



Ecological predictors of lateral line asymmetry in stickleback (*Gasterosteus aculeatus*)

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Abstract

Threespine stickleback exhibit a row of superficial neuromasts that project through the bony plates on each side of the trunk and which constitute an important sensory modality for detection of near-field water motion. Previously, we have shown that numbers of neuromasts on each structural plate are highly variable among populations. In the current paper, we expand this study to evaluate the extent of deviation from bilateral symmetry of 4344 fish in 57 natural and three transplant populations of threespine stickleback from lakes, streams and oceanic habitats of coastal British Columbia, predicting that neuromasts would be largely bilaterally symmetrical for optimal detection of external stimuli. In contrast, we found asymmetry in all populations, the greatest amount occurring on the anterior buttressing lateral plates and on populations with the fewest neuromasts. We found no consistent trends of signed (directional) asymmetry (SA) among the populations while relative absolute asymmetry (RAA) is lower in dystrophic (stained) habitats than in clearwater habitats ($p < 0.001$), except for fish with few neuromasts. Sexual dimorphism in RAA is also greater in dystrophic habitats ($p < 0.001$). Transplants from stained lakes to unstained ponds resulted in a 0.1% to 14% difference in RAA from the source population in less than 12 generations but varied in direction among experiments. Our data suggest a widespread tendency for populations exposed to reduced photic information to exhibit reduced asymmetry in their lateral line system, which can change rapidly in response to a new environment.

Keywords Threespine stickleback · Lateral line · Asymmetry · Water spectra · Geographic variability · Adaptation

Introduction

The lateral line is a primary sensory modality of many fishes, essential for their ability to process stimuli in the diversity of ecological conditions that they encounter. Present on both sides of the body, the neuromast receptors of this organ detect near-field water movement from external stimuli (Mogdans 2019). Similar to other sensory modalities, these receptors are typically bilaterally symmetrical as this facilitates omnidirectional

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detection of approaching prey and predators. Yet expanding observations on a diversity of invertebrate and vertebrate taxa indicate small to large bilateral asymmetries in sensory receptors for chemotaxis, audition and vision (Hart et al. 2000) as well as asymmetric behavioural responses to different stimuli (Werner and Seifan 2006; Lychakov et al. 2006, 2008; Anfora et al. 2011; Frasnelli et al. 2012; Rogers 2017; Krings et al. 2019). The influence of structural asymmetry on the symmetry of ecologically important behaviours such as habitat navigation and predator evasion (Westin 1998; Lippolis et al. 2009) may be a cause of disparate results among studies using asymmetry as an indicator of developmental instability (Markow and Clarke 1997; Houle 1997; Moller 1997; Beasley et al. 2013; Niemeier et al. 2019). The extent to which the lateral line of fishes exhibits bilateral asymmetry and whether such asymmetry, if present, has ecological relevance is not well known.

Lateral line morphology is highly variable among ecological contexts, making it an ideal system to assess if asymmetry occurs in sensory structures and if so, how ecological landscapes affect asymmetry. The lateral line is comprised of many neuromasts, clusters of stereocilia encased in a gelatinous cupula, found in linear arrays on the head and the lateral flank of fishes and amphibians (Coombs et al. 2014). Neuromasts can be classified into two major sub-modalities, velocity detecting superficial neuromasts and acceleration detecting canal neuromasts, the former being found on the surface of armor/skin/scales and the latter being housed in fluid filled canals. Lateral line morphology, whether it be neuromast counts, pattern or internal structure, is highly variable among species due to its diversity of functions (Coombs et al. 2014; Schmitz et al. 2014). Many life history traits are associated with the lateral line, such as predator prey interaction (Coombs and Patton 2009; Junges et al. 2010; Schwalbe et al. 2012), rheotaxis (Baker and Montgomery 1999; Suli et al. 2012; Brown and Simmons 2016; Jiang et al. 2017), schooling (Middlemiss et al. 2017; Mekdara et al. 2018) and conspecific interaction (Butler and Maruska 2016). There is also a growing body of evidence showing that lateral line morphology is variable within species, particularly among populations in different habitats (Wark and Peichel 2010; Trokovic et al. 2011; Fischer et al. 2013; Planidin and Reimchen 2019). This intraspecific divergence presents a useful model system to study the mechanisms driving the diversification of the lateral line system.

Threespine stickleback, *Gasterosteus aculeatus* (Linnaeus 1758), exhibit highly variable numbers of neuromasts (Wark and Peichel 2010; Wark et al. 2012; Planidin and Reimchen 2019; Ahnelt et al. 2021). The lateral line of threespine stickleback is comprised entirely of superficial neuromasts, which differ in their number in association with habitat characteristics and life history. On average, neuromast counts are greater in freshwater than oceanic populations and benthic versus limnetic population pairs (Wark and Peichel 2010; Ahnelt et al. 2021). Furthermore, populations subject to peat-stained waters develop fewer neuromasts on their lateral plates, as do females relative to males (Planidin and Reimchen 2019).

Asymmetry is prevalent in stickleback morphology. Bony lateral plates covering the entire flank of oceanic threespine stickleback and ranging from fully plated, to low-plated (3–8 plates), to ‘naked’ fish in freshwater systems, exhibit wide spread changes in their bilateral asymmetry dependent on ecological context and life history (Moodie and Reimchen 1976; Bergstrom and Reimchen 2000). Fish with asymmetries of lateral plates are less common in populations exposed to puncturing predators (Moodie and Reimchen 1976), exhibit increased parasite load (Reimchen and Nosil 2001; Bergstrom and Reimchen 2005) and have better young survival (Moodie and Moodie 1996). Furthermore, within a population, left biased plate asymmetry increases from littoral to limnetic habitats but also the frequencies vary with respect to collecting conditions such as temperature and windspeed

(Reimchen and Bergstrom 2009). Lateral plate asymmetry appears to be integrated to the functional ecology of threespine stickleback.

Threespine stickleback morphology can change quickly in response to a new environment. Following transplant from a stained lake to an unstained pond, stickleback have undergone changes in many traits over the course of just eight generations, representing one third of the difference observed between natural lake and natural pond populations (Spoljaric and Reimchen 2007; Leaver and Reimchen 2012). This transplant population has also undergone genome wide changes of a similar degree, particularly in regions associated with phenotypic traits (Marques et al. 2018). Rapid change has also occurred in the neuromasts on the lateral plates of two transplant pond experiments, which underwent an increase or decrease in number following transplant to a pond, depending on the source population phenotype (Planidin and Reimchen 2019).

We scored fish for their number of neuromasts occurring on the left and right lateral plates of threespine stickleback from 57 localities from the coast of British Columbia, Canada. These populations spanned major habitat types such as oceanic, lake, stream and pond populations, as well as ecological landscapes from clear, partially-stained and deeply stained clarity regimes. We then explored associations between neuromast count asymmetry and habitat characteristics. A subset of 34 lakes for which spectra, area and predation regime data were available were further tested for the influence of biophysical and life history traits on neuromast asymmetry, as well as two lake-stream pairs. We also examined the extent of asymmetry in three transplant populations in relation to the source populations. While non-directional asymmetries of the lateral line have been previously assessed as a component of multi-metric studies of fluctuating asymmetry in several fish species, including ninespine stickleback (Almeida et al. 2008; Trokovic et al. 2012), here we present a direct investigation of lateral line asymmetry among a diversity of ecological contexts and its potential rate of change.

Materials and methods

Sample and biophysical data collection

Threespine stickleback and biophysical measurements from 57 localities were previously collected as part of continuing research on the Haida Gwaii archipelago (48) and Dewdney-Banks archipelago (9) (review in Reimchen and Nosil 2006; Reimchen et al. 2013; Fig. 1; Table 1). Threespine stickleback were captured using minnow traps baited with aged cheddar cheese, anaesthetized with MS22 or Clove oil, preserved in formalin and stored in ethanol. Localities were selected to encompass the breadth of biodiversity found on the Haida-Gwaii archipelago and the Dewdney-Banks archipelago, as well as reference oceanic populations, *i.e.* stickleback caught nearshore during breeding season. Three transplant populations were also investigated. Roadside Pond and Bevan's Pond were established in 1993 and 1995 respectively using the giant black stickleback from Mayer Lake as the source population (Leaver and Reimchen 2012). For Roadside Pond, we scored generations two, six and twelve and for Bevan's Pond, generations one and ten in addition to multiple year samples (1982, 1997, 2002, 2003) from the source population. The third transplant population was at Drizzle Pond which was established in 1997 by 16 adult fish from the giant black stickleback at Drizzle Lake. We scored generations three, four, nine and twelve

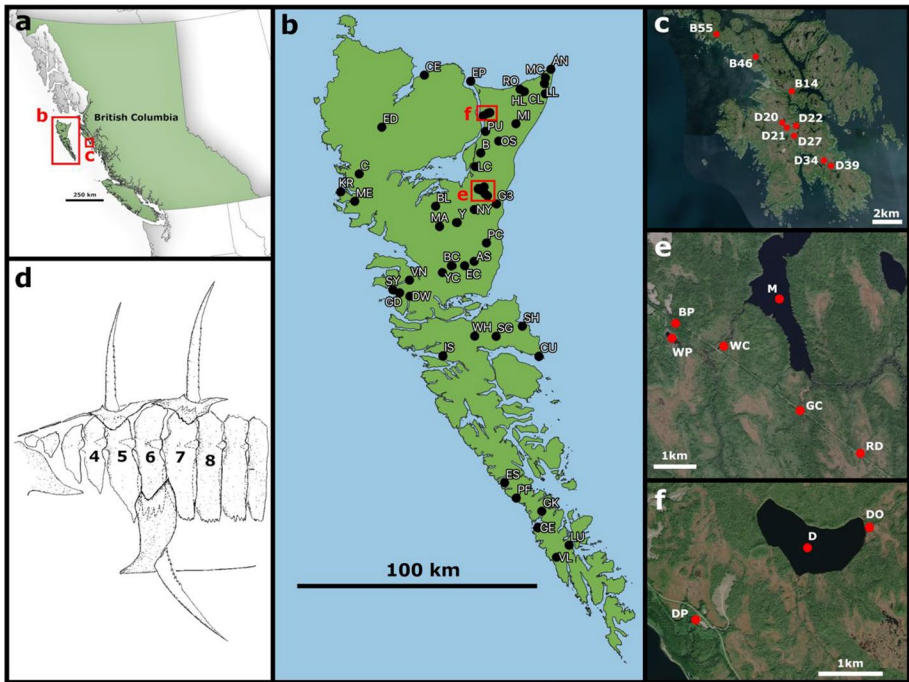


Fig. 1 Map of study localities (a–c, e, f) and diagram of butressing lateral plates (d). (a) British Columbia, (b) Haida Gwaii, (c) Dewdney-Banks archipelago. (e, f) transplant experiment populations (D to DP; M to RD; M to BP) and lake-stream pairs (D and DO; M and GC). See table 1 for population details

of the transplant population, as well as fish sampled in 1979, 1981, 1983, 1987, 1988, 1989 and 2015 from the source population.

Lab work

We scored the number of neuromasts and neuromast pores on the fourth through eighth lateral plates (hereafter referred to as butressing lateral plates) on both sides of the trunk (Fig. 1d) under a dissecting microscope, and determined sex, standard length and lateral plate counts on both sides for all individuals (see Planidin and Reimchen 2019). Repeatability was tested on seven populations, Drizzle Lake, Drizzle Pond, Mayer Lake, Roadside Pond, Gudal Lake, Swan Lake and Lake D21. These were selected to span the breadth of morphologies observed in the study. Repeat scoring of neuromasts was done in the reverse sequence (right side first) relative to the initial scoring (left side first) to ensure that order of scoring did not impart any bias in results. Effect size of measurement error relative to biological asymmetry was compared according to Palmer and Strobeck (1986).

Describing asymmetry among populations

Only positions with plates present on both sides were included in analysis. R is the number of neuromasts on the right side and L is the number of neuromasts on the left of a plate pair for a given position. Initially, signed asymmetry ($SA=R-L$) and absolute asymmetry

Table 1 Summary of sampling sites

Locality	Abrev.	F	M	Avg. Plates	Region	Habitat	Clarity	1400	Area (h)	Predators
Anderson South	AS	20	20	4.2	Haida Gwaii	Lake	Clear	90.5	14	CT
Anser	AN	35	45	3.7	Haida Gwaii	Lake	Stained	76	18	CT
B46	B46	20	20	3.9	Dewdney-Banks	Lake	Clear	86.5	2.9	IA
B55	B55	20	20	3.8	Dewdney-Banks	Lake	Clear	83.1	7.9	CT
BA14	B14	20	20	3.9	Dewdney-Banks	Lake	Clear	83.8	4.6	CT
Boulton	B	55	22	2.5	Haida Gwaii	Lake	Partial-Stain	78.1	15	IA
Brent Creek	BC	17	37	4.4	Haida Gwaii	Stream	Partial-Stain	-	-	-
Blackwater Creek	BL	9	20	4.9	Haida Gwaii	Stream	Clear	-	-	-
Cedar	CE	15	25	3.9	Haida Gwaii	Lake	Stained	-	-	-
Clearwater	CL	30	11	1.4	Haida Gwaii	Lake	Partial-Stain	-	-	-
Coates	C	23	27	5	Haida Gwaii	Lake	Clear	94.5	90	RT
Cumshewa	CU	10	36	4.2	Haida Gwaii	Lake	Stained	-	-	-
D20	D20	20	20	3.9	Dewdney-Banks	Lake	Clear	-	-	-
D21	D21	20	20	3.7	Dewdney-Banks	Lake	Clear	92.7	0.6	CT
D22	D22	20	20	3	Dewdney-Banks	Lake	Clear	81.3	1.6	CT
D27	D27	20	20	4.2	Dewdney-Banks	Lake	Partial-Stain	70.6	6.6	RT
D34	D34	20	20	3.6	Dewdney-Banks	Lake	Clear	86.2	10.7	IA
D39	D39	20	20	1.6	Dewdney-Banks	Lake	Clear	85.6	0.8	IA
Dawson	DW	19	19	4.6	Haida Gwaii	Lake	Clear	82	2.4	CT
Drizzle	D	361	628	2.9	Haida Gwaii	Lake	Stained	67	97	CT
Drizzle Outlet	DO	21	22	3.3	Haida Gwaii	Stream	Stained	-	-	-
Eden	ED	20	20	4.8	Haida Gwaii	Lake	Clear	-	-	-
Elk Creek	EC	10	46	4.6	Haida Gwaii	Stream	Partial-Stain	-	-	-
Entry Point	EP	8	13	5	Haida Gwaii	Oceanic	Clear	-	-	-
Escarpment	ES	49	42	4.6	Haida Gwaii	Lake	Clear	93.6	97	RT
Geikie	GE	15	19	2.6	Haida Gwaii	Stream	Stained	-	-	-
Gold Creek	GC	20	20	4.1	Haida Gwaii	Stream	Stained	-	-	-

Table 1 (continued)

Locality	Abrev.	F	M	Avg. Plates	Region	Habitat	Clarity	t400	Area (h)	Predators
Goski	GK	18	22	4.7	Haida Gwaii	Lake	Clear	88.1	10	RT
Gowgaia East	GE	19	19	5	Haida Gwaii	Lake	Partial-Stain	77.9	25	RT
Gudal	GD	19	20	4.5	Haida Gwaii	Lake	Clear	95	25	RT
Harelda Lower	HL	44	21	0.2	Haida Gwaii	Lake	Stained	-	-	-
Inskip Lagoon	IS	21	28	5	Haida Gwaii	Oceanic	Clear	-	-	-
Krajina	KR	20	20	4.6	Haida Gwaii	Lake	Clear	86.5	16	RT
Loon Creek	LC	21	35	2.6	Haida Gwaii	Stream	Stained	-	-	-
Lummi	LL	20	20	2.9	Haida Gwaii	Lake	Stained	-	-	-
Lutea	LU	14	36	4.7	Haida Gwaii	Lake	Clear	93.9	3	RT
Marie	MA	15	20	4.3	Haida Gwaii	Lake	Clear	89.1	36	CT
Mayer	M	170	118	4.7	Haida Gwaii	Lake	Stained	57.1	373	CT
Mercer	ME	16	24	4.6	Haida Gwaii	Lake	Clear	-	-	-
Mica	MC	20	20	3.8	Haida Gwaii	Lake	Stained	-	-	-
Middle	MI	12	23	0.3	Haida Gwaii	Lake	Stained	-	-	-
New Year	NY	20	20	2.6	Haida Gwaii	Lake	Partial-Stain	68.6	6.9	CT
Otter South	OS	21	18	4.5	Haida Gwaii	Lake	Stained	44.5	36	CT
Pontoon	PC	20	20	4	Haida Gwaii	Lake	Partial-Stain	88.3	1	IA
Puffin	PF	20	20	5	Haida Gwaii	Lake	Clear	82.7	4.6	RT
Pure	PU	18	20	4	Haida Gwaii	Lake	Partial-Stain	65.9	34	CT
Rouge	RO	68	43	0.2	Haida Gwaii	Lake	Partial-Stain	68.1	2	IA
Sheldon	SH	16	16	4.9	Haida Gwaii	Oceanic	Clear	-	-	-
Skidegate	SG	2	20	4.8	Haida Gwaii	Lake	Clear	94.4	734	CT
Stiu	SY	17	36	5	Haida Gwaii	Lake	Clear	92.8	24	RT
Van Inlet	VN	16	16	4.7	Haida Gwaii	Lake	Clear	-	-	-
Victoria Lower	VL	18	20	5	Haida Gwaii	Lake	Clear	94.8	149	RT

Table 1 (continued)

Locality	Abrev.	F	M	Avg. Plates	Region	Habitat	Clarity	t400	Area (h)	Predators
White Swan	WH	20	17	4.3	Haida Gwaii	Lake	Clear	90.7	0.6	CT
Woodpile Creek	WC	20	20	4.1	Haida Gwaii	Stream	Stained	-	-	-
Woodpile	WP	20	20	4.1	Haida Gwaii	Lake	Stained	60.8	4	CT
Yakoun Lake	Y	20	20	4.5	Haida Gwaii	Lake	Clear	94.6	790	CT
Yakoun River	YC	9	34	4.6	Haida Gwaii	Stream	Clear	-	-	-
Drizzle Pond	DP	180	141	3.5	Haida Gwaii	Transplant	Partial-Stain	88	0.1	IA
Roadside Pond	RD	133	101	4.7	Haida Gwaii	Transplant	Partial-Stain	79	0.2	IA
Bevan's Pond	BP	40	40	4.3	Haida Gwaii	Transplant	Partial-Stain	87	0.1	IA

Abrev. are abbreviations presented in Fig. 1, F is female sample size, M is male sample size, and Avg. Plates is the average number of buttressing plate pairs with at least one neuromast scored for the population

($AA=|R-L|$) were summarized among all populations, to assess if asymmetry was present in buttressing plate neuromast counts and if such asymmetry differed among populations. Due to the dependence of AA on the number of neuromasts per plate (NPP) (Poisson GLMM: $\chi^2_1=46.3$, $p < 0.001$) and thus the heterogeneity of variance in SA among populations, all modeling and statistical testing was done on the corrected metrics relative signed asymmetry ($RSA = \frac{R}{R+L}$) and relative absolute asymmetry ($RAA = \frac{|R-L|}{R+L}$). Note that RAA can be converted to the commonly used metric for fluctuating asymmetry $\frac{|R-L|}{(R+L)}$ by multiplying it by two (Palmer and Strobeck 1986). Since it is unclear whether RAA or AA is a better predictor of functional consequences in asymmetry, model predictions of RAA were converted to AA by multiplying it by the total number of neuromasts on each plate pair and presented in figures, however no modeling was done on AA (Figs. 1–3).

Testing for differences among buttressing plate positions

Both RSA and RAA were modeled using a binomial GLMM with a logit link, with the total number of neuromasts on each plate pair ($R+L$) included as weights (number of binomial trials). For initial testing of the presence of RSA and RAA Eq. 1 was used (Table 2). Interaction effects are justified, as buttressing plate NPP varies with sex and plate position and since asymmetry in other traits also exhibit sexual dimorphism in threespine stickleback (Reimchen et al. 2008, 2016; Reimchen and Bergstrom 2009; Planidin and Reimchen 2019). This model and all subsequent models, underwent backward stepwise model selection using $\alpha=0.05$, a conservative estimate of model complexity relative to other methods such as AIC, although it is still subject to selection bias (Zucchini 2000; Murtaugh 2009). Nested models were compared using a Wald test. All continuous predictors were rescaled by subtracting the mean and dividing by two standard deviations prior to model fitting. All non-significant test statistics presented were calculated by adding the predictor back into the final selected model. All statistical analysis was done in R 3.6.3 using *lme4* and *emmeans* (Bates et al. 2015, p. 4; Lenth 2019; R Core Team 2020). As only RAA was found to differ among populations, RSA was dropped from subsequent analysis.

Testing the effect of ecology on RSA

We assessed the differences in RAA among major geographic regions (Haida Gwaii and Dewdney-Banks), habitat types (oceanic, lake and stream) and regimes of water clarity (stained, partially-stained, clear) among 57 populations (3709 fish; 14,613 plate pairs). These habitat characteristics were assessed with Eq. 2, with two-way interactions between habitat characteristics, NPP and sex kept to determine sex specific effects and to account for potential covariation between NPP and ecological factors (Engqvist 2005; Table 2). Our dataset is notably imbalanced both in samples sizes within populations and among ecological regimes e.g. three oceanic populations versus 45 lakes. While GLMMs are robust to imbalance, the power of the pairwise comparison is limited by the smaller group (Cnaan et al. 1997).

To further understand why RAA differed among clarity regimes a subset of 34 lakes (2671 fish; 10,881 plate pairs) for which more complete environmental data were available (Table 1), were tested for additional habitat and morphological predictors of asymmetry, as were two parapatric lake-stream pairs. Clarity code is correlated with changes in water spectra, predation regime and lake area (Reimchen 1989). Therefore, t400 (percent

transmission at 400 nm), log(lake area) (hectares) and predation regime (invertebrate/avian, cutthroat/avian, rainbow trout/avian) were tested as predictors of differences in RAA among lakes, as well as standard length (SL) (mm), log(lateral plate count) log(LP) and absolute lateral plate asymmetry (LP_{R-L}), Eq. 3 (Table 2). Random slopes by population were included for each morphological trait and covariance between random slopes was removed for model convergence (Harrison et al. 2018; Table 2). Covariation between RSA and lateral plate count is of interest due to the association between NPP and lateral plate count (Planidin and Reimchen 2019), as well as the common genetic loci controlling lateral plate and neuromast development (Wark et al. 2012). The effects of lateral plate asymmetry on RAA are of interest given the contentions around correlations in asymmetry among different traits (Leung et al. 2000). Lateral plate asymmetry is not dependent on lateral plate count ($\chi^2_1 = 0.282$, $p = 0.596$). Intrapopulation effects of SL on RAA were tested in the two populations sampled across multiple years, Drizzle Lake (986 individuals; 3872 plate pairs) and Mayer Lake (284 individuals; 1343 plate pairs), Eq. 4 (Table 2). Lake-stream pairs, Drizzle Lake and Drizzle Outlet (43 individuals; 143 plate pairs) and Mayer Lake and Gold Creek (40 individuals; 165 plate pairs) were analyzed using Eq. 5 (Table 2).

Transplant experiments

Three experimental transplant ponds, Roadside Pond (234 individuals; 1026 plate pairs) and Bevan's Pond (80 individuals; 346 plate pairs) from Mayer Lake and Drizzle Pond (321 individuals; 1133 plate pairs) from Drizzle Lake, were tested for differences in RAA relative to their source populations, using Eq. 5 (Table 2). If significant differences between a transplant and its source population were found, the transplant population was then tested for intergenerational change in RAA using Eq. 6 (Table 2). RAA in all ponds was plotted against the expected value given the final reduced model from Eq. 3 (Table 2).

Repeatability

Measurements were highly repeatable. Replicate and side had no significant effect on number of neuromasts observed, with a 95% CI on directional measurement error being 6.8% of the standard deviation of average neuromast SA, ± 0.04 versus ± 0.55 (replicate: $\chi^2_1 = 0.05$, $p = 0.818$; side: $\chi^2_1 = 1.19$, $p = 0.276$). Furthermore, the relative rate of measurement error does not increase with number of neuromasts and the 95th quantile of relative measurement error is only 5.1% of the average RAA, 0.02 versus 0.39 ($\chi^2_1 = 1.47$, $p = 0.225$).

Results

Presence of asymmetry

Asymmetry in buttressing plate neuromast counts is prevalent. Frequencies of neuromast asymmetries range from 70.8% at LP4, 60.3% at LP5, 53.3% at LP6, 51.1% at LP7 and 48.9% at LP8 and overall, 93.3% of fish are asymmetric in neuromast count at one or more plate positions. AA ranges from 0 to 7 per plate and with population means ranging from 0 to 1.13, 95%CI=[1.04, 1.22]. Per plate SA is also variable, ranging from -7 to 6 and with population means ranging from -0.18 [-0.34, 0.07] to 0.22 [-0.03, 0.37].

RAA differs among populations, sexes, among buttressing plate positions and with NPP (Table 2). RAA estimated marginal means (emms) [95% CI] range from 0.093 [0.080, 0.108] to 0.875 [0.614, 0.969] among populations (population: $\chi^2_{56} = 2380$, $p < 0.001$). Anterior positions exhibit greater RAA than posterior positions and males have significantly greater RAA than females on all plate positions other than the fourth (sex: $\chi^2_1 = 38.4$, $p < 0.001$; position: $\chi^2_4 = 238$, $p < 0.001$; sex \times position: $\chi^2_4 = 10.13$, $p = 0.038$; Table 3). RAA declines with NPP, with the most rapid decline occurring on the sixth plate and a steeper decline in males than females (NPP: $\chi^2_1 = 95.3$, $p < 0.001$; NPP \times position: $\chi^2_4 = 69.5$, $p < 0.001$; NPP \times sex: $\chi^2_1 = 7.77$, $p = 0.005$; Table 3).

Population average RSA ranged from 0.42 [0.18, 0.69] to 0.66 [0.47, 0.80], with 22 left biased populations and 35 right biased populations (binomial test: $p = 0.056$), however 95% CIs for all population means contained 0.5 (equal proportion of right and left neuromasts). RSA does not significantly vary among populations (population: $\chi^2_{56} = 19.4$, $p = 1.000$), between sexes, among buttressing plate positions or with NPP, including two-way interactions (all trait effects: $\chi^2_{1-4} \leq 1.49$, $p \geq 0.25$; Table 2).

Differences among major ecological regimes

RAA differs among geographic regions and clarity regimes but not habitat types (Table 2). Haida Gwaii stickleback have less RAA than Dewdney-Banks stickleback (Haida Gwaii emm [95% CI] = 0.121 [0.113, 0.130]; Dewdney-Banks emm [95% CI] = 0.142 [0.123, 0.162]; region: $\chi^2_1 = 4.77$, $p = 0.03$), but sexual dimorphism in RAA and the relationship between NPP and RAA do not differ between regions (All $\chi^2_1 \leq 0.107$, $p \geq 0.74$). Stained localities exhibit reduced RAA for fish with many NPP, but fish with few neuromasts have comparable RAA among all clarity regimes (clarity: $\chi^2_2 = 24.15$, $p < 0.001$; NPP \times clarity: $\chi^2_2 = 22.9$, $p < 0.001$; Fig. 2). Sexual dimorphism in RAA differs among clarity regimes, with the greatest sexual dimorphism occurring in stained localities (sex \times clarity: $\chi^2_2 = 23.7$, $p < 0.001$; Fig. 2). There is no significant difference in RAA, sexual dimorphism in RAA or the relationship between NPP and RAA among habitat types (All $\chi^2_3 \leq 6.50$, $p \geq 0.090$). RAA did not differ between either lake-stream pair (Drizzle Lake-Drizzle Outlet: $\chi^2_1 = 0.3$, $p = 0.583$; Mayer Lake-Gold Creek: $\chi^2_1 = 1.32$, $p = 0.251$).

Differences among lake stickleback

t400 and standard length are the best predictors of RAA among lake populations (Table 2). RAA increases with t400, RAA sexual dimorphism is greater in longer fish and RAA is greater in smaller lakes (NPP \times t400 $\chi^2_1 = 5.63$, $p = 0.018$; sex \times standard length $\chi^2_1 = 11.9$, $p < 0.001$; area $\chi^2_1 = 4.21$, $p = 0.040$; Fig. 3). The variance captured by population random slopes for standard length converged to zero and there are no significant effects of standard length within Drizzle Lake or Mayer Lake (Drizzle lake log-odds = 0.08 ± 0.13 , $\chi^2_1 = 0.39$, $p = 0.530$; Mayer Lake log-odds = 0.22 ± 0.13 , $\chi^2_1 = 0.3$, $p = 0.583$; Table 2). Predation regime, lateral plate count and lateral plate asymmetry did not have a significant effect on RAA, including sex and NPP interactions (predation regime: $\chi^2_1 = 4.75$, $p = 0.093$; all other $\chi^2_{1-2} \leq 3.86$, $p \geq 0.145$).

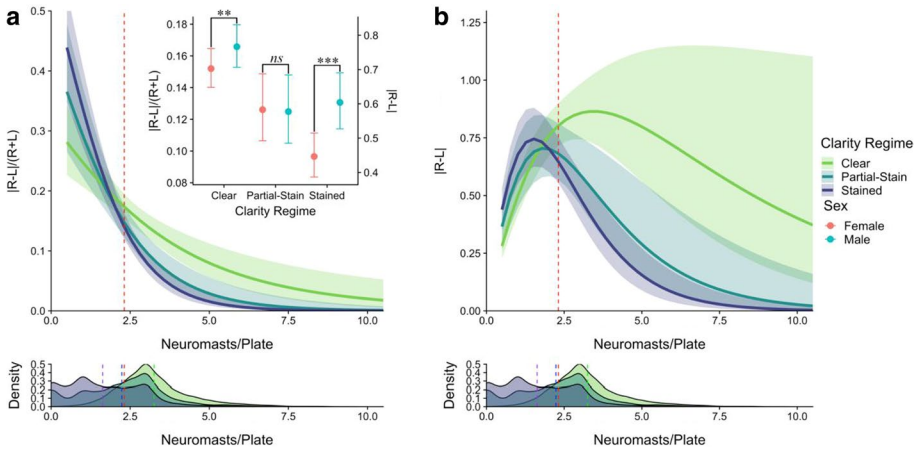


Fig. 2 Decline in (a) relative absolute asymmetry and (b) absolute asymmetry, of buttressing plate neurocasts in stained water populations relative to clear water populations. Trend lines are marginal mean model estimates, averaged over sexes, plate positions and geographic region, shaded regions are a 95% CI. Inset figure in (a) displayed sexual dimorphism at the average neuromast count marked by the vertical dashed line. Points on inset are estimated marginal means with 95% CI error bars, averaged over, plate positions and geographic region. Significance level of marginal mean contrast of $p < 0.001$ ***, $p < 0.01$ **.

Transplant populations

The transplants from the deeply stained Mayer Lake to unstained Roadside Pond resulted in a reduction in RAA for fish with few neurocasts and an increase in RAA for fish with many NPP (NPP \times population: $\chi^2_1 = 10.0$, $p = 0.002$; population : $\chi^2_1 = 0.880$, $p = 0.348$; Table 2; Fig. 4a, b). There was no change in sexual dimorphism in RAA (sex \times population: $\chi^2_1 = 0.64$, $p = 0.424$). This trend also grows stronger in subsequent generations of Roadside Pond (NPP \times generation: $\chi^2_1 = 4.62$, $p = 0.032$; Fig. 4a).

RAA increased from Mayer Lake to Bevan’s Pond, with fish with many NPP tending to have undergone a greater increase (population: $\chi^2_1 = 4.27$, $p = 0.039$; NPP \times population: $\chi^2_1 = 3.54$, $p = 0.060$; Table 2; Fig. 4c,d). Sexual dimorphism in RAA remaining the same (sex \times population: $\chi^2_1 = 0.62$, $p = 0.432$). RAA increased in subsequent generations of Bevan’s pond, further diverging from Mayer Lake (generation: $\chi^2_1 = 7.35$, $p = 0.007$; Fig. 4c).

The transplant from the deeply stained Drizzle Lake to the unstained Drizzle Pond resulted in a significant increase in RAA but no changes to sexual dimorphism or the relationship between neurocast count and RAA (population: $\chi^2_1 = 20.76$, $p < 0.001$; sex \times population: $\chi^2_1 = 1.77$, $p = 0.183$; NPP \times population: $\chi^2_1 = 1.75$, $p = 0.186$; Table 2; Fig. 4e,f). There was no significant intergenerational change in RAA in Drizzle Pond ($\chi^2_1 = 0.46$, $p = 0.499$; Fig. 4e).

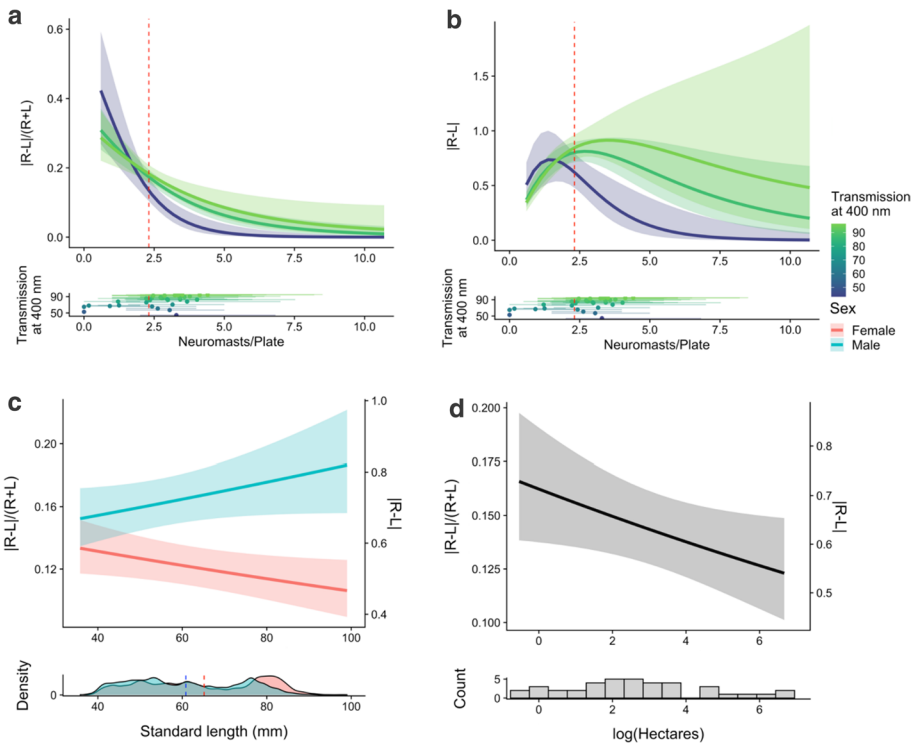


Fig. 3 Reduction of RAA (**a**) and AA (**b**) of buttressing plate neuromasts with increased transmission at 400 nm. (**c**) Increase in asymmetry sexual dimorphism with standard length. (**d**) Reduction in asymmetry with lake surface area. Trend lines are marginal means, averaged over sex, plate position and all other habitat and morphological predictors. Trends in (**c**, **d**) are for fish with an average neuromast count, indicated by the vertical dotted line (**a**, **b**). Populations with the minimum, maximum and median spectra are shown to indicate the range of variation observed. Scatter plots show the distribution of neuromast counts with spectra, error bars span between 2.5th and 97.5th quantiles for a given population. Density plot shows the distribution of neuromast counts for each sex, with dashed vertical lines indicating the mean. Histogram shows distribution of lake sizes

Discussion

The buttressing plate neuromasts of coastal British Columbia threespine stickleback, exhibit a wide degree of individual and population-level signed (SA) and absolute (AA) asymmetry. Frequencies of asymmetrical fish averaged 93% among the 61 populations and while there is a marginal excess of right-biased populations, there was no evidence for any significant departures from a mean of zero in any population. AA increases with the number of neuromasts per lateral plate (NPP), at least for buttressing plates. Relative absolute asymmetry (RAA) is smaller in stained water localities, except for fish with few NPP. Furthermore, sexual dimorphism in RAA is elevated in these stained water localities, which is associated with an increase in body length. Despite the role of the lateral line in rheotaxis and other behaviours expected to differ among major ecological regimes, we found no statistical differences in RAA between allopatric lakes and streams, between parapatric lake-stream pairs, or between oceanic and freshwater fish. However, we did observe both increases and decreases in RAA in populations transplanted from stained lakes to unstained

Table 2 Model equations for binomial GLMMs used throughout analysis

Eq.	Selection	Model structure
1	Full	$RSA \text{ or } RAA = (NPP + \textit{sex} + \textit{position})^2 + (NPP \mid \textit{local}) + (1 \mid \textit{local} : \textit{individual ID})$
	Reduced	$RAA = (NPP + \textit{sex} + \textit{position})^2 + (NPP \mid \textit{local}) + (1 \mid \textit{local} : \textit{individual ID})$
	Reduced	$RSA = 1$
2	Full	$RAA = (NPP + \textit{sex}) * (\textit{position} + \textit{region} + \textit{habitat} + \textit{clarity}) + (NPP \mid \textit{local}) + (1 \mid \textit{local} : \textit{individual ID})$
	Reduced	$RAA = (NPP + \textit{sex}) * (\textit{position} + \textit{clarity}) + \textit{region} + (NPP \mid \textit{local}) + (1 \mid \textit{local} : \textit{individual ID})$
3	Full	$RAA = (NPP + \textit{sex}) * (\textit{position} + \log(LP) + SL + LP_{IR-L} + t400 + \log(\textit{area}) + \textit{predators}) + (NPP + \log(LP) + LP_{IR-L} \parallel \textit{local}) + (1 \mid \textit{local} : \textit{individual ID})$
	Reduced	$RAA = (NPP + \textit{sex}) * (\textit{position}) + SL * \textit{sex} + t400 * NPP + nn (NPP \mid \textit{local}) + (1 \mid \textit{local} : \textit{individual ID})$
4	Full	$RAA = (NPP + \textit{sex}) * (\textit{position} + SL) + (1 \mid \textit{date} / \textit{individual ID})$
	Red. D	$RAA = NPP * \textit{position} + \textit{sex} + (1 \mid \textit{date} / \textit{individual ID})$
	Red. M	$RAA = NPP * \textit{position} + (1 \mid \textit{date} / \textit{individual ID})$
5	Full	$RAA = (NPP + \textit{sex}) * (\textit{position} + \textit{population}) + (1 \mid \textit{date} / \textit{individual ID})$
	Red. M-GC	$RAA = NPP * \textit{position} + (1 \mid \textit{date} / \textit{individual ID})$
	Red. D-DO	$RAA = NPP * \textit{position} + (1 \mid \textit{date} / \textit{individual ID})$
	Red. M-RD	$RAA = NPP * (\textit{position} + \textit{population}) + \textit{sex} + (1 \mid \textit{date} / \textit{individual ID})$
	Red. M-BP	$RAA = (NPP + \textit{sex}) * \textit{position} + \textit{population} + (1 \mid \textit{date} / \textit{individual ID})$
	Red. D-DP	$RAA = NPP * \textit{position} + \textit{sex} + \textit{population} + (1 \mid \textit{date} / \textit{individual ID})$
6	Full	$RAA = (NPP + \textit{sex}) * \textit{position} + \textit{generation} + (1 \mid \textit{individual ID})$
	Red. M-RD	$RAA = NPP * \textit{generation} + \textit{sex} + \textit{position}$
	Red. M-BP	$RAA = NPP * \textit{position} + \textit{generation} + (1 \mid \textit{individual ID})$
	Red. D-DP	$RAA = NPP * \textit{position} + (1 \mid \textit{individual ID})$

Eq.=equation number, *NPP*=neuromasts per plate, *LP*=lateral plate count, *LP_{IR-L}*=absolute lateral plate asymmetry, *SL*=standard length. Italicized words indicate categorical factors whereas other predictors are continuous. Square(²) indicates all two way interactions, (|) indicates random effects with a 1 on the left being random intercepts and a continuous predictor on the left, e.g. *NPP* being random slopes, (:) indicates an interaction, (||) are random slopes that have been assumed to lack covariance and (/) is a nested random effects structure. For models applied to multiple populations, i.e. Equations 4–6, the reduced model structure for each population/population pair is given along with the population’s abbreviation (Table 1; Fig. 1), e.g. red. *D-DO*=reduced model for Drizzle Lake and Drizzle Outlet.

ponds over the course of several generations, suggesting that changes in lateral line asymmetry observed across Haida Gwaii and the Dewdney Banks stickleback populations could have developed quickly following initial post-glacial colonization to the lakes.

Our findings suggest that the requirement of the lateral line to respond to stimuli from all sides overwhelms the advantage of having greater sensitivity on one side, even in habitats with limited light availability. In contrast, Mexican tetras which have adapted to survive in the absence of light (blind Mexican cavefish), exhibit a left bias in superficial neuromast counts and mechanosensory behavior not seen in their surface dwelling counterparts (Burt de Perera and Braithwaite 2005; Gross et al. 2016; Fernandes et al. 2018). The differences between the species may be due to the difference in response stimuli. The lateral line of Mexican cavefish is specialized for prey localization and navigation (Holzman et al. 2014;

Table 3 Summary of RAA by sex and plate position

Sex	Position	Average RAA		Neuromast interaction	
		% RA	Log-odds	Odds	Log-odds
Female	Four	0.18 [0.17,0.2]	-1.5 [-1.61,-1.39]	0.26 [0.19,0.37]	-1.34 [-1.68,-1]
	Five	0.14 [0.12,0.15]	-1.84 [-1.95,-1.73]	0.18 [0.12,0.25]	-1.74 [-2.09,-1.39]
	Six	0.1 [0.09,0.11]	-2.16 [-2.27,-2.05]	0.16 [0.11,0.23]	-1.82 [-2.19,-1.46]
	Seven	0.1 [0.09,0.11]	-2.2 [-2.31,-2.09]	0.26 [0.18,0.37]	-1.37 [-1.73,-1]
	Eight	0.1 [0.09,0.12]	-2.15 [-2.29,-2.01]	0.39 [0.26,0.59]	-0.93 [-1.34,-0.53]
Male	Four	0.2 [0.18,0.22]	-1.41 [-1.53,-1.29]	0.22 [0.16,0.31]	-1.51 [-1.85,-1.17]
	Five	0.17 [0.15,0.19]	-1.59 [-1.7,-1.48]	0.15 [0.1,0.21]	-1.9 [-2.26,-1.55]
	Six	0.13 [0.12,0.15]	-1.86 [-1.97,-1.75]	0.14 [0.1,0.2]	-1.99 [-2.35,-1.62]
	Seven	0.12 [0.11,0.13]	-2.01 [-2.12,-1.9]	0.22 [0.15,0.31]	-1.53 [-1.9,-1.16]
	Eight	0.12 [0.11,0.13]	-2 [-2.13,-1.86]	0.33 [0.22,0.5]	-1.1 [-1.51,-0.69]

Average RAA values are estimated marginal means [95% CI] for fish with an average number of neuromasts per lateral plate (2.3). Neuromast interaction values are estimated marginal trends. Note that RAA increases with fewer neuromasts multiplicatively rather than linearly.

Yoshizawa et al. 2015), whereas threespine stickleback use their lateral line for rheotaxis (Jiang et al. 2017) and schooling (Greenwood et al. 2013). We have also examined trunk neuromasts which are associated with escape response (Faucher et al. 2006), rather than facial neuromasts which are used for feeding (Bleckmann et al. 1989) and which exhibit directional asymmetry in Mexican cavefish (Gross et al. 2016). Given that prey emit oscillations from a single point, whereas abiotic flow and schools provide a diffuse stimulus, lateralization may be less advantageous for stickleback. Furthermore, it is likely that a predatory fish is more capable of selecting which side to approach prey with, than which side abiotic flow or conspecifics stimulate. Lastly, cavefish live in an aphotic environment whereas stained water stickleback live in a low-light environment. While sub-modalities of the lateral line may exhibit SA under the right circumstances, we do not see evidence of this in the anterior trunk neuromasts of threespine stickleback.

RAA is highly variable in threespine stickleback, reflecting a diversity of ecologies and life histories and a functional trade-off between visual and mechanosensation modalities. As far as we are aware, Trokovic et al. (2012) have conducted the only other study of habitat-dependent changes in lateral line asymmetry, finding that nine spine stickleback in ponds have much greater asymmetry than oceanic fish which was interpreted as due to relaxed selection. Our data suggest an alternative mechanism, we found that much of the variation in RAA is due to changes in NPP, we suggest that changes in RAA observed by Trokovic et al. (2012) may be in part due to changes in neuromast count rather than developmental instability. This is supported by the similarity in RAA between oceanic and freshwater populations, among predation regimes and the loss of buttressing plate neuromasts in low-plated compared to fully-plated threespine stickleback (Planidin and Reimchen 2019). As we found smaller lakes which likely have a smaller population size also have elevated RAA, our findings suggest that genetic heterogeneity is also a factor influencing RAA. We find that water clarity is more important in determining RAA than predation by puncturing predators, with stained water populations having reduced RAA, except for fish with very few NPP. As tannin concentration causes low pH in tandem with reducing transmission of light, we cannot entirely rule out pH as a mechanism acting on RA,

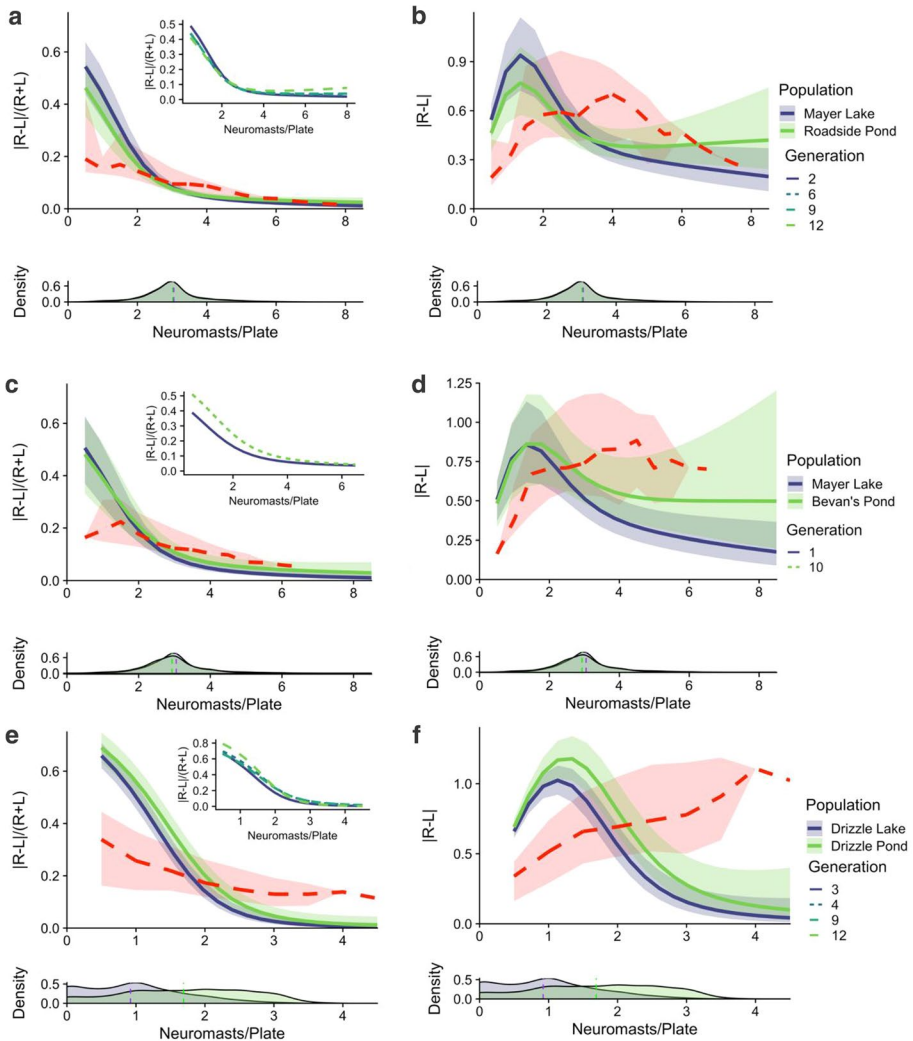


Fig. 4 Changes in RAA (**a**, **c**, **e**) and AA (**b**, **d**, **f**) following transplant from stained lakes and unstained ponds. Mayer Lake to Roadside Pond (**a**, **b**). Mayer Lake to Bevan's Pond (**c**, **d**). Drizzle lake to Drizzle Pond (**e**, **f**). Trend lines are as in previous figures. Red line is the expected RAA for a pond population given its environmental and morphological characteristics. Stochasticity in expected RAA is due to differences in average standard length for a given neuromast count within pond samples and the red shaded region spans the 2.5th and 97.5th quantiles of the model estimates. Inset figures indicate intergenerational change within pond populations and density plots show the distribution of neuromasts counts within the source and transplant populations, with vertical dashed lines indicating the population means.

especially given the inhibition of lateral line development for larval fish reared in low pH (Lin et al. 2019). However, as low pH inhibits the development of neuromasts we would expect that it increases RAA, whereas light limitation in these same environments would decrease RAA, due to a shift from visual to mechanosensation mediated behaviour (Liao 2006; Schwalbe et al. 2012; York and Bartol 2014), which may restrict the development of asymmetry in the lateral line. This suggests that spectral regime has a stronger influence

on RAA than pH for fish with more than ~2 NPP, whereas pH has a greater influence on RAA for populations with few NPP. Our geographic survey that there is a functional trade-off between available visual sensory information and lateral line RAA, in addition to the effects of population heterogeneity.

Similar to our findings comparing allopatric lakes and streams across Haida Gwaii, RAA is conserved in parapatric lake-stream pairs, suggesting that flow regime does not affect lateral line asymmetry. The paired lake and stream populations are genetically distinct, reproductively isolated and have undergone changes in phenotype including lateral plate and defense traits Deagle et al. (2012) there appear to be no consistent changes in neuromast count between lakes and streams (Wark and Peichel 2010; Jiang et al. 2017; Kelley et al. 2017; Planidin and Reimchen 2019). This is particularly interesting, given the role that the lateral line has in mediating rheotaxis behaviour in threespine stickleback and other species of fish (Montgomery et al. 1997; Suli et al. 2012; Jiang et al. 2017). Given similar RAA between lake-stream pairs, we can infer that rheotaxis is not associated with differences in asymmetry of the lateral line.

In our analysis of our transplant experiments from the stained Drizzle Lake and Mayer Lake to the unstained Drizzle Pond, Roadside Pond and Bevan's Pond, the observed changes in RAA in the transplants were relatively consistent with the expected effect of changes in clarity. All three transplants underwent an increase in RAA for fish with more than three NPP as expected, however only Roadside Pond fish showed a reduction in RAA for fish with fewer than three neuromasts. A possible explanation for this difference of outcome may be an increase in nocturnal foraging behaviour and benthic prey consumption by Roadside Pond fish (Leaver 2010), however behavioural changes in Drizzle Pond and Bevan's pond are unknown. The Drizzle Pond transplant suggests phenotypic plasticity in RAA is possible, given the abrupt increase in RAA seen in the first generation and the similarity among subsequent generations. However, the degree of change in RAA is much smaller than changes in NPP (Planidin and Reimchen 2019) and as expected by changes in clarity regime and standard length, suggesting that the population has not yet reached an equilibrium within twelve generations. In contrast, the Roadside Pond and Bevan's Pond transplants suggest a genetic mechanism, undergoing small directional changes in subsequent generations. Drizzle Lake is relatively deep with a small littoral zone mostly lacking vegetation (Reimchen 1990), whereas Mayer Lake is shallow with a large and vegetated littoral zone (Moodie 1972), thus the spatial heterogeneity of Mayer Lake is more similar to the transplant ponds and may be a cause for the slower change observed in its transplant populations. We also see in all transplants that fish with fewer neuromasts tend to be more variable in RAA, which may be why Drizzle Lake fish exhibited greater changes in RAA than Mayer Lake fish following transplant to a pond.

Independence of neuromast asymmetry and lateral plate count and asymmetry is surprising given their developmental link and overlapping ecological functions. Feeding behaviour and predator avoidance are associated with lateral line morphology (Mesa and Warren 1997; Coombs and Patton 2009; Junges et al. 2010; Schwalbe et al. 2012) and lateral plate asymmetry in threespine stickleback (Moodie and Reimchen 1976; Reimchen and Nosil 2001), suggesting that these traits would be associated. Furthermore, the common genetic loci and closely linked in developmental ontogeny of lateral plates and neuromasts (Wada et al. 2010; Wark et al. 2012; Mills et al. 2014) also suggests that asymmetry in these traits would be related. However, given the contention around the genetic basis of asymmetry in many species (Markow and Clarke 1997; Møller and Thornhill 1997; Houle 1997; Pomiankowski 1997), it is not completely unexpected that neuromast count asymmetry is independent of lateral plate count and

asymmetry. While asymmetry in neuromast count and lateral plate count are common and despite their developmental and functional association, they seemingly interact with life history independently.

Given the importance of the lateral line for mechanosensation, it would be reasonable to predict that population average RAA would decrease over ontogeny as lower fitness asymmetric individuals are lost at a higher rate (Beasley et al. 2013). However, we saw no decline in RAA over ontogeny within populations. The same trend has also occurred in lateral plate asymmetry (Reimchen and Bergstrom 2009) suggesting that asymmetry in some traits may not reduce fitness (Lens et al. 2002; Lajus et al. 2019).

Sexual dimorphism in threespine stickleback is dependent on a variety of ecological and life history traits (Reimchen et al. 2016). Sexual dimorphism in RAA is greater in stickleback populations that exhibit increased adult body length, suggesting that RAA changes with certain aspects of life history. Increased sexual dimorphism in RAA with greater body size may be due to a greater difference in reproductive strategy, exposure to predation or trophic niche between the sexes in long stickleback populations (Moodie 1972; Oravec and Reimchen 2013; Reimchen et al. 2016). Males from giant stickleback populations also feed on more benthic prey, whereas females are predominantly limnetic (Reimchen and Nosil 2004). As the lateral line is important in the detection of zooplankton (Montgomery 1989), this niche partitioning may be conserving symmetry in females and driving increased asymmetry in males. The relationship between length and RAA suggests that asymmetry in the lateral line plays a more complex role in life history than indicating reduced fitness.

Given the high fidelity required of the lateral line to detect subtle mechanosensory stimuli in the environment, one would expect that a symmetrical lateral line would be common in most fishes. However, we find that for threespine stickleback, asymmetry in the lateral line is widespread and highly variable. This suggests that differences in stimuli sensitivity between the two sides may impart additional sensory information in different ecological contexts. Asymmetry in neuromast counts may allow fish with very few neuromasts to gain more complete coverage of their body surface and glean more sensory information with fewer receptors. Sensory structure asymmetry may also be advantageous if it plays a role in the development of behavioural laterality, which can allow individuals to respond more rapidly to certain environmental stimuli such as the vibrations produced by prey (Fernandes et al. 2018) or attacks by predators (Cantalupo et al. 1995). While we found no evidence that there is a directional bias at the population level, individuals may still exhibit lateralized behavioural syndromes associated with greater asymmetry in neuromast count. We also found that lateral line asymmetry is highly responsive to ecological change, in addition to changes in neuromast count. These findings show that asymmetry in the lateral line system can change rapidly in response to colonization of an ecological divergent habitat, warranting future investigation into the functional ecology of lateral line asymmetry.

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Availability of data and material The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflicts of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval This research was undertaken and approved through the University of Victoria Animal Use for Research Protocol 2019–021(1), which follows the guidelines set by the Canadian Council for Animal Care.

Consent to participate Not applicable.

Consent for publication Not applicable.

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