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Sensory trait variation contributes to biased dispersal of threespine stickleback in flowing water

Running title: Sensory variation affects stickleback dispersal

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Abstract

Gene flow is widely thought to homogenize spatially separate populations, eroding effects of divergent selection. The resulting theory of 'migration-selection balance' is predicated on a common assumption that all genotypes are equally prone to dispersal. If instead certain genotypes are disproportionately likely to disperse, then migration can actually promote population divergence. For example, previous work has shown that threespine stickleback (*Gasterosteus aculeatus*) differ in their propensity to move up- or down-stream ('rheotactic response'), which may facilitate genetic divergence between adjoining lake and stream populations of stickleback. Here, we demonstrate that intraspecific variation in a sensory system (superficial neuromast lines) contributes to this variation in swimming behavior in stickleback. First, we show that intact neuromasts are necessary for a typical rheotactic response. Next, we showed that there is heritable variation in the number of neuromasts, and that stickleback with more neuromasts are more likely to move down-stream. Variation in pectoral fin shape contributes to additional variation in rheotactic response. These results illustrate how within-population quantitative variation in sensory and locomotor traits can influence dispersal behavior, thereby biasing dispersal between habitats and favoring population divergence.

Keywords: adaptive divergence; *Gasterosteus aculeatus*; lateral line; neuromasts; nonrandom gene flow; rheotaxis.

Introduction

Animal dispersal promotes gene flow between populations, which may constrain among-population divergence and local adaptation (Endler, 1973; Slatkin, 1985; 1987; Garcia-Ramos & Kirkpatrick, 1997; Lenormand, 2002). This opposition between migration and

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selection rests on an assumption that dispersing individuals are random samples from their source populations. However, this assumption has been challenged recently by empirical evidence that genotypes differ with respect to dispersal probability, distance, direction, or destination (Bolnick *et al.*, 2009; Garant *et al.*, 2004; Postma & van Noordwijk, 2004; Thomas & Singer, 1987). This variation in dispersal can be due to many factors including habitat preference *sensu stricto*, variation in local fitness, variation in energetic or biomechanical capacity to disperse, exclusion by social groups, etc. When genotypes differ in their dispersal behavior, gene flow carries a non-random sample of alleles (Garant *et al.*, 2004; Armsworth & Roughgarden, 2005; Postma & van Noordwijk, 2005; Edelaar *et al.*, 2008; Shine *et al.*, 2011; Edelaar & Bolnick, 2012; Bolnick & Otto, 2013). Such biased dispersal may actually facilitate population divergence and local adaptation (Bolnick & Otto, 2013). Consequently, there is growing interest among evolutionary biologists in identifying instances of biased dispersal, and understanding the underlying phenotypic cause of this bias (Gilbert & Singer, 1973; Haag *et al.*, 2005; Edelaar *et al.*, 2008; Phillips *et al.*, 2010; Shine *et al.*, 2011; Edelaar & Bolnick, 2012). Note that phenotype-dependent dispersal can occur as well, and may be a result of genetic variation or may be induced by learning/imprinting, or phenotypic plasticity.

The mechanistic basis of biased movement remains underexplored, having been identified in few systems, mostly insects (Thomas & Singer, 1987; Jaenike & Holt, 1991; Clobert, 2001; Duckworth & Badyaev, 2007; Edelaar *et al.*, 2008; Hanski, 2011; Clobert *et al.*, 2012). Here, we identify phenotypes that contribute to biased dispersal in threespine stickleback (*Gasterosteus aculeatus*) from a lake-stream ecotone. Parapatric lake and stream populations of stickleback on Vancouver Island exhibit extensive ecological, morphological and genetic divergence and consequently have been very intensively studied over the past

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decade to understand the genomic and phenotypic basis of rapid parapatric diversification (Reimchen *et al.*, 1985; Lavin & McPhail, 1993; Hendry & Taylor, 2004; Moore *et al.*, 2007; Berner *et al.*, 2008; Hendry *et al.*, 2009; Berner *et al.*, 2009l Roesti *et al.* 2012). Often, this phenotypic divergence between lake and stream populations happens across clines spanning tens to hundreds of meters (Berner *et al.* 2009; Bolnick *et al.*, 2009; Weber *et al.*, In press). This fine-scaled divergence is too abrupt to be plausibly explained by migration-selection balance alone, given sticklebacks' capacity for movement (Bolnick & Otto, 2013).

Previous work suggests that adaptive divergence between lake and stream stickleback may be facilitated by non-random dispersal. Experimentally displaced lake and stream stickleback were disproportionately likely to return to their native environment: inlet stream fish swam back upstream, and lake fish swam down-current back to the lake (Bolnick *et al.*, 2009). The 10% of fish that switched habitats were morphologically predisposed to do so: their body shape disproportionately resembled the natives of their newly adopted habitat. This non-random movement of lake and stream stickleback may be due to differences in the swimming behavior of individuals in flowing water ('rheotactic response'). Rheotaxis can be positive (swimming up-current or seeking current) or negative (swimming down-current or seeking still water) (Lyon, 1904; Arnold, 1974; Montgomery *et al.*, 1997; Pavlov *et al.*, 2010). Examining the same lake-stream pair that showed biased dispersal, Jiang *et al.* (2015) demonstrated that lake stickleback are more likely to move down-current (negative rheotaxis) compared to stream fish. Specifically, stream fish more effectively used low-flow boundary microenvironments and spent a large fraction of time facing up-current, thereby remaining approximately stationary. In contrast, lake fish used high-flow areas and faced downstream more often, resulting in greater net displacement downstream ('negative rheotaxis') despite repeated bursts of up-current swimming. Note that this lake-stream difference in rheotaxis

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was only observed in wild fish caught during the breeding season, but not in lab-reared or wild stickleback outside the breeding season (Jiang *et al.*, 2015). These lake-stream differences in rheotaxis could minimize migration between habitats (at least in the breeding season) and maintain population divergence (Bolnick *et al.*, 2009).

At present, little is known about the mechanistic and phenotypic basis of rheotactic variation within populations. One potentially relevant trait is the lateral line system, which is a major sensory modality in aquatic vertebrates (Bleckmann, 1986; Münz, 1989; Bleckmann & Bullock, 1989). The lateral line system is composed of neuromasts, which are clusters of mechanoreceptive hair cells that detect local water displacement over the body surface (Dijkgraaf, 1963; Coombs *et al.*, 2013). There are two types of neuromasts: superficial neuromasts reside on the surface of the skin and detect water velocity along the body surface, and canal neuromasts reside in fluid-filled enclosed canals below the skin surface and detect water acceleration and deceleration (Coombs & Montgomery, 1994; Coombs *et al.*, 2013).

Rheotaxis is thought to be mediated by superficial neuromasts but not canal neuromasts (Montgomery *et al.*, 1997; Baker & Montgomery, 1999a). Chemical ablation of superficial neuromasts has been shown to drastically increase the flow rate threshold needed to induce the rheotactic response in many fish species (Montgomery *et al.*, 1997; Baker & Montgomery, 1999a; b; Suli *et al.*, 2012). However, this association is not universally accepted: one of the pioneers of lateral line research had suggested that vision and touch (which we do not investigate here) may be more important for rheotaxis than the lateral line system (Dijkgraaf 1963). For instance, in Mexican blind cave fish, lateral line function was not necessary for rheotaxis (Van Trump & McHenry, 2013). Thus, the contributions of these different sensory modalities to rheotaxis in different species of fish remain an open question.

Threespine stickleback only have superficial neuromasts, which are organized into twelve lines (Fig. 1) (Wark & Peichel, 2010). We hypothesized that quantitative variation in

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lateral line anatomy (specifically, the number of neuromasts per line) might give rise to variation in stickleback rheotactic response. To test this hypothesis, we experimentally ablated stickleback neuromasts to determine whether they are necessary for a typical rheotactic response. Then, using both wild-caught and lab-reared lake and stream stickleback from a single lake-stream pair that was previously shown to exhibit biased dispersal and differences in rheotactic reponses (Bolnick *et al.*, 2009; Jiang *et al.*, 2015), we tested for correlations between neuromast number and rheotactic responses. We also evaluated covariance between rheotaxis and body size, body shape, and pectoral fin morphology, which have previously been associated with stickleback swimming performance in stickleback (Walker, 1997; Walker & Westneat, 2002) and differ between lake and stream ecotypes. The trait-locomotion associations that we document here may help explain non-random movement of lake and stream fish across the habitat boundary in our focal lake-stream pair, and the resulting fine-scale genetic divergence in this particular study system.

Materials and methods

Study system, fish collection and care

We measured rheotactic response and morphology of threespine stickleback (*Gasterosteus aculeatus*, Linneaus) from Blackwater Lake (Northern Vancouver Island, B.C., Canada) and its inlet stream. Previous studies demonstrated that stickleback from this lake and inlet stream exhibit nonrandom dispersal behavior (Bolnick *et al.*, 2009) and divergent rheotactic responses (Jiang *et al.*, 2015).

Here, we analyze three distinct samples of stickleback from the Blackwater lake and inlet stream that address distinct questions (summarized in Table 1 and a schematic diagram in Fig. S1). These are the same samples (and same animals) described in Jiang et al. (2015). That paper focused on lake versus stream differences in rheotaxis, whereas here we focus on

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phenotypic covariates of rheotactic response within populations. In chronological order (but not the order presented in the Results), the three samples are:

Sample 1: Common-garden rearing (June 2010): We collected reproductively mature lake and stream stickleback, and used *in vitro* fertilization to generate 15 lake families and 12 stream families. Eggs were shipped to the University of Texas at Austin (UT) within six days post-fertilization, and reared in still-water aquaria at 16-17°C (water temperature) and 16 hours of light. After swim-up, fry were fed twice daily on freshly hatched brine shrimp nauplii until they reached 1.0 cm standard length, then fed pelleted trout chow and freeze-dried blood worms. As adults, the fish were transitioned to winter conditions at 13°C and 8 hours of light. Individuals from different families were reared in separate tanks to keep track of their family identities, and kept at similar densities (~10 per 20L aquarium as adults). The tanks had no directional flow, but some turbulence from an air supply. In fall 2011, we measured rheotaxis and morphology (body shape and lateral line) on these non-breeding lab-reared fish, to test for heritable phenotypic differences between lake and stream fish (Question I), and rheotaxis-trait correlations (Questions III and IV). We used one fish per family to avoid bias from genetic pseudo-replication or tank effects. Note that common-garden rearing experiments such as this are a standard method to test for heritable between-population differences, but strictly speaking this study design cannot rule out trans-generational epigenetic effects (e.g., maternal, paternal, or even grand-parental effects).

Sample 2: **Wild-caught fish.** (June 2011): We trapped wild lake and stream stickleback during the breeding-season (but not exclusively gravid individuals), and immediately measured their rheotactic response. Subsequently, these fish were measured for body shape to test for correlations between rheotaxis and body shape (Question IV). We did not measure lateral line morphology, lacking a fluorescent microscope in the field.

Sample 3: Superficial neuromast ablation. (April 2013): We trapped wild-caught

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pre-breeding lake and stream stickleback and transported them to the Fred Hutchinson Cancer Research Center (FHCRC). These adult fish were used to test for rheotactic effects of neuromast ablation (Question II) and rheotaxis-trait correlations (Questions III and IV).

All animals used in this study were collected with permission from the British Columbia Ministry of Forests, Lands and Natural Resources Operations (permits NA11-7031 and NA13-85103). Wild-caught fish used for behavioral assays and lateral line ablations were transferred to the FHCRC with the permission from the British Columbia Ministry of Forests, Lands and Natural Resources Operations (VI13-86478). All procedures were approved by the Institutional Animal Care and Use Committees of the University of Texas (#AUP-2010-00059 and #AUP-2013-00027), and the FHCRC (#1575).

Rheotactic response assay

We used a circular flow tank assay (Fig. 2A, illustrated in Supporting Information Video S1) to quantify rheotactic responses of individuals. The circular tank allows fish to swim freely up- or down-current indefinitely. The flow tank was equipped with two aquarium pumps generating uni-directional circular flow. Flow rates were within the natural range observed in the Blackwater inlet stream (Jiang *et al.*, 2015). The flow rate was the fastest in the outer part of the tank and the slowest in the inner part of the tank, allowing fish to chose among flow microenvironments.

Each fish was acclimated to still water in the tank for 15 minutes, followed by a five minute trial with current videotaped by an overhead webcam. The order of fish, and direction of flow (clockwise or counter-clockwise), was randomized across trials. A researcher who was blind to fish identity extracted frames from each trial video (3.4 frames/second) and manually tracked the coordinates for individuals' anterior and posterior ends (caudal peduncle). We quantified the following four distinct measures of rheotactic response (see

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Jiang *et al.*, 2015 for details):

1) Net displacement: an individual's net movement up- or down-current (+ and – values) from the beginning to the end of the trial, including any full circuits.

2) Cumulative upstream movement: the total upstream path length during the five-minute current trial.

3) Upstream orientation: the fraction of time an individual faced into the current (<45º angle relative to the tangent of circular flow at the midpoint of the fish).

4) Flow regime: we divided the tank into concentric rings corresponding to increasing flow and recorded which area the fish was in per frame (innermost = 0, inner = 1, middle = 2, and outermost = 3). The average value represents individuals' use of flow microenvironments, with larger scores indicating more time in the periphery where water velocities were higher. Random use of flow regimes corresponds to a mean score of 1.98, accounting for each region's surface area (e.g., ring 3 is largest, inflating the null score). Video files are available upon request.

A novel aspect of our study is the use of four measures of rheotactic response. Most prior experimental studies of rheotaxis measure a single response variable: the minimal flow rate at which fish begin to face upstream in linear flow tanks (Montgomery *et al.*, 1997; Baker & Montgomery, 1999a; Suli *et al.*, 2012). Our four metrics are not statistically redundant, and they confer distinct biological interpretations including net progress upstream, total swimming effort (cumulative movement upstream), orientation, and microhabitat use.

Counting superficial neuromasts

We visualized the 12 lines of superficial neuromasts of live stickleback with a fluorescent dye, 2-[4-(dimethylamino) styryl]-N-ethylpyridinium iodide (DASPEI). Following Wark and Peichel (2010), we incubated individuals for 20 minutes in an aerated 0.4L container with

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0.025% DASPEI solution in aquarium water. The fish was then anaesthetized (two minute immersion in 500 mg/L MS-222) then rinsed with fresh aquarium water. We immediately counted neuromasts in each of the 12 lines on the left side of the fish, using a FITC filter set on a Leica fluorescent dissecting scope (Leica Microsystems Inc., Bannockburn, IL, USA). We focus our analyses on variation in the number of neuromasts per line, not the location, orientation, or innervation of these lines.

Question I: Is variation in neuromast number heritable?

We counted neuromast numbers (see above) on 22 stream and 27 lake fish, using the lab-reared fish from Sample 1. Due to non-normality of the residuals of the neuromast counts, we used nonparametric Wilcoxon rank-sum tests to compare neuromast numbers for lake versus stream fish, for each the 12 lines of neuromasts. We used a weighted Z test (Whitlock, 2005) to combine the independent tests from each of the twelve lines into a single test of whether these lake and stream fish differ in neuromast numbers across all lines when raised in a common garden, which would confirm there is heritable divergence. We sampled approximately one individual per family within each ecotype, so among-family variation is redundant with the residual variation in our analysis. We also tested for significant correlations between total neuromast numbers and standard length, using Spearman's rank correlation. Because we found no correlation, we did not use body size as a covariate in other analyses. Likewise, we counted the number of lateral armor plates on each fish (left and right side) and used Spearman’s rank correlation to test for a correlation between neuromast number and plate number (both on the left side of the fish). Plate number did not explain any significant variation in neuromast number, so we do not include this in later analyses.

We next tested whether the heritable differences observed in common-garden stickleback are representative of variation present in wild-caught stickleback. We counted

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neuromasts of 22 wild-caught lake and 22 stream fish from Sample 3, and repeated the Wilcoxon rank-sum tests for individual lines and weighted Z test of overall differences. We then used a single analysis to test whether (i) lines differ between lab-raised and wild-caught stickleback, and (ii) lake-stream divergence is comparable between lab and wild samples. For each neuromast line we used a two-way ANOVA with effects of population (lake vs. stream) and rearing environment (wild vs. lab-reared) and their interaction. We used permutation tests to calculate p-values when residuals were non-normal. As in Sample 1, size and armor plate number were not significant covariates.

Question II: Are neuromasts required for typical rheotactic response?

To establish a causal role of neuromasts in sticklebacks' rheotactic response, we compared rheotactic measures of fish with and without functional neuromasts. Our intent here is to examine general effects on rheotactic response, not to explain any difference between lake and stream ecotypes. Consequently, we used non-breeding wild-caught fish (Sample 3), because these non-breeding lake and stream sticklebacks were previously shown to exhibit no significant differences in rheotactic response (Jiang *et al.*, 2015).

We used neomycin to induce mechanoreceptive hair cell death and temporarily inhibit lateral line function. Lateral line hair cells regenerate quickly once neomycin is removed (Harris *et al.*, 2003, Kaus, 1987), within 3-4 days following pharmacological ablation of stickleback neuromasts (Catherine Peichel, pers. obs.). We used this approach to eliminate neuromasts of 15 lake and 15 stream fish. We held individual fish overnight (10 hours) in an aquarium with 5mM neomycin, then rinsed them for one minute before returning them to an aquarium. Control fish (22 per habitat) were treated identically, except that neomycin was not added to their aquarium water. Rheotactic response was measured between one to six hours after neomycin exposure was complete. After the assay, DASPEI staining confirmed

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complete ablation of all neuromasts. For each of the four rheotactic measures (net displacement, cumulative upstream movement, upstream orientation, and flow regime), we tested for differences between ablated and control fish, using a Wilcoxon rank sum test. In these tests, we ignored source population because it does not affect rheotactic response of the non-breeding stickleback used in this analysis (Jiang *et al.*, 2015).

Question III: Is neuromast number correlated with rheotactic response?

As described in detail in Jiang *et al.* (2015), these non-breeding lake and stream stickleback did not differ in rheotactic response. We therefore proceeded to instead analyze phenotypic variation among individual stickleback irrespective of their source population. We used canonical correlation analysis (CCA) to test for correlations between neuromast numbers and rheotactic response, in both lab-reared stickleback (Sample 1, N=13 lake and 12 stream) and wild-caught stickleback (Sample 3, N = 22 lake and 22 stream). CCA loadings can identify the subset of the 12 neuromast lines involved in rheotactic response, allowing us to account for the fact that the 12 neuromast lines are not functionally interchangeable. We omitted flow regime use from the matrix of response variables, because this rheotactic response trait was unaffected by neuromast ablation (see Results). We used permutation tests (10,000 runs) to estimate the statistical significance for each canonical variate (CV) using Wilks' λ as the test statistic. For each significantly correlated CV pair, we used corresponding structure coefficients (r_s) to identify important dependent and independent variables ($r_s > 0.3$), and used the standardized canonical function coefficients (coef) to infer the direction of correlation between each combination of important dependent and independent variables in each pair of CV axes. CCA does not readily allow for fixed effect factors such as ecotype. Instead, we accounted for individuals' ecotype as follows. For each pair of significantly correlated CVs, we obtained individuals' axis scores for the x and y eigenvectors driving the

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correlation. We then ran a linear regression of y (rheotaxis CV) on x (neuromast CV), while including a fixed factor effect of fish ecotype (lake vs. stream) and an interaction between ecotype and neuromast CV. This allows us to test whether lake versus stream population differences drive the observed neuromast-rheotactic response association.

The CCA was applied separately to data from lab-reared versus wild-caught stickleback (Samples 1 and 3). To test whether the lab- and wild-sample CCA axes are mutually consistent, we calculated a test statistic by correlating the CCA structure coefficients (trait loadings) from the lab versus wild fish datasets. This correlation between loadings was compared against 10,000 null values obtained by shuffling the wild/lab identity to simulate a single pooled population. The p-value is the fraction of null values whose correlation is less than our observed value (the null expectation being a high correlation coefficient).

Question IV: Is body shape correlated with rheotactic response?

After measuring rheotactic response, each fish was euthanized and fixed in 10% formalin. We subsequently measured the following univariate traits: body mass, standard length, pelvic width, body width at the pectoral fin, and body width at the preoperculum. Pectoral fin area and aspect ratio were calculated from photographs measured using Image J. For geometric morphometrics, we photographed the right side of each fish and digitized 20 homologous landmarks using tpsDIG2 (Rohlf, 2007) following Berner et al. (2009) but adding the caudal tip of the posterior process of the pelvic girdle, the posterior tip of the ectocoracoid, the anterior edge of the eye, the base of the last pectoral fin ray, and the tip of the pelvic spine. We used tpsUtil to remove the effects of specimen bending owing to preservation and calculated relative warps (RWs) of all specimens using tpsRelw (Rohlf 2007). We generated

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RWs separately for each of the three samples, using the top 5 axes (each > 5% of total shape variance) for subsequent analyses.

CCAs tested whether any of the three sets of morphological traits (univariate traits, fin shape, body shape) covaried with rheotactic response, analyzed separately for Samples 1, 2, and 3. We used the matrix of morphological traits as predictor variables (ignoring lake/stream distinctions), and all four measures of rheotaxis as criterion variables. As described above for the neuromast-rheotaxis CCA, we then tested for any effect of fish ecotype (lake versus stream) in driving the observed morphology-rheotaxis covariance. For each pair of significantly correlated CVs in each CCA, we used an ANCOVA to verify the effect of a given morphological CV axis on the rheotaxis CV axis, including a main effect of ecotype (lake vs. stream), and an ecotype*morphology interaction. All analyses were done using the R statistical language (Venables & Ripley, 2002; R Core Team, 2013; Legendre *et al.*, 2014).

Results

I: Heritable differences in neuromast number between lake and stream stickleback

In wild-caught stickleback (Sample 3), lake natives had more total neuromasts than did stream natives (Table 2). After correcting for multiple comparisons, this difference was only significant for one neuromast line (Mp). Given the substantial within-population variance in neuromast numbers, and our small sample size per population, we had relatively low power to detect significant differences for any single lateral line. However, when we took a multivariate approach and considered all neuromast lines together, we found a clear tendency for lake fish to have more neuromasts than stream fish. This trend held for 10 of 12 lines of superficial neuromasts, which represents a significantly consistent trend (combined Z, P=0.025) despite our limited power. These between-habitat differences are small compared to within-habitat standard deviations. Standard length had no effect on neuromast number

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(ρ=0.005 and 0.13, P=0.98 and 0.57 for lake and stream fish respectively; sample sizes for all tests are in Table 1).

Lake-stream differences in neuromast numbers were also observed in first-generation lab-reared stickleback (Sample 1), suggesting that neuromast differences are heritable. Lake stickleback had significantly more neuromasts than did stream fish for the infraorbital (IO), mandibular (MD) and otic (OT) lines (Table 2). All other neuromast lines exhibited non-significant tendencies for lake fish to have more neuromasts. As a result, a multivariate analysis across all lines presented clear evidence for more neuromasts in lake than stream fish (combined Z, P<0.001), recapitulating the results from wild-caught fish (above). Again, total neuromast numbers are independent of body size (ρ=-0.16 and 0.11, P=0.43 and 0.61 for lake and stream fish respectively). The number of bony armor plates (counted on the same side as the neuromasts) showed no significant correlation with neuromast counts of any single line, or total counts (Spearman correlation tests all P>0.25 before Bonferroni correction).

Lab-reared stickleback systematically had more neuromasts than their wild-caught relatives (Sample 1 vs. 3, P<0.001) for the Mp, MD, OT, and IO lines. The same trend held, but was not significant, for all other lines. The magnitude of the difference between wild-caught and lab-reared fish was similar for lake and stream stickleback. The lower number of neuromasts in wild fish may be due to greater abrasion, as their neuromast lines were often patchy whereas lab-reared fish exhibited complete rows of evenly-spaced neuromasts.

II. Neuromast ablation alters rheotactic response in stickleback

Ablating the lateral line system of wild-caught stickleback altered three of our four measures of rheotactic response. Control lake and stream stickleback were displaced down-current during the swimming trials (Fig. 2B). By contrast, neuromast-ablated fish exhibited remained

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stationary in the current resulting in zero net displacement on average. The difference in net displacement between ablated and control fish was significant for lake natives (*W*=95, *P*=0.03) but not stream natives (*W*=148, *P*=0.86), though the trend was in the same direction. Using a nonparametric 2-way ANCOVA (*sm.ancova* in the *sm* package in R), we found no significant difference in how lake versus stream fish responded to ablation (interaction effect P = 0.502).

Control fish exhibited substantial cumulative- (but not net-) upstream movement due to alternating down- and up-current swimming. In contrast, lateral line-ablated stickleback exhibited little cumulative up-stream movement (lake fish: *W*=295, *P*<0.0001 and stream fish: *W*=263, *P*=0.0002; Fig. 2C; ; *sm.anova* test for a ecotype*ablation interaction P = 0.94). To remain stationary, neuromast-ablated fish spent a greater fraction of their time facing upstream, compared with control fish (Fig. 2D; lake fish: *W*=69.5, *P*=0.003; stream fish tended in the same direction: *W*=111, *P*=0.16; *sm.anova* test for a ecotype*ablation interaction P = 0.34). Lateral line ablation had no significant effect on use of flow regime(s) in either lake or stream fish (Fig. 2E, *W*=175 and 121, *P*=0.77 and 0.29, respectively). All pre-breeding wild-caught lake and stream fish distributed randomly in currents regardless of treatment (*P*>0.4 for lake and stream, control or ablated groups).

III: The number of neuromasts is correlated with rheotactic response in stickleback

Both wild-caught and lab-reared sticklebacks exhibit multivariate correlations between neuromast numbers and rheotactic response (Fig. 3A&C). Wild-caught stickleback (Sample 3) exhibited a single canonical variate axis of rheotactic-neuromast correlation (Fig. 4A, Table S1). Fish with more neuromasts in the Mp and caudal fin (CF) lines (the two most posterior lines) exhibited more cumulative upstream movement and more upstream orientation (Table 1). Net displacement did not load significantly on CV1. Neuromast CV1

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remains a significant predictor of rheotactic CV1 when controlling for fish origin ($P<0.001$). Although the ecotypes differed in neuromast numbers for particular lateral lines (as noted above), the combination of lines involved in rheotaxis does not differ between wild-caught lake and stream fish. Specifically, lake and stream fish do not differ for the CV1 of neuromast number ($P=0.53$), and there is no significant neuromast CV1*ecotype interaction ($P=0.45$) affecting the rheotactic CV1. We conclude that rheotaxis variation in these non-breeding fish is attributable to neuromast variation that mostly segregates within rather than between populations.

Lab-reared stickleback (Sample 1) exhibited two orthogonal canonical axes of neuromast-rheotactic correlation (Fig. 4B, Table S2). CV1 indicates that fish with fewer anterior pit (AP) and more oral (OR) and supratemporal (ST) line neuromasts (all in the head) exhibited more positive net displacement, less cumulative upstream movement, and more frequent upstream orientation (Tables 1 and S2). CV2 had a weaker but significant effect (Table S2) indicating that fish with more neuromasts in the cranial preopercular (PO), ethmoid (ET), and ST lines were displaced further downstream (negative net displacement), and showed less cumulative upstream movement and more frequent upstream orientation (Fig. 4C, Tables 1 and S2). Both neuromast CV1 and CV2 remain significant predictors of rheotactic CV1 and CV2, respectively (both $P<0.001$) when controlling for fish ecotype (both $P>0.2$) and ecotype*neuromast interaction (both $P>0.3$). Again, it seems that neuromast variation within rather than between lake and stream populations is the primary cause of rheotactic variation. We found no significant difference in CCA axis structure between the wild-caught and lab-reared fish data sets ($P=0.668$).

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IV: Fin morphology is correlated with rheotactic response in stickleback

In lab-reared stickleback (Sample 1), we found significant canonical correlations between rheotaxis and pectoral fin morphology (Figs. 3B&D and 5, Table S3). Stickleback whose fins were longer with lower-surface area exhibited longer cumulative distance upstream, less frequent upstream orientation, and a tendency to stay in areas with slower flow regimes (Table 1). We observed no heritable divergence in pectoral fin shape or area between lab-reared ecotypes (W=76, P=0.94 and W=78.5, P=1). Because ecotype does not affect fin morphology, fin CV1 is significantly associated with rheotactic CV1 (P=0.002) when fish origin is included in the model. Pectoral fin size and neuromast counts are not correlated (Wilks' λ=0.22, P=0.47). We found no significant correlations between rheotaxis and body size (Table S4), body shape (Table S5), or bony armor plate number (Spearman rank correlations P > 0.1 for all rheotactic measures within all samples).

The fin-rheotaxis correlation is corroborated by a sample of breeding-season wild stickleback (Sample 2; Fig. 3). CCA revealed significant correlations for both CV1 and CV2 (Fig. 5B, Table S6). Similar to trends seen in lab-reared fish, CV1 showed that fish with longer fins with less surface area had more negative net displacement, less upstream orientation, and used slower flow regimes. The CV1 of pectoral fin morphology remains a significant predictor of the CV1 of rheotaxis (P=0.01) after controlling for the non-significant ecotype effect on pectoral fin CV1 (P=0.91) and ecotype*CV1 interaction (P=0.82). We did not find divergence in pectoral fin length (W=138, P=0.46) or area (W=133, P=0.37) between lake and stream fish. Moreover, neither body size (Table S7) nor body shape (Table S8) were significantly correlated with rheotaxis in Sample 2. In contrast, non-breeding wild-caught stickleback (Sample 3) exhibited no significant CCA between rheotaxis and any morphology, for both control and lateral line-ablated fish (Tables S9-S14).

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Discussion

Phenotype-dependent dispersal can profoundly alter evolutionary processes (Garant *et al.*, 2004; Postma & van Noordwijk, 2005; Edelaar *et al.*, 2008; Edelaar & Bolnick, 2012). If different phenotypes sort themselves into different habitats, then migration can actually facilitate rather than undermine population divergence and local adaptation (Postma & van Noordwijk, 2005; Garant *et al.*, 2007; Bolnick & Otto, 2013; Jiang *et al.*, 2015). Prior studies indicated that such non-random dispersal occurs in stickleback (Barrett *et al.*, 2009) and facilitates adaptive divergence between lake and stream populations (Bolnick *et al.*, 2009; Jiang *et al.*, 2015). Here, we identify phenotypic traits (lateral line and fin morphology) that contribute to this non-random dispersal behavior (though we do not rule out effects of additional traits such as vision). More generally, our results provide support for the hypothesis that quantitative variation in superficial neuromast number influences among-individual variation in fish rheotactic response. Rheotactic response, in turn, is likely to influence the propensity of an individual to disperse up-or down current and thus into a stream or lake habitat. The implication is that genes contributing to neuromast variation may facilitate among-population divergence between parapatric flow regimes.

Lateral line function

The lateral line system, vision and tactile senses are the three major sensory modalities that fish employ to orient and respond to currents (Montgomery *et al.*, 1997; Coombs *et al.*, 2013). In multiple fish species, ablating neuromasts reduces sensitivity to currents, as indicated by a drastically higher minimum current required to observe rheotaxis (Montgomery *et al.*, 1997; Baker & Montgomery, 1999a; Suli *et al.*, 2012). This effect does not hold for all fish, however (Van Trump & McHenry 2013), for instance if neuromast number evolves to facilitate predator evasion rather than locomotion per se (e.g., Fischer *et*

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al., 2013). Our ablation experiment supported the notion that neuromasts affect rheotactic response, but in a surprising manner. Stickleback lacking neuromasts exhibited stronger position-holding behavior in currents, compared to control fish, which were displaced farther downstream.

Why should loss of a sensory modality actually increase the ability of sticklebacks to hold their position in a current? We speculate that the answer entails a distinction between sensing a current, and subsequent dispersal decisions. Ablating neuromasts removes a source of information about water velocity required to make a decision. When stickleback detected currents via superficial neuromasts, they moved downstream. By eliminating this source of information, fish lost a sensory input needed to make such a decision. We posit that neuromast-ablated fish resorted to using visual cues and remained stationary to stabilize the image of their surroundings (Coombs *et al.*, 2013).

Experimental ablation results in binary traits (neuromasts present or absent) that have little bearing on the quantitative lateral line variation we observe within and between stickleback populations. This quantitative variation has previously been studied in across-species comparative studies, revealing that the number and type of neuromasts covaries with habitat use (Dijkgraaf, 1963; Vischer, 1990; Wark & Peichel, 2010; Trokovic *et al.*, 2011; Vanderpham *et al.*, 2012; Coombs *et al.*, 2013; for a counter example, see Beckmann *et al.*, 2010). Species with more superficial neuromasts tend to live in slower-moving water, or are less active swimmers (Montgomery *et al.*, 1995; Coombs *et al.*, 2013). To explain this negative correlation, some researchers suggest that more neuromasts confer greater sensitivity to water disturbance, which is beneficial in still water but is overloaded in turbulent flowing water or frequently-moving fish (Engelmann *et al.*, 2000; 2002; Coombs *et al.*, 2013). Low neuromast number in stream fish may therefore represent an adaptation to

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mitigate sensory over-stimulation. However, this conclusion is based largely on across-species correlations, and remains experimentally unverified.

Comparatively few studies have examined quantitative variation in lateral line morphology within species (Wark & Peichel, 2010; Yoshizawa *et al.*, 2010; Trokovic *et al.*, 2011). A few papers also document among-population lateral line divergence associated with flow regime, focusing on superficial neuromasts (Wark & Peichel, 2010; Trokovic *et al.*, 2011) and/or canal neuromasts (Trokovic et al., 2011; Vanderpham *et al.*, 2012). However, the directions of these associations were inconsistent. This literature focuses on neuromast numbers (as we do), ignoring potentially relevant variation in their placement, orientation, innervation, and sensitivity.

To summarize, both among- and within-species comparisons previously indicated that fish lateral line traits are often divergent between alternative habitats (Dijkgraaf, 1963; Vischer, 1990; Guarnieri *et al.*, 1993; Wark & Peichel, 2010; Trokovic *et al.*, 2011; Vanderpham *et al.*, 2012; Coombs *et al.*, 2013), implying that natural selection acts on this phenotype. The cause of this selection remains ambiguous, because the lateral line system may influence locomotion in hydrodynamic environments, predator avoidance, or sociality (Wark & Peichel, 2010; Greenwood *et al.*, 2013; Coombs *et al.*, 2013). Consequently, there remains a need to identify the behavioral effects of variation in lateral line traits including (but not limited to) the number of neuromasts. For instance, to our knowledge no prior study has tested for an effect of within-species quantitative variation in the lateral line system (neuromast numbers) on rheotactic response.

Our results therefore clarify the relationship between neuromast number and rheotactic response in this one lake-stream pair of stickleback. We found a significant correlation between neuromast number and rheotaxis. Individuals with more neuromasts tended to exhibit stronger negative rheotactic response (displaced further down-stream,

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spending more time swimming up- and down-stream, and spending less time facing up-stream). The canonical correlations suggest that multiple neuromast lines jointly contributed to a composite measure of rheotactic response, but not all lines contributed equally to this effect. This correlation is irrespective of the native population (lake or stream) of individuals, so the association represents among-individual rather than between-population differences. We observed similar correlations using both lab-reared and wild-caught samples of stickleback, indicating that these conclusions are robust to rearing environment. Depending on which sample we examined (wild-caught, or lab raised), different neuromast lines had the largest effect on rheotactic response, but this discrepancy was not statistically significant. As far as we are aware, the results reported here represent the first evidence that intraspecific quantitative variation in neuromast number influences rheotactic response. However, we must emphasize that these results were obtained from a single lake-stream pair of stickleback. Any generalization regarding the effects of neuromasts should be treated with caution until our findings are replicated in additional populations, preferably from disparate geographic regions. That said, we believe our results will prove to be general, because other (across-species) studies also found fewer superficial neuromasts in high-flow fish (Dijkgraaf, 1963; Vischer, 1990; Guarnieri *et al.*, 1993; Coombs *et al.*, 2013).

All four distinct metrics of rheotactic response were correlated with lateral line morphology (to varying degrees), and three metrics were affected by neuromast ablation. The observed correlations between neuromast number and all four measures of rheotaxis are qualitatively concordant with our ablation results, which confirmed that neuromasts have a causal effect on rheotactic response. Control fish (intact neuromasts) showed stronger negative rheotactic response than did ablated fish (neuromasts absent), which held their position in flow and faced up-stream more often. Thus, fish with fewer neuromasts tended to resemble individuals with ablated neuromasts. This is an encouraging finding, implying that

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ablation experiments (which are comparatively easy) recapitulate the effect of quantitative neuromast variation (which is difficult to experimentally manipulate). Our simultaneous use of an ablation experiment (experimentally rigorous but less natural) with a correlative observation (less controlled, but more biologically relevant) greatly strengthens our inference that stickleback with more neuromasts exhibit more negative rheotactic response.

Pectoral fin shape

Rheotactic response was also correlated with pectoral fin morphology, but not with body size or body shape. Stickleback use their pectoral fins for sculling-style ('labriform') locomotion. Longer pectoral fins with a smaller surface area were associated with rheotactic responses that mostly facilitate downstream dispersal (i.e. more cumulative movement, more time facing downstream, using areas with slower currents). Because of the greater cumulative movement, fish with relatively long thin fins will likely use relatively more energy to remain in flowing water (though we have not directly measured energetic costs). The association between pectoral fin morphology and rheotactic response is consistent with biomechanical studies suggesting that longer pectoral fins with smaller surface area favor prolonged swimming (Walker & Westneat, 2002), and tend to be associated with lake habitats (Hendry *et al.*, 2011). Shorter pectoral fins with larger surface area have been suggested to favor maneuvering (Walker & Westneat, 2002), and are more common in stream habitats (Hendry *et al.*, 2011). However, the correlation between pectoral morphology and rheotactic response only exists when the lateral line is functional, and disappeared in lateral-line ablated fish. Thus, variance in rheotactic response was not merely a result of sensory system variation, but also reflected variation in locomotor morphology, which required sensory input to have an effect.

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Evolutionary implications

We identified differences in neuromast number between lab-reared lake and stream stickleback, implying that lateral line variation is heritable within the focal populations. Lake fish had more neuromasts per line, generally; a phenotype that conferred stronger negative rheotaxis. One caveat is that we cannot rule out trans-generation maternal effects with our study design. However, prior evidence for genetic control of neuromast number in stickleback (Wark *et al.*, 2012) strongly implies that our common-garden experiment results reflect heritable rather than environmental or epigenetic differences.

This heritable divergence in lateral line morphology occurred between the lake and stream sampling sites that are only 350 meters away from each other with no physical barrier to dispersal. Stickleback can readily swim this distance within a few days (Bolnick et al., 2009), so we would typically expect high gene flow to homogenize these populations. How, then, is divergence maintained in the face of gene flow? The results presented here suggest an intriguing answer. Variation in phenotypes such as neuromast number and fin shape (and perhaps other unmeasured traits) confer variation in swimming performance in flowing water (though they may also affect predator detection). This variation in dispersal capacity is likely to cause phenotype-dependent non-random movement (and non-random gene flow for any relevant genes), deterministically sorting traits into different habitats. Specifically, lake phenotypes (more neuromasts) are less likely to swim up-current to join a stream population (Bolnick et al., 2009; Jiang et al., 2015). Thus, dispersal can actually be a means of population differentiation, rather than a maladaptive opposing force (Garant *et al.*, 2004; Postma & van Noordwijk, 2005; Bolnick & Otto, 2013). Our results therefore suggest a promising candidate mechanism for non-random dispersal to facilitate adaptive divergence to lake versus stream hydrodynamic environments.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1: A schematic diagram illustrating the relationship among the three samples of stickleback, phenotypic measurements, and biological questions.

Table S1 – S14: Summary of canonical correlation analyses of rheotactic response associations with lateral line morphology and body shape.

Video S1: Side-by-side videos of the rheotactic response assay with a group of wild-caught threespine stickleback from Blackwater Lake (left side), and from the inlet stream to Blackwater (right side).

Data deposited at Dryad: doi: TBD

Table 1. A summary of the samples of stickleback used in this study and the major inferences about rheotactic response gained in each sample. We list the samples in chronological order (as presented in the Methods). See Supplementary Information Figure S1 for a schematic diagram of the relationship among samples, measurements, and questions. For each sample we list the origin (wild-caught or lab-reared), sample size, sample date (or rearing period for lab-reared fish), and the measurements taken from the resulting specimens.

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For rheotactic response, we list the location where we conducted the assay (FHCRC, UT, or the field in BC). We then list the analyses conducted on each sample (multiple analyses from a given sample are listed on separate rows). For each analysis we indicate the independent (predictor) effect being tested. In cases where the predictor is a CCA axis, we indicate the neuromast lines (or fin shape traits) that load most strongly on the CCA axis, and the direction of these loadings (+/-). Neuromast line abbreviations are as in Fig. 1. The next columns summarize the direction of any correlations between the independent variable and each of four measures of rheotactic response (net displacement, cumulative upstream movement, upstream orientation, or flow regime use). Non-significant effects (NS) and untested effects (NT) are indicated. We do not provide rows describing the non-significant tests of body size or body shape effects on rheotactic response. A summary column indicates, verbally, the behavioral implications inferred from a given CCA analysis.

Table 2. Number of neuromasts in all twelve lines for both wild-caught and lab-reared common-garden lake and stream stickleback. We provide the two-tailed P-values of Wilcoxon rank-sum tests for lake-stream comparisons. Mean (standard deviation) number of neuromasts is shown for each of the twelve lines for each population. Neuromast numbers that significantly differ between lake and stream stickleback are indicated by asterisks.

Fig. 1 The lateral line system of threespine stickleback. A) A schematic of the lateral line system of threespine stickleback, modified from Wark and Peichel 2010. The twelve neuromast lines are the infraorbital (IO), oral (OR), mandibular (MD), preopercular (PO), otic (OT), supratemporal (ST), main trunk line anterior (Ma), main trunk line posterior (Mp), caudal fin (CF), ethmoid (ET), supraorbital (SO) and anterior pit (AP). Neuromast lines with known quantitative trait loci (QTL; Wark et al. 2012) affecting the numbers of neuromasts

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are indicated by an underscore. B) An image of DASPEI-labeled neuromasts on the head of a lake stickleback.

Fig. 2 Effect of neuromast ablation on stickleback rheotactic response. A) An overhead schematic of the circular flow tank, reproduced from Jiang et al. 2015. A side-by-side comparison of B) net displacement (in meters), C) cumulative upstream movement (in meters), D) upstream orientation (% of time) and E) flow regime in wild-caught non-breeding lake and stream stickleback that were unmanipulated (control; dark-gray) and lateral-line ablated (neomycin-treated; light gray). The height of each bar is the group mean and the error bars are the standard errors. Sample sizes are provided in Table 1. Wilcoxon rank-sum tests revealed significant effects of ablation on three of four measures of rheotactic response (panels B-D), see text for W-statistic and P-values.

Fig. 3 Correlation between rheotactic response and neuromast number or fin shape. Scatterplots of the canonical correlation analysis results, for the relationships between rheotaxis canonical variate 1 as a function of: A) canonical variate (CV) 1 of neuromast counts of wild-caught unablated (control) stickleback from Sample 3, $P<0.001$; B) fin shape CV1 of wild-caught stickleback from Sample 2, $P=0.01$; C) neuromast count CV1 of lab-reared fish from Sample 1, $P<0.001$; and D) fin shape CV1 of lab-reared fish from Sample 1, $P=0.002$. Trait loadings on each predictor and dependent axes are given in Figures 4 and 5. Sample sizes are provided in Table 1.

Fig. 4 Correlation between rheotactic response and neuromast number. Helio plots of structural coefficients of: A) the first canonical function predicting rheotactic response using neuromast numbers in non-breeding wild-caught fish (Sample 3); B) the first canonical

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function and C) the second canonical function predicting rheotactic response using neuromast numbers in non-breeding lab-reared fish (Sample 1). Data are displayed in radial bars, with positive trait loadings orienting outward and negative trait loadings pointing inward. The length of the bar reflects the importance of the variable in driving a correlation between neuromast counts and rheotactic response. Significant variables ($P<0.05$) are indicated by asterisks. Sample sizes are provided in Table 1. Significance of the canonical correlations is indicated in the text and in the legend for Fig. 3.

Fig. 5 Correlation between rheotactic response and fin shape. Helio plot of structural coefficients of the first canonical function predicting rheotactic response using pectoral fin size in A) lab-reared non-breeding fish (Sample 1) and B) wild-caught breeding fish (Sample 2). Data are displayed in radial bars, with larger positive values orienting outward and smaller negative values pointing inward. The length of the bar reflects the importance of the variable. Significant variables ($P<0.05$) are indicated by asterisks. Sample sizes are provided in Table 1. Significance of the canonical correlations is indicated in the text and in the legend for Fig. 3.

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Table 1. Summary of three samples used in this study, with sample sizes, source, measurements, analyses, and interpretations.

Sample	Origin	Sample date and sample size (Lake, Stream)	Measurements: Rheotactic response	Lateral line	Morphology	Analysis	Independent effect / CCA axis	Rheotatic response variables: Net Displacement	Cumulative Upstream Movement	Upstream Orientation	Flow Regime	Behavioral Implication
1	Lab-reared	June 2010 - October 2011 (non-breeding)	UT	yes	yes	Test for neuromast-rheotactic response correlation (Q III)	AP (-), OR (+), ST (+)	+	-	+	NT	Fewer AP neuromasts, more OR and ST neuromasts confer greater upstream net displacement, with lower energy expenditure in currents. (Fig. 4B)
		Lateral Line: N = 27L, 22S Rheotaxis and Morphology: N = 15L, 15S				Test for neuromast-rheotactic response correlation (Q III)	PO (+), ET (+), ST (+)	-	-	-	NT	After controlling for CV1, more neuromasts confer greater downstream net displacement with low energy expenditure (Fig. 4C)
						Test for morphology-rheotactic response correlation (Q IV)	Fin length (+), fin area (-)	NS	+	-	-	Long narrow fins have higher energy expenditure in currents, face downstream more, use areas with slower currents (Fig. 5A)
2	Wild-caught	June 2011 (breeding) N = 18L, 18S	BC	no	yes	Test for morphology-rheotactic response correlation (Q IV)	Fin length (+), fin area (-)	-	NS	-	-	Long narrow fins are displaced farther downstream, select areas with slower currents (Fig. 5B)
3	Wild-caught	April 2013 (non-breeding)	FHCRC	yes	yes	Neuromast ablation experiment (Q II)	Ablated group	+	-	+	NS	Neuromasts confer negative rheotaxis (downstream net displacement, higher energy expenditure, less orientation into current; Fig. 2)

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	Ablation: N = 15L, 15S Control: N = 22L, 22S				Test for neuromast-rheotactic response correlation (Q III)	MP (+), CF (+)	NS	+	+	NT	More neuromasts confer higher energy expenditure in currents (Fig. 4A)

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Table 2. Summary of between-sample differences in neuromast counts for the various neuromast lines (see Fig. 1 for line locations). For each sample and line we provide the mean (with standard error) of the number of neuromasts.

	Wild-caught lake (N=22)	Wild-caught stream (N=22)	*P*	Lab-reared lake (N=27)	Lab-reared stream (N=22)	*P*
IO	20.8(6.3)	23.7(8.5)	0.2	37(6)	33.8(4.9)	0.0082*
OR	5.6(4.1)	4.7(3.6)	0.45	7.3(1.7)	6.6(1.5)	0.26
MD	16.5(7.4)	18.5(9.3)	0.50	43(4.7)	39.7(4.7)	0.021*
PO	9.4(4.8)	8.6(4.2)	0.51	21.4(6)	20.4(3.6)	0.78
OT	6.4(3.6)	5.8(3.6)	0.58	12.2(2.1)	10.7(1.8)	0.022*
ST	9.5(3.7)	8.0(3.4)	0.14	18.6(4.1)	16.8(3.1)	0.11
Ma	18.7(8.7)	17(4.8)	0.35	25.7(6.8)	23.2(5.8)	0.20
Mp	60.2(23.3)	45(25.9)	0.036*	91.9(26.8)	81.8(29.6)	0.14
CF	3.3(2.3)	2.5(2.2)	0.29	6.4(3.3)	5.6 (2.5)	0.47
ET	5.9(2.6)	5.5(3.1)	0.56	7(1.2)	6.7(1.4)	0.27
SO	18.6(7.3)	17.5(6.8)	0.63	32.3(4.8)	30.5(5.7)	0.22
AP	8.5(4.4)	6.8(2.6)	0.13	10.9(2.1)	10.7(2.8)	0.224

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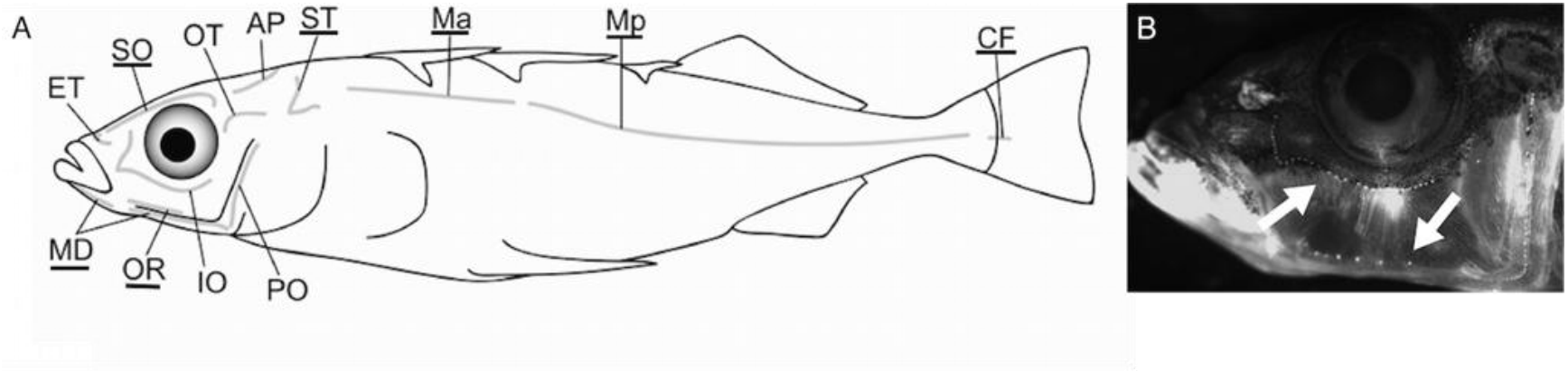

Figure 1

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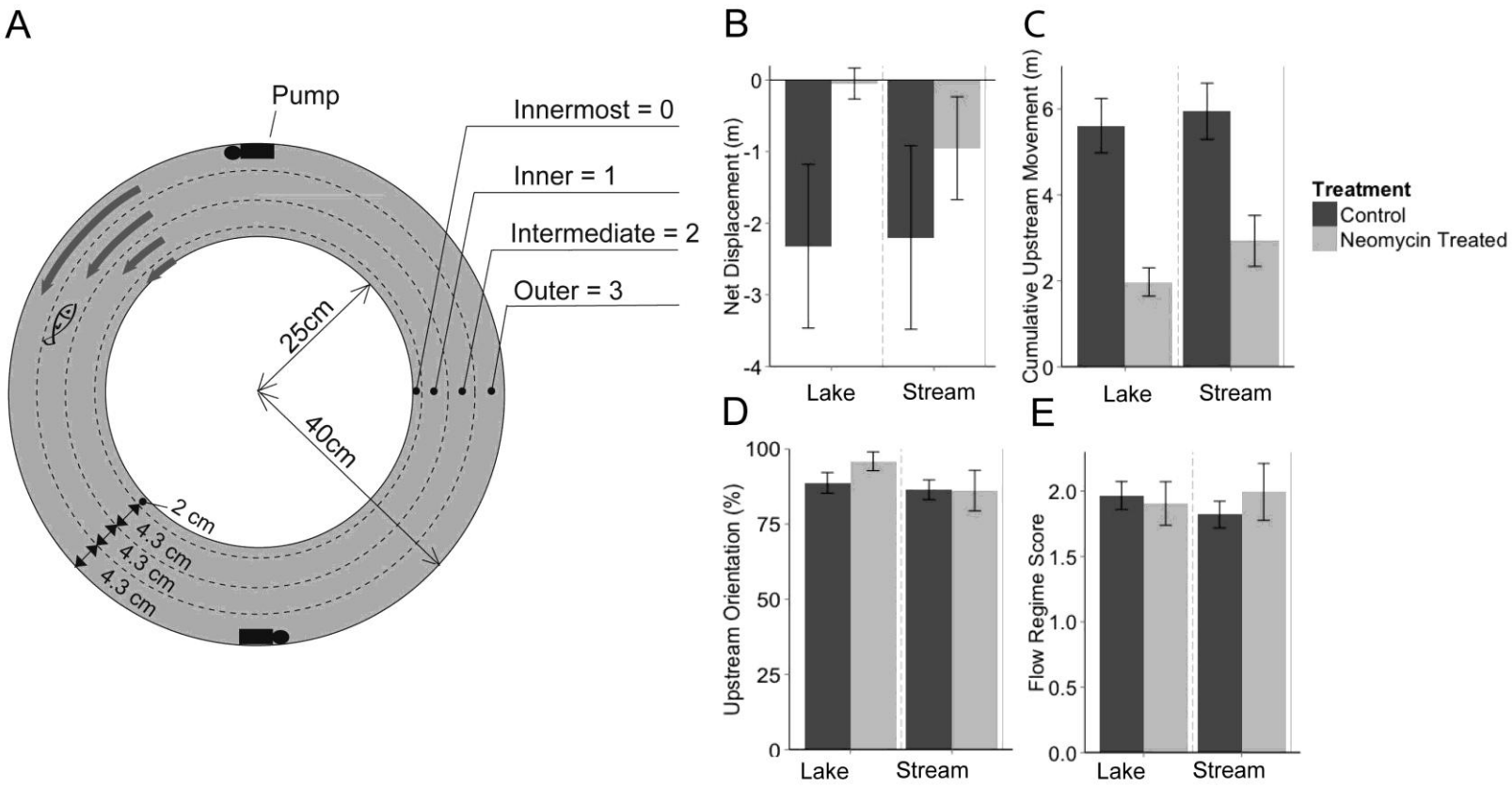

Figure 2

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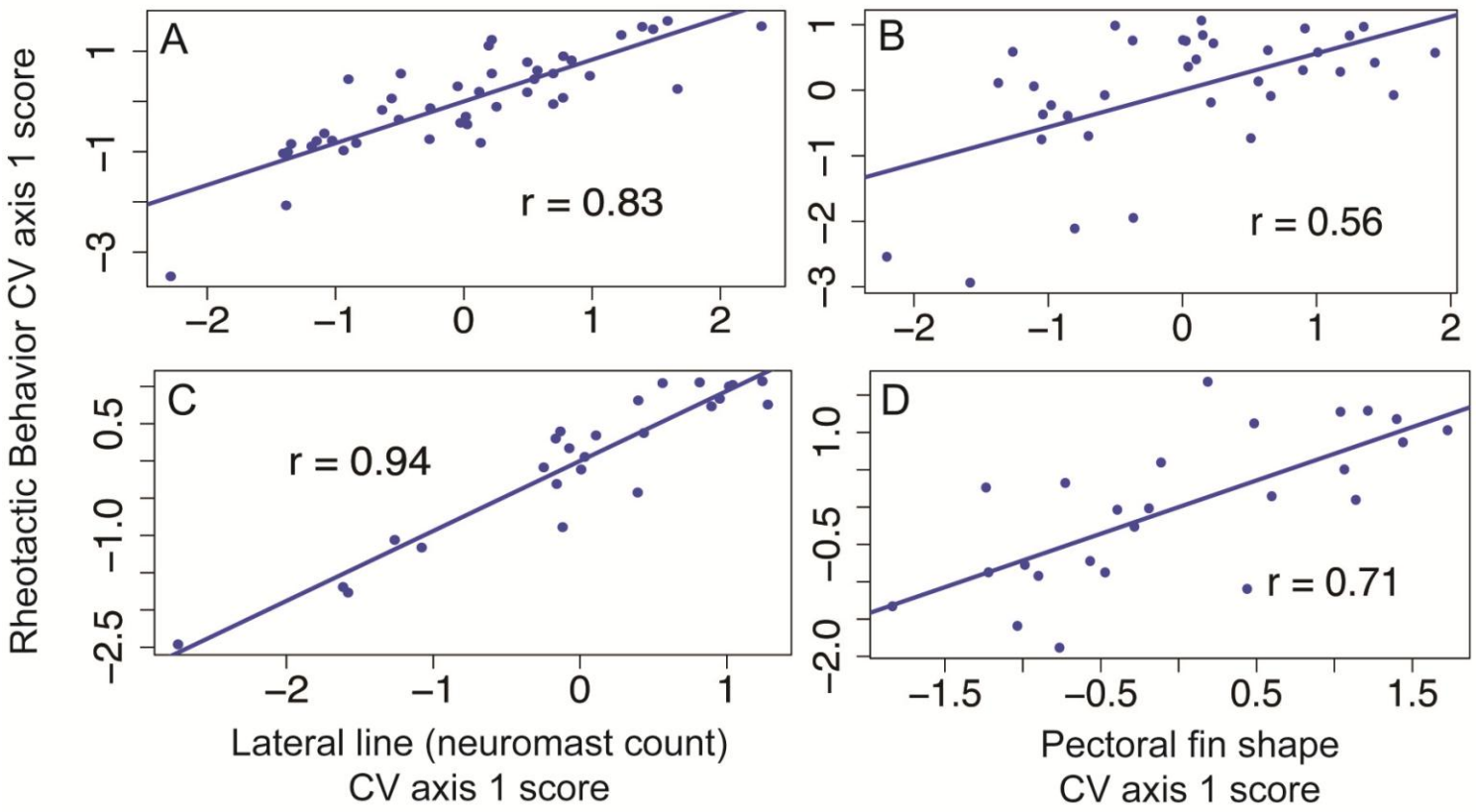

Figure 3

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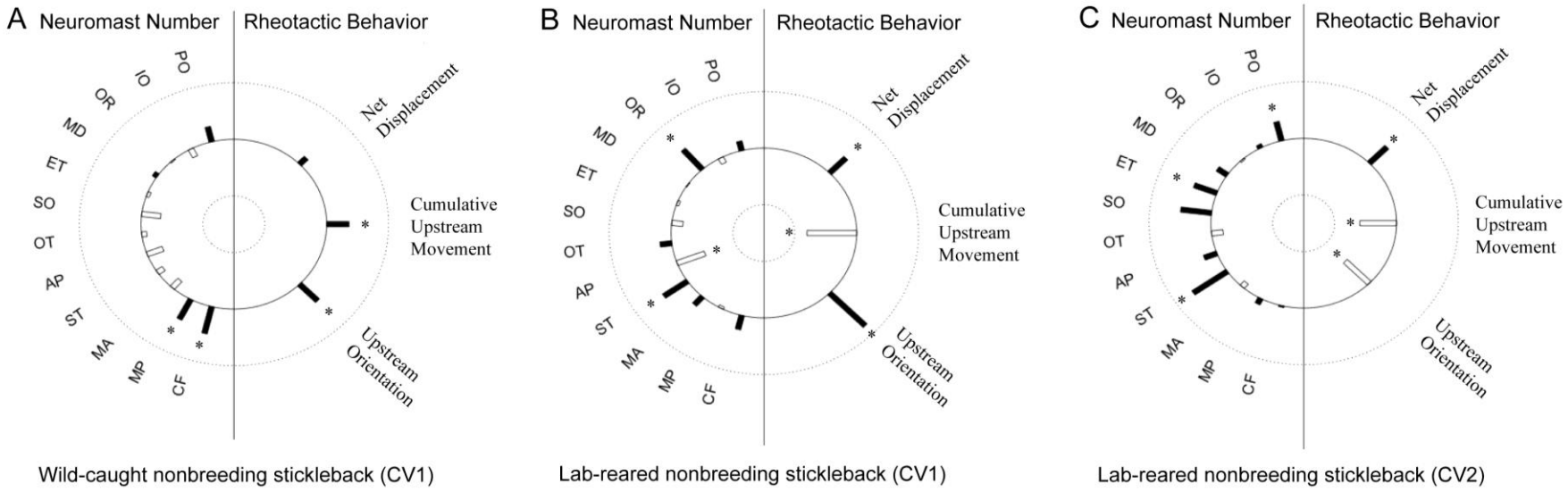

Figure 4

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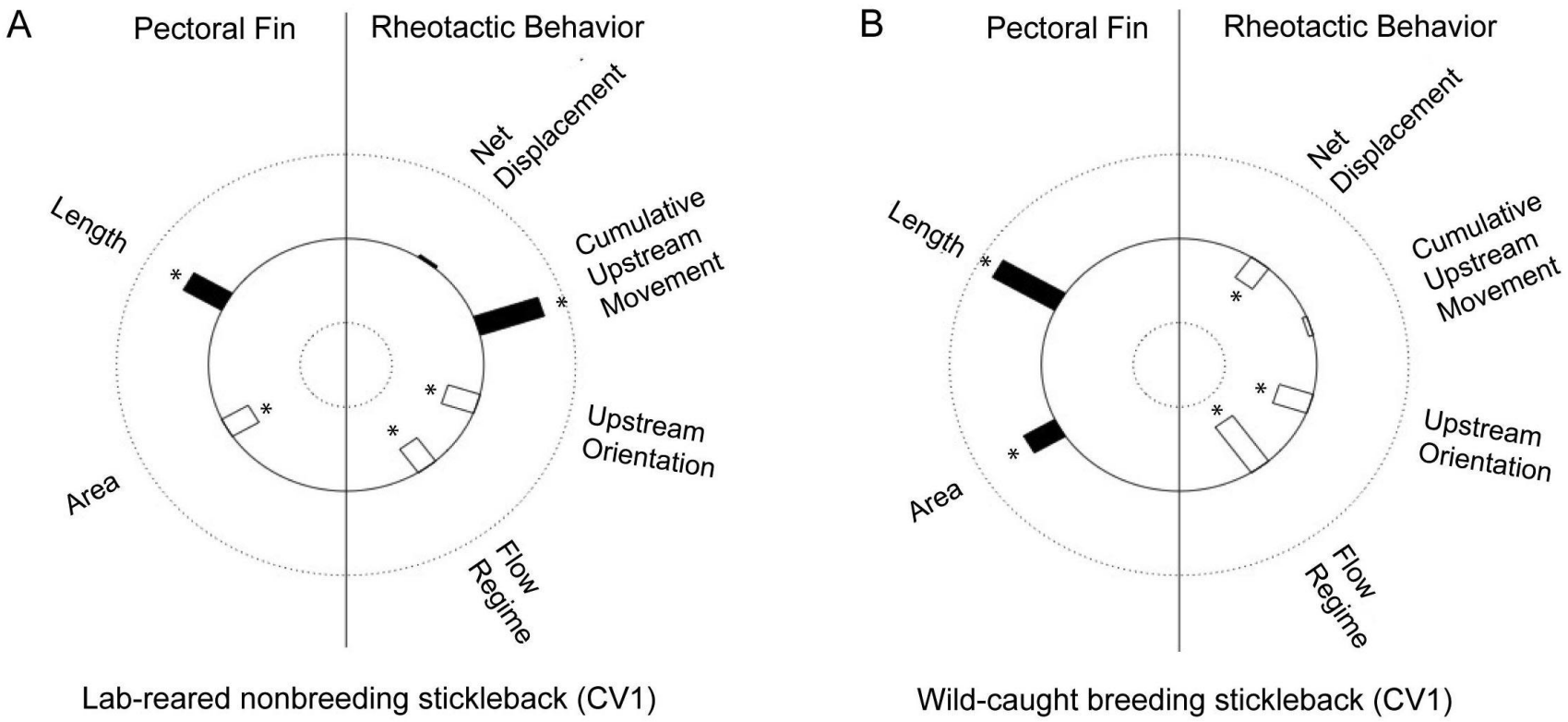

Figure 5

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