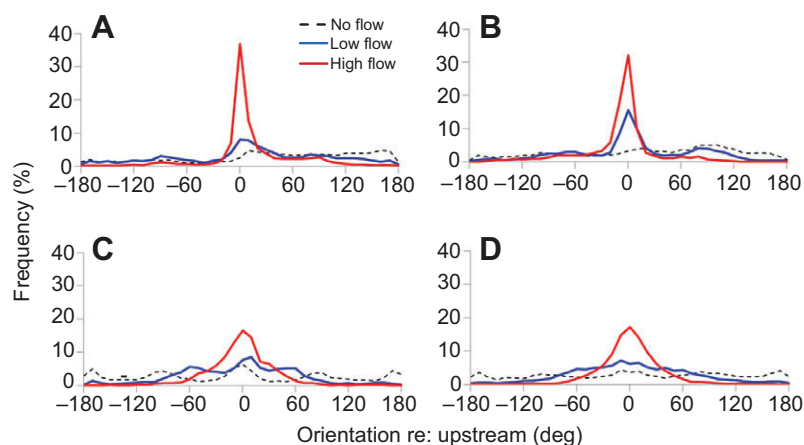

Fig. 6. Frequency distributions of fish headings for the lateral line-enabled (LL+; A,C) and lateral line-deprived (LL–; B,D) treatment groups, each tested under vision-enabled (V+; A,B) and vision-disabled (V–; C,D) conditions. Each treatment group was also tested at three flow speeds: the no-flow control condition (dashed black line), the low (3 cm s^{-1}) flow speed condition (blue solid line) and the high (7 cm s^{-1}) flow speed condition (red solid line). Each frequency distribution was normalized to represent the percentage of the total sample.
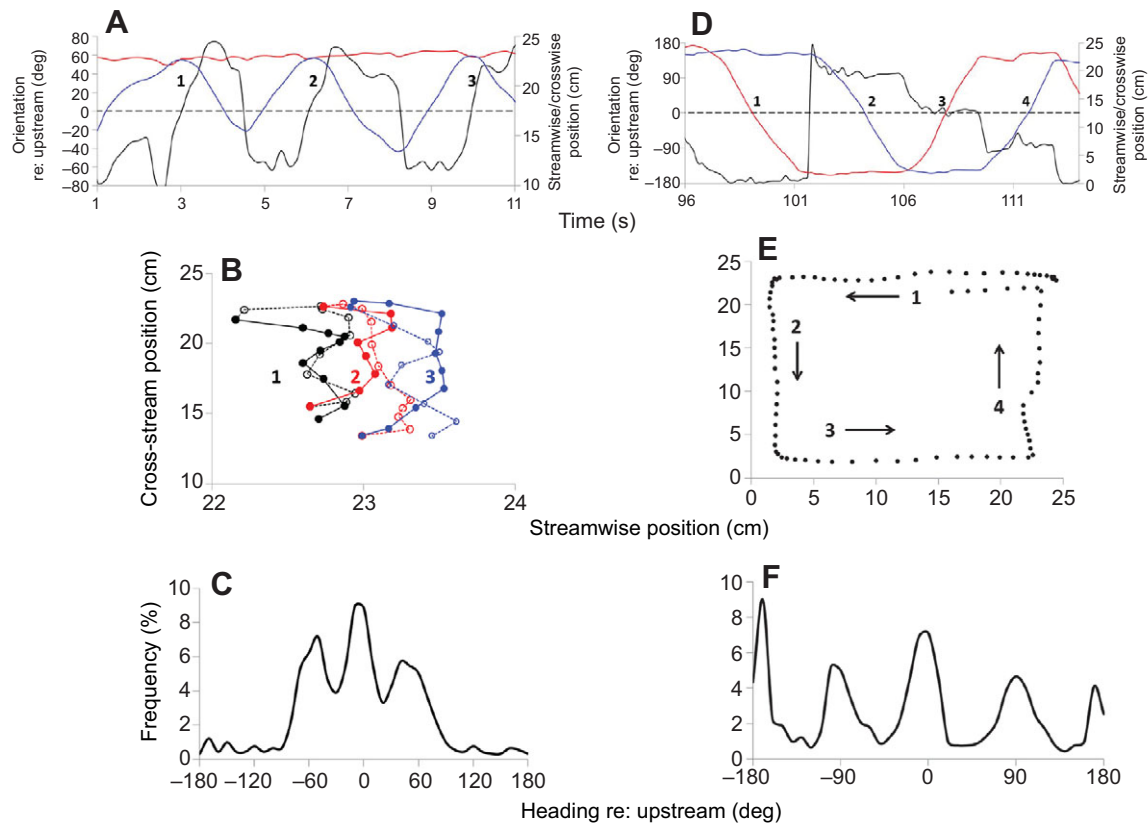
Fig. 7. Cross-stream sweeping (A–C) and wall-following (D–F) behaviors associated with trimodal (C) and quadramodal (F) frequency distributions of fish headings; illustrations were drawn from a single individual in each case. Temporal plots (A,D) illustrate how fish heading (black line, left-hand *y*-axis), and streamwise and crosswise (red and blue lines, respectively, right-hand *y*-axis) positions change over time during three sweep cycles (A) and during one trip around the test arena (D). Spatial plots (B,E) track the movements of the fish's approximate center of mass in the horizontal plane of the tank during each of the three sweep cycles (B) and during one trip around the test arena (E). Each color represents one full sweep cycle, whereas solid and dashed lines with filled and open symbols, respectively, delineate one half of each cycle. Although this example appears to show that the fish is advancing towards the upstream barrier from one sweep cycle to the next, the size of the apparent advancement (~1 cm) with respect to potential depth-of-field measurement errors does not allow us to distinguish between whether the fish is advancing in the horizontal plane or moving upward in the vertical plane (see Materials and methods, 'Data collection and analysis'). Both sweeping and wall-following behaviors were observed in visually deprived fish. Whereas wall-following behaviors occurred in the no-flow condition, sweeping behaviors were prominent at the low speed flow condition.

The multisensory nature of rheotaxis

As fish are quite naturally and routinely challenged with loss of sensory information, it is perhaps unsurprising that even bimodal (lateral line and visual) sensory ablation failed to alter rheotactic performance. While there is much evidence to suggest that both vision and the lateral line play an important role in rheotaxis (reviewed in Arnold, 1974; Montgomery et al., 1997), fish are clearly able to compensate for the loss of such sensory information. Under these conditions, fish likely utilize their vestibular system in conjunction with touch. Unfortunately, it is difficult to examine the role of vestibular cues, as blocking the vestibular system affects the balance of fish and makes it difficult for them to behave normally.

Given the well-known synergistic effects of multisensory integration (i.e. multisensory enhancement) (Stein and Meredith, 1993), there is a noteworthy lack of evidence for enhanced rheotaxis (i.e. higher RI) when all senses are present compared with when one or two senses are blocked. While RA was higher in V– fish compared with V– fish (Table 4), it is hard to determine whether this finding results from multisensory enhancement or from different behavioral strategies for sensing the environment (see 'Compensatory sensorimotor strategies in the absence of vision',

below). According to the principle of 'inverse effectiveness' (Stein and Meredith, 1993), the degree of multisensory enhancement is inversely proportional to stimulus strength, with near-threshold stimuli producing the greatest multisensory enhancement. Thus, the absence of any appreciable multisensory enhancement in this study is predictable, given that flow speeds are sufficiently above threshold for eliciting rheotaxis.

The effects of lateral line deprivation on rheotactic behavior

In stark contrast to previous studies indicating the necessity of the lateral line at low flow speeds (Montgomery et al., 1997; Baker and Montgomery, 1999a; Baker and Montgomery, 1999b), our results with giant danio show no effect of lateral line deprivation in visually enabled or disabled fish. Furthermore, for fish deprived of both visual and lateral line information, vestibular and/or tactile stimuli remain the only plausible cues for rheotaxis. In this regard, it is interesting to note that out of all four possible sensory conditions, only the LL–/V– condition resulted in rheotactic performance that was negatively correlated with positional variability (Fig. 12D, Table 5). As positional variability could, in theory, stimulate the vestibular system in such a way as to interfere with the use of inertial cues in determining water-current direction, such an effect might be
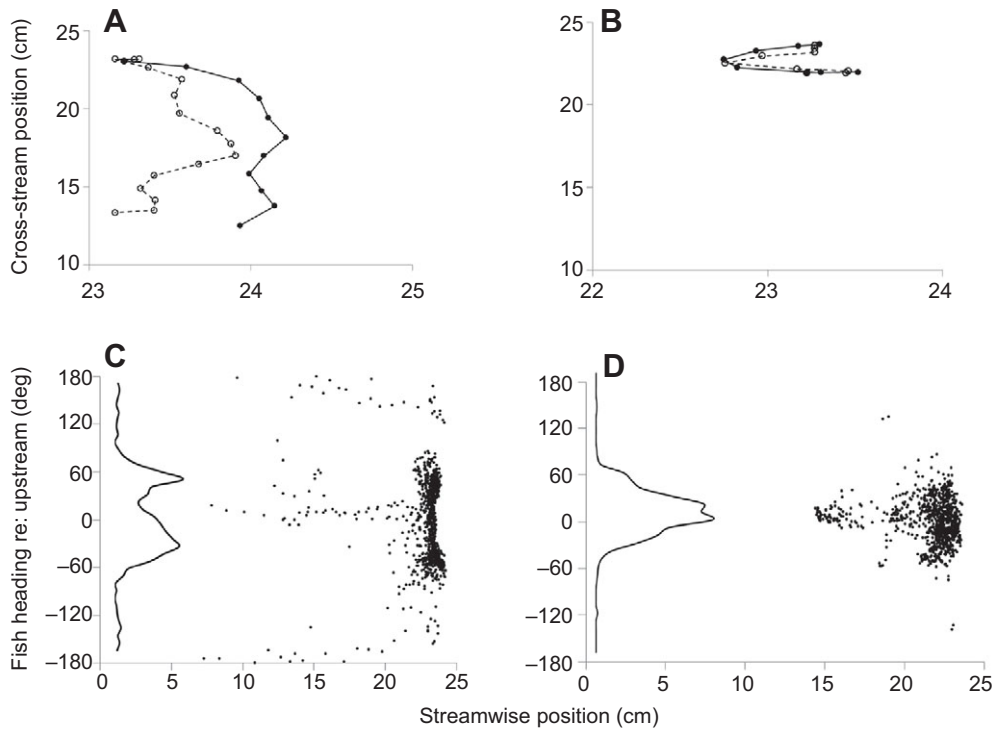
Fig. 8. Spatial plots of fish movements for brief periods of time (0.2 s between data points) (A,B) compared with plots of fish heading *versus* streamwise position for the entire 3 min test period in the same fish (C,D). Plots in A and C are from the low (3 cm s^{-1}) flow speed condition, whereas those in B and D are from the high (7 cm s^{-1}) flow speed condition. Movements in A correspond to one sweep cycle in which the fish moves from the left to the right (dashed line with open symbols) and back again (solid line with filled symbols) in front of the upstream barrier. Movements in B depict two downstream drift/upstream recovery cycles, the first indicated by the dashed line with open symbols and the second by the solid line with filled symbols. During each sweep cycle in A, the fish's heading is maintained near ±60 deg, as illustrated by the bimodal frequency distribution of fish headings in C. During each downstream/upstream cycle in B, the deviation of the fish's heading from upstream is much smaller, resulting in a nearly unimodal distribution of heading in D.

expected if fish were relying heavily on vestibular information. If true, this argument raises the possibility that vestibular cues were somehow stronger or more salient in the current study than in previous studies, thus reducing the reliance on (or need for) lateral line information.

The current and previous studies differed in at least two ways that may have affected the utility of vestibular cues: (1) the number of fish tested and (2) the shape of the test arena. Previous studies were conducted with groups of four or five fish, tested at the same time, whereas the current study tested fish individually. Compared with solitary fish, grouped fish face the additional challenge of having to avoid collisions with each other while negotiating the flow. In principle, the constant reshuffling of positions could lead to decreased utility of the vestibular system for the purpose of rheotaxis and, hence, greater reliance on lateral line cues. Moreover, given

that the lateral line plays an important role in preventing collisions between neighboring fish in a school and in maintaining school structure (Partridge and Pitcher, 1980), it is reasonable to suggest that groups of lateral line-deprived fish might have greater difficulty maintaining position with respect to each other, as well as with respect to a current, in a confined test area.

In terms of tank shape, the rectangular shape used in the previous studies could have led to an orientation bias in the streamwise direction for lateral line-enabled but not -disabled groups of fish. This differential effect may have been even further amplified by the packing density of grouped fish in a confined area. Alternatively, the square-shaped arena in the present study could have increased the probability of wall-following behavior (thigmotaxis), which might arguably compete with rheotactic tendencies, especially at low speed flows. Lateral line-enabled wall-following behavior was indeed observed in
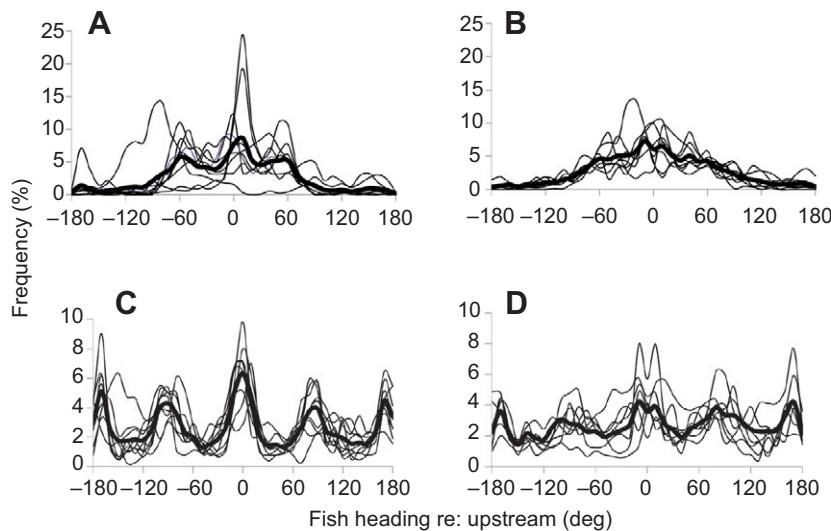

Fig. 9. Frequency distributions of fish headings in the low flow (A,B) and no-flow (C,D) conditions in the vision-disabled (V–) condition with either the lateral line-enabled (LL+; A,C) or -disabled (LL–; B,D) fish. Each thin line represents the frequency distribution from one fish; each thick line represents the mean of frequency distributions from eight fish. Trimodal distributions in A reflect sweeping behaviors (see Results), whereas quadramodal distributions in C reflect wall-following behaviors. Both behaviors are suppressed after the lateral line system is blocked.
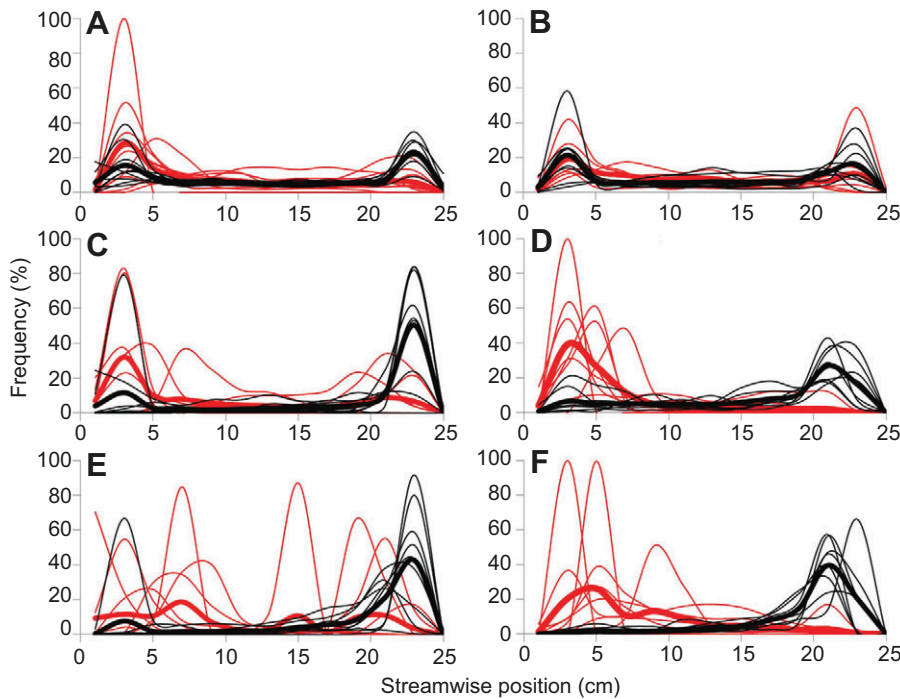
Fig. 10. Frequency distributions of streamwise positions in no-flow (A,B), low flow (3 cm s^{-1}; C,D) and high flow (7 cm s^{-1}; E,F) conditions in lateral line-enabled (LL+; A,C,E) and disabled (LL−; B,D,F) treatment groups. Within each treatment group, results from visually enabled (V+) and disabled (V−) conditions are plotted as red and black functions, respectively.

the no flow condition, but there was little evidence for this kind of behavior in either low or high speed conditions. Rather, cross-stream sweeping behaviors (Fig. 7A–C) emerged at low flow speeds and these were quite distinct from wall-following behaviors (Fig. 7D–F).

A third possible difference between the studies is the nature of the flow itself. Previous studies used pumps to create a unidirectional (Baker and Montgomery, 1999a) or circular flow around a tank

divider (Baker and Montgomery, 1999b) in the fish's home tank. Although honeycomb channels were used to reduce turbulence and straighten out the flow in the first case, the spatial characteristics of the flow were not measured or quantified in either case. In contrast, this study employed a flow tank modeled after Vogel and LaBarbera (Vogel and LaBarbera, 1978) that was designed specifically to minimize spatial non-uniformities of the flow field.
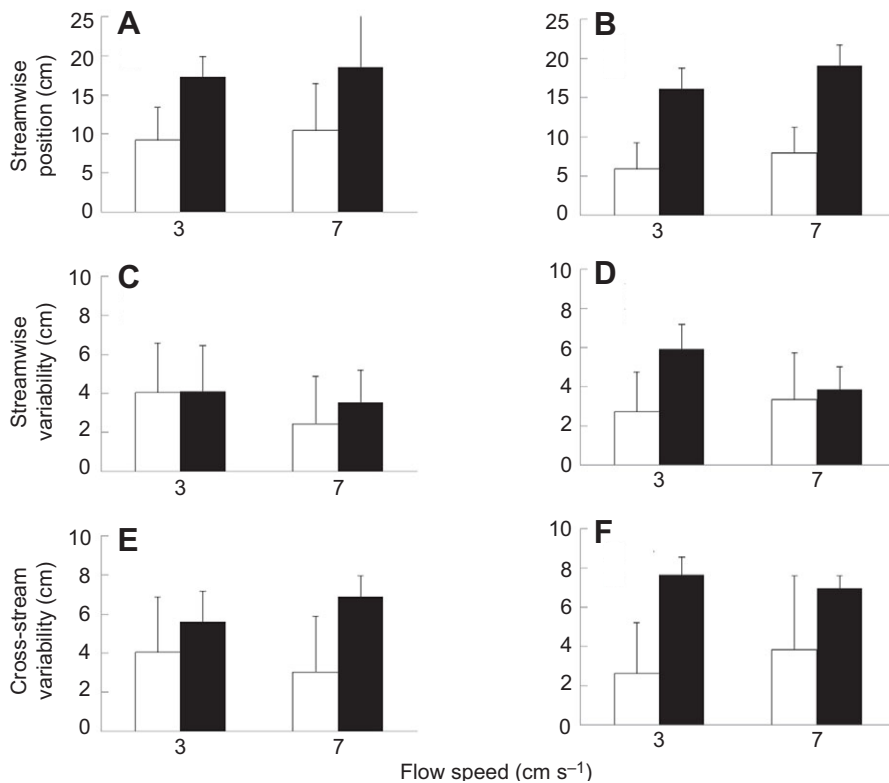

Fig. 11. Bar graphs comparing the mean streamwise position (A,B) and mean variability of streamwise (C,D) and cross-stream position (E,F) across the different flow speed and sensory conditions: lateral line-enabled (LL+; A,C,E) and lateral line-deprived (LL−; B,D,F) treatment groups, tested under vision-enabled (V+, white bars) and vision-disabled (V−, black bars) conditions.
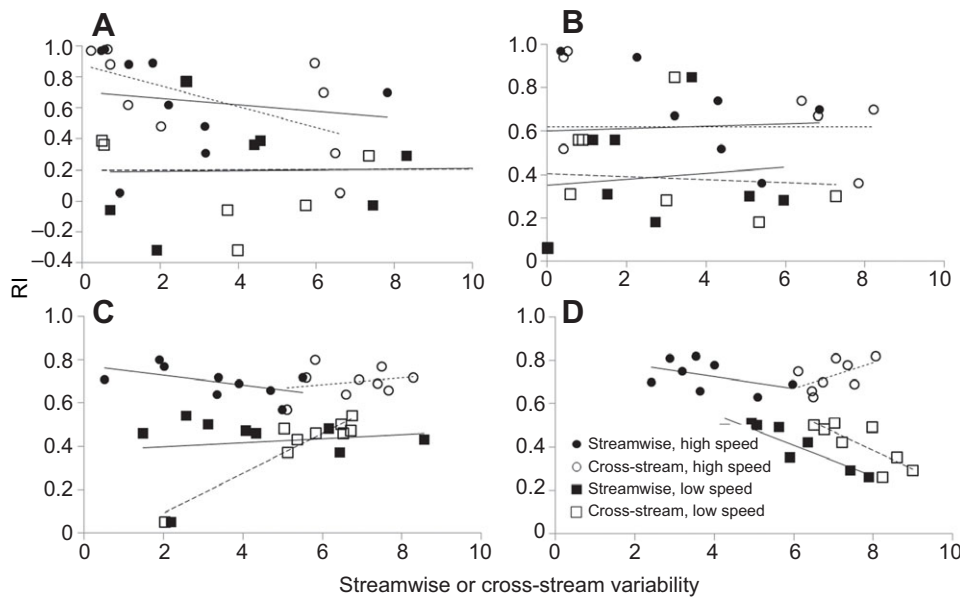
Fig. 12. Relationship between rheotactic strength (RI) and the variability in streamwise or cross-stream position (s.d.) under four sensory conditions: both vision and lateral line enabled (V+/LL+; A); vision enabled/lateral line disabled (V+/LL–; B); vision disabled/lateral line enabled (V–/LL+; C); and both vision and lateral line disabled (V–/LL–; D).

Moreover, flow speed measurements at many different horizontal and vertical locations in the tank confirmed the overall spatial uniformity. While it is impossible to know the spatial characteristics of the flow in all the various previous studies, it is conceivable that the flow in the present study was more spatially uniform than that in previous studies. One possible consequence of this difference arises from the likelihood that fish in a spatially uniform flow will be displaced directly downstream as opposed to being buffeted around in other directions, as might happen in a spatially non-uniform (or turbulent) flow. Thus, body-motion signals from the vestibular system about water-current direction are likely to be less informative in spatially non-uniform flows than in uniform flows. Degraded vestibular performance might thus decrease reliance on vestibular cues while increasing reliance on lateral line cues, leading to a more pronounced effect of lateral line deprivation in spatially non-uniform flows.

Finally, one major difference between the present study and the work of Montgomery and colleagues is the species tested (Montgomery et al., 1997; Baker and Montgomery, 1999a; Baker and Montgomery, 1999b). It might be argued that some of the previously tested species have evolved under conditions that favor heavy reliance on non-visual information and, thus, lateral line deprivation would be expected to cause larger behavioral deficits

than those expected from fish that rely more heavily on visual senses. While some species-specific differences likely exist, unpublished data from this lab (J.B.-C. and S.C.) have failed to show an effect of lateral line ablation on solitary blind cave fish, suggesting that species choice alone does not explain the observed lack of a lateral line effect. It remains possible that species-specific differences or other, methodological, factors act alone or in concert to produce the lateral line deprivation effect seen in previous studies but not observed in the present study.

Despite potential differences between studies that may explain the absence of a lateral line effect, there is little doubt that the lateral line system was effectively blocked in this study. DASPEI uptake by lateral line neuromasts in streptomycin-treated fish was almost completely absent compared with sham-treated fish and, moreover, several behavioral effects of lateral line blockage were observed, including the disappearance of cross-stream sweeping (Fig. 9A,B) and wall-following (Fig. 9C,D) behaviors. The discriminant analysis furthermore showed that the rheotactic behavior of lateral line-enabled and -disabled fish could be significantly discriminated, but only under a very limited set of conditions, i.e. when fish were tested in the dark at the low flow speed. In this case, the three strongest predictors of lateral line condition were RI and variability in both streamwise and cross-stream position, all of which were higher in lateral line-deprived fish than lateral line-enabled fish. This result can best be understood in terms of the subtle alterations in the spatiotemporal dynamics of rheotactic behavior (i.e. sweeping behaviors near the upstream barrier) that emerged in visually deprived fish at the low flow speed (Fig. 7A–C, Fig. 8A,C). Though the functional significance of this behavior is unknown, it could represent a compensatory sensorimotor strategy for gathering information about water-current direction and/or the fish's position in space with respect to a stationary frame of reference.

Compensatory sensorimotor strategies in the absence of vision

The fact that the sweeping behavior emerged in the dark at low speeds and was abolished when the lateral line system was blocked suggests that this behavior may function as a compensatory strategy for acquiring information in the absence of vision. Interestingly, blind cavefish unilaterally deprived of lateral line information are able to

Table 5. Spearman's correlation statistics for RI *versus* positional variability

Condition	Streamwise		Cross-stream	
	ρ	P	ρ	P
High flow				
V+/LL+	−0.227	0.59	−0.694	0.06
V+/LL−	0.25	0.56	−0.33	0.42
V−/LL+	0.26	0.54	−0.19	0.66
V−/LL−	0.64	0.09	−0.42	0.31
Low flow				
V+/LL+	−0.14	0.74	−0.48	0.23
V+/LL−	0.56	0.15	−0.10	0.82
V−/LL+	−0.26	0.53	−0.64	0.090
V−/LL−	−0.90	**0.002****	−0.81	**0.016***

Bold *P*-values indicate significant correlations (**P*<0.01* and ***P*<0.01).

turn in the correct upstream direction, but only when their unblocked side is facing upstream (Baker and Montgomery, 1999a). In light of this finding, it is possible that the sweeping behavior observed in giant danio represents a sensorimotor strategy for using lateral line information to determine flow direction and overall heading. The directional sensitivity of lateral line hair cells combined with the velocity sensitivity of superficial neuromasts make this submodality an ideal candidate for this purpose (Montgomery et al., 1997).

A non-zero angle of attack may increase the information in two ways: (1) by increasing the velocity of the water relative to the fish's skin surface and (2) by promoting bilateral comparison because of flow separation. A fish maintaining an angle of attack with respect to upstream would be forced to translate in the cross-stream direction. This would increase the flow across its upstream side, which is a combination of the bulk flow and the fish's own self-generated flow. As the lateral line responds to the relative movement between the fish and the surrounding water (Kalmijn, 1988; Kalmijn, 1989; Denton and Gray, 1983), this motion would presumably increase the stimulus to the lateral line. To remain in the vicinity of a stable cross-stream position, fish would be forced to tack back and forth about this position in a sweeping motion. Additionally, the flow separation on the upstream side of a zig-zagging fish is less than that at the downstream side. As a result, information about flow direction is retained on the upstream side, but obscured on the downstream side by the presence of shed vortices (or a turbulent wake). Fish may even compare these differences bilaterally, as the difference between the upstream and downstream side should contain some information about bulk-flow direction.

The effects of sensory condition on spatial position

In this study, fish with vision were able to maintain their streamwise position for up to 3 min (maximum test period) at different locations along the upstream/downstream axis of the tank (red functions in Fig. 9E) and without reference to any upstream bluff body, as previously reported for obstacle-entrainment behaviors (Sutterlin and Waddy, 1975; Montgomery et al., 2003; Liao et al., 2003; Przybilla et al., 2010). Visual, tactile and/or lateral line senses can all theoretically inform fish about their body position with respect to some external spatial reference. Ideally, information about body position in space is combined with vestibular information about self-motion to yield information about body motion through space, thus enabling fish to withstand the destabilizing influences of the oncoming current and to maintain posture, balance, spatial position and an upstream orientation.

When deprived of vision, the streamwise position of fish was dramatically shifted upstream (black functions, Fig. 10E,F). A likely explanation for this shift is that fish switched from using a visual frame of reference for position holding to a tactile and/or lateral line frame of reference. That is, fish with all senses available were likely relying on stationary features in their visual surroundings. Given that the side walls of the tank were translucent and the top of the tank completely open, it is conceivable that fish could discern stationary features outside the confines of the test arena at considerable distances. In contrast, visually deprived fish are more likely to rely on close-range stationary features within the tank, namely the floor or walls, detectable *via* touch and/or touch-at-a-distance (the lateral line). Indeed, there are several reports of fish using tactile contact with the substrate as a stationary frame of reference in rheotaxis (Lyon, 1904; Dykgraaf, 1933; Montgomery et al., 1997). Whether this upstream shift in position has any biological relevance is unknown. However, fish in the wild have been observed to migrate to the banks of a river at night (Arnold, 1974), perhaps in search of a more stable frame of

reference, among other reasons. An alternative explanation is that visual deprivation causes fish to overestimate the flow speed needed to maintain a fixed streamwise position – in other words, it is symptomatic of an overshoot error due to the lack of visual feedback for swim speed control.

Effects of flow speed on rheotactic performance

This study extends and confirms the general finding that the strength of rheotactic performance increases with flow speed (Champalbert et al., 1994; Montgomery et al., 1997; Baker and Montgomery, 1999a; Baker and Montgomery, 1999b; Kanter and Coombs, 2003; Coombs and Grossman, 2006). The effects of flow speed on rheotaxis can be understood in terms of at least three interrelated factors: the magnitude of sensory stimuli that elicit the rheotactic response, the lift and drag forces on the fish's body, and the momentum of the fish as it swims against the current. Momentum is likely to play an increasing role in stabilizing the fish's heading as flow speed (and, thus, the swim speed of fish) increases, because it is much easier for fish to stabilize body orientation (pitch, roll and yaw) when momentum (which is proportional to swim speed) is high than when it is low (Webb et al., 2010). The fact that the RA of giant danio was lower at the low flow speed than at the high flow speed is consistent with this general idea. An additional, non-sensory explanation of reduced rheotactic performance (both RI and RA) at low speeds is simply that fish may have been less motivated to rheotact at lower speeds because the lift and drag forces that tend to displace the fish downstream are much lower. That is, the energetic costs of not rheotacting are lower in low speed flow.

It is also reasonable to expect that flow speed will have a direct impact on the sensory cues that elicit and maintain rheotaxis. Both optic flow and vestibular cues rely on downstream displacement, which should increase as a direct function of flow speed. If a fish is displaced by a water current and there is no net movement between the fish and the current, the lateral line will receive no information about current direction. However, if the fish is able to stabilize its position using tactile or vestibular cues, the stimulus to the lateral line will increase as a function of flow speed. Furthermore, the lateral line could detect the slip of the substrate past the ventral surface of an epibenthic fish, as it passively drifts downstream. Thus, the magnitude and nature of the lateral line stimulus will depend on flow speed, but also on whether fish are moving with or against the current.

For fish that are actively swimming against the current, there is another factor that is likely to reduce the effectiveness of lateral line stimuli. Vigorous swimming movements are known to activate the octavolateralis efferent system, which has a descending inhibitory influence on lateral line hair cells (Russell and Roberts, 1974; Roberts and Meredith, 1989). In effect, the efferent system is thought to reduce the sensitivity of lateral line hair cells to self-generated flow noise. Taken together, these theoretical considerations predict that lateral line stimuli are likely to be most effective as rheotactic cues at low flow speeds, a prediction supported by a number of different studies (Montgomery et al., 1997; Baker and Montgomery, 1999a; Baker and Montgomery, 1999b; Suli et al., 2012).

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