

Spatial, sexual, and rapid temporal differentiation in neuromast expression on lateral plates of Haida Gwaii threespine stickleback (*Gasterosteus aculeatus*)

N.P. Planidin and T.E. Reimchen

Abstract: Lateral lines, a major sensory modality in fishes, are diverse among taxa, but their intraspecific variation has received limited attention. We examined numbers of superficial neuromasts on the buttressing lateral plates (LP) of 1910 threespine stickleback (*Gasterosteus aculeatus* Linnaeus, 1758) from 26 ecologically and morphologically diverse populations on the Haida Gwaii archipelago, western Canada. Extending from previous studies, we predicted that (i) highly stained dystrophic localities would have threespine stickleback with elevated numbers of neuromasts per plate due to a greater reliance on non-visual sensory modalities and (ii) that LP count and neuromast numbers per plate would functionally covary with predatory assemblage. We found that there were no differences in neuromast count across major habitats (marine, lake, stream), but clear-water populations and those with predatory fish had significantly more neuromasts per plate than most populations in highly stained dystrophic lakes, the effects being accentuated on the first buttressing plate (LP4). We also report the first evidence that neuromast counts per plate are sexually dimorphic, with males having a greater density of neuromasts in most populations. Two transplant experiments between ecologically opposite habitats indicate that within 12 generations, neuromast counts per plate can rapidly shift in response to a change in habitat.

Key words: adaptation, threespine stickleback, lateral line, mechanosensory, neuromasts, predation, dystrophic, sensory modality, *Gasterosteus aculeatus*.

Résumé : Si les lignes latérales, une importante modalité sensorielle chez les poissons, varient d'un taxon à l'autre, leur variabilité intraspécifique a reçu peu d'attention. Nous avons examiné le nombre de neuromastes superficiels sur les plaques latérales (PL) de soutien de 1910 épinoches à trois épines (*Gasterosteus aculeatus* Linnaeus, 1758) provenant de 26 populations différentes des points de vue écologique et morphologique dans l'archipel Haida Gwaii (Ouest canadien). À la lumière d'études antérieures, nous avons prédit que (i) des localités dystrophiques fortement colorées présenteraient des nombres élevés de neuromastes par plaque en raison d'un plus grand recours à des modalités sensorielles non visuelles et (ii) le nombre de PL et les nombres de neuromastes par plaque covarieraient fonctionnellement avec l'assemblage de prédateurs. Nous avons constaté qu'il n'y a pas de différence du nombre de neuromastes entre les principaux habitats (marin, lacustre, fluvial), mais que les populations vivant en eau claire et celles ayant des poissons comme prédateurs comptaient significativement plus de neuromastes par plaque que la plupart des populations dans des lacs dystrophiques fortement colorés, ces effets étant accentués sur la première plaque de soutien (PL4). Nous faisons aussi état des premières preuves de dimorphisme sexuel des nombres de neuromastes par plaque, les mâles présentant une plus grande densité de neuromastes dans la plupart des populations. Deux expériences de transplantation entre habitats opposés sur le plan écologique indiquent que, en 12 générations ou moins, les nombres de neuromastes par plaque peuvent changer rapidement en réponse à un changement d'habitat. [Traduit par la Rédaction]

Mots-clés : adaptation, épinouche à trois épines, ligne latérale, mécano-sensoriel, neuromastes, prédation, dystrophique, modalité sensorielle, *Gasterosteus aculeatus*.

Introduction

The lateral line is the major bilateral sensory modality of fishes, detecting flow velocity and pressure perturbation in a three-dimensional environment (Coombs et al. 1988). This sensory system plays a major role in many behaviours for fish, such as predator-prey interaction, habitat navigation, and schooling (Pohlmann et al. 2004; McHenry et al. 2009; Junges et al. 2010; York et al. 2016; Mekdara et al. 2018). Dispersed on the body, the lateral line consists of a series of neuromasts, an array of stereocilia and kinocilia encased in a gelatinous cupula, that when displaced can determine the direction of a stimulus. (Coombs et al. 2014). This

mechanosensory mode is divided into two major submodalities, the detection of pressure changes by canal neuromasts and flow sensing by superficial neuromasts. This functional distinction is derived from differences in the structure of the two types of neuromast, with canal neuromasts being contained within a fluid-filled canal and superficial neuromasts being exposed to direct water flow, their height above the body surface affecting sensitivity (Teyke 1990; McHenry et al. 2008). The number of lateral-line pores, which transmit environmental stimuli to canal neuromasts, is often species-specific and is generally associated with habitat (Voronina 2007; Trokovic et al. 2011; Vanderpham et al. 2016). Superficial neuromast are also highly variable among and

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within species (Cernuda-Cernuda and Garcia-Fernández 1996) with nocturnal or crepuscular taxa tending to have more neuromasts and greater sensitivity to perturbations than related diurnal taxa (Teyke 1990; Jeffery et al. 2000; Yoshizawa et al. 2010).

Lateral-line morphology is dependent on the context of sensory input, which varies with habitat and niche space (Carton and Montgomery 2004; Vanderpham et al. 2016). Species living near the surface, such as flying fish (Exocoetidae), have ventrally positioned lateral lines because the major sensory input is from below, whereas benthic species, such as midshipman (Batrachoididae), have dorsally positioned lateral lines because the major input is from above (Kasumyan 2003). Most fish have a lateral line positioned along the mid-trunk, presumably because they inhabit the water column and are subject to a three-dimensional sensory input.

Intraspecific variation in the position and number of lateral-line neuromasts can also occur when populations inhabit different niche space (Fischer et al. 2013; Vanderpham et al. 2016). Such variation can reflect heritable differences among individuals (Trokovic et al. 2011; Wark et al. 2012), as well as phenotypic plasticity (Kelley et al. 2017). The mechanism driving this differentiation is not well established, but it is a reasonable inference that most differences observed among individuals or populations, whether heritable or environmentally induced, will result in differential reception of signals to which the fish are exposed (Kasumyan 2003).

Threespine stickleback (*Gasterosteus aculeatus* Linnaeus, 1758) typically have bony lateral plates on the trunk that vary in number and which overlay the lateral-line canal. Distinctive pores may occur on the lateral plates through which superficial neuromasts of the lateral line are expressed. Recent studies indicate that the *Eda* gene, the major controller for number of bony plates, has larger pleiotropic effects in that number and position of neuromasts covaries with development of lateral plates (Mills et al. 2014) and with schooling behaviour (Mekdara et al. 2018).

Stickleback neuromast counts vary among populations. Although there is extensive overlap in counts among habitats, recent studies in southern British Columbia, Canada, indicate that marine threespine stickleback, the ancestors of freshwater populations, have fewer neuromasts than stream and lake threespine stickleback, whereas benthic lake fish have more neuromasts than sympatric limnetic lake fish and this occurs across all regions of the lateral line (Wark and Peichel 2010). Such population-specific variation in a major sensory modality offers novel insight in ecological and behavioural interactions.

In this study, we examined lateral-plate pores (hereafter referred to as pores) and neuromast variation in the lateral plates of threespine stickleback from the Haida Gwaii archipelago, western Canada. Ecological differences among the lakes, streams, and estuaries have created a complex selective landscape resulting in a diversity of stickleback morphologies, ranging from unarmored stickleback in small ponds to small and giant armored limnetic fish in small and large lakes, most associated with distinctive biophysical conditions including predation regimes, lake size, and aquatic spectra (Reimchen et al. 2013). One of the most prominent post-capture adaptations of stickleback are their bony lateral plates. Populations experiencing high predation pressure from puncture piscivores, such as salmonids, develop greater numbers of lateral plates, whereas populations exposed to compression piscivores, such as diving birds, experience plate reduction and those with minimal vertebrate predation have few if any lateral plates (Reimchen 1994, 1995; Bergstrom 2002). We chose to examine the neuromasts on the lateral plates buttressing the dorsal and pelvic spines. These buttressing plates are often the only plates retained in most freshwater populations of Haida Gwaii (Reimchen 1983; Reimchen et al. 2013). This captures only part of the sensory breadth of the lateral-line system, but it allows potentially useful comparisons within and among populations for this

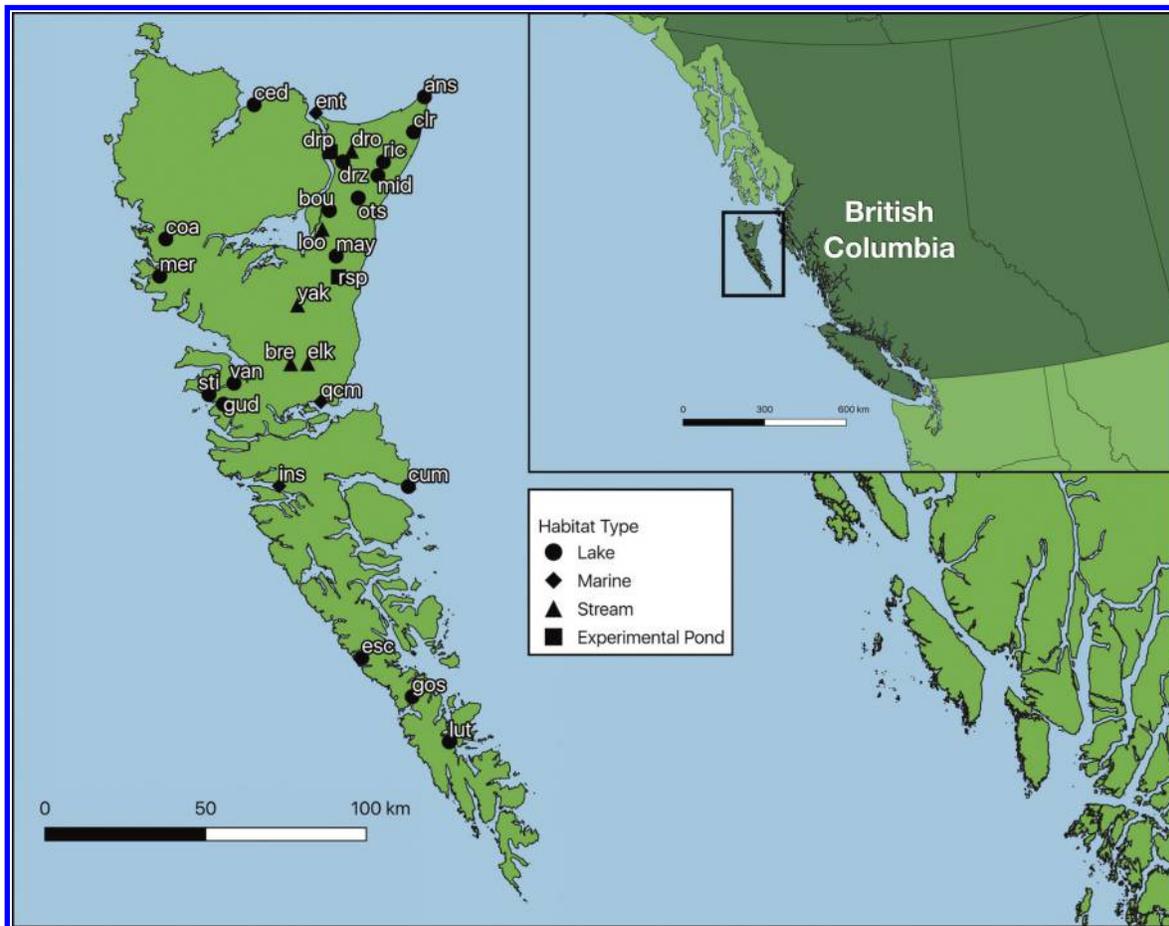
component of the sensory modality. As with Wark and Peichel (2010), we hypothesize that environments with greater exposure to predatory vertebrates will have greater numbers of neuromasts per plate and that stream stickleback will have more neuromasts per plate than marine forms. Because the Haida Gwaii lakes can be dystrophic with deeply stained waters, we also predict that neuromasts should be accentuated in these lakes where visual cues are more limiting relative to clear-water lakes, but predation levels are also high (Reimchen 1994). Additionally, we examine plate neuromasts in two field transplant experiments in which large-bodied limnetic stickleback from two dystrophic lakes (stained water, low conductivity) with a diverse assemblage of predatory fish and predatory birds were transplanted to two shallow small ponds that were ecologically opposite to the source lakes (unstained, higher conductivity, benthic, lacking predatory fish, avian piscivores rare) (Leaver and Reimchen 2012). Natural pond populations tend to have reduced expression of multiple defense traits, as well as occupying more benthic life histories (Reimchen et al. 2013), so if there are any changes in the neuromasts of the transplant populations, we predict a reduction in numbers per plate compared with the source populations.

Materials and methods

We used samples collected during ongoing studies (1975 to the present) of stickleback from the Haida Gwaii archipelago. The fish were preserved in formalin and later transferred to ethanol (review in Reimchen et al. 2013). Twenty-six populations were used in the current study (Fig. 1), chosen to exemplify the full range of morphological diversity of stickleback in the archipelago from a broad assemblage of habitats including marine ($n = 3$), stream ($n = 5$) and lakes ($n = 18$). The two experimental transplant ponds are Roadside Pond (0.07 ha) established in 1993 with 100 individuals from Mayer Lake (373 ha) (Leaver and Reimchen 2012) and Drizzle Pond (0.15 ha) established in 1997 with 16 individuals from Drizzle Lake (97 ha) (T.E. Reimchen, unpublished data). All fish were measured for standard length (SL). Those less than 40 mm SL were not scored because the lateral plates on smaller fish are often incomplete, reducing reliable scoring of the pores and associated neuromasts. Fish were sexed and the number of pores and cupula on their lateral plates were counted. These populations vary in mean number of lateral plates (range = 0 to 35; mode = 5) and we restricted our scoring to the buttressing plates (positions 4 thru 8; Fig. 2a). These plates were chosen because they are often the only plates present in many populations and because the plates provide protection of neuromast cupulae from abrasion that commonly effects superficial neuromasts on the naked trunks. Pores on the lateral plates are readily identifiable within which the distinctive cupula are visible by their shape, contrasting sheen, texture, and colour from the rest of the lateral plate (Figs. 2b, 2c, 2d). To evaluate repeatability, 50 fish were re-scored a month later, without reference to fish identity, resulting in 96.2% repeatability. One or more neuromasts could be found within each pore. Occasionally, neuromasts within a pore were separated by a calcified wall and these were treated as separate pores (Fig. 2d). Plates on both sides of the trunk were scored but in the current study, we only analyze those on the left side of the body (LP).

For statistical analyses and hypotheses testing, we used linear mixed-effects models. Repeat measures, i.e., multiple fish sampled from a locality and multiple neuromast counts on each fish, were modeled as random effects. For individual plate analysis, data were not normally distributed, so generalized linear mixed-effects models (GLMM) with Poisson-distributed residuals were used. The significance of all parameters was determined by a likelihood ratio test between nested models, with the parameter of interest being removed, shown as a χ^2 statistic. We computed mean number of neuromasts for each plate and a mean number of neuromasts for the five buttressing plates. If the analysis being

Fig. 1. Map of collection sites for threespine stickleback (*Gasterosteus aculeatus*) on Haida Gwaii, British Columbia, Canada. Each point represents a locality. Abbreviations are as follows: ans, Anser 1987 ($n = 35$ females, 45 males); bou, Boulton 1982 ($n = 57$ females, 22 males); bre, Brent Creek 1986 ($n = 17$ females, 37 males); ced, Cedar 1991 ($n = 15$ females, 25 males); clr, Clear Water 1978 ($n = 14$ females, 12 males); coa, Coates 1986 ($n = 23$ females, 27 males); cum, Cumshewa 1992 ($n = 10$ females, 36 males); drz, Drizzle 1979, 1981 spring, 1981 summer, 1983, 1987, 1988, 1989, and 2015 ($n = 170$ females, 245 males); drp, Drizzle Pond 2003 and 2013 ($n = 99$ females, 142 males); dro, Drizzle Outlet 1982 ($n = 21$ females, 22 males); elk, Elk Creek 1986 ($n = 10$ females, 46 males); ent, Entry Point 1983 ($n = 8$ females, 13 males); esc, Escarpment 1986 ($n = 49$ females, 42 males); gos, Goski 1986 ($n = 18$ females, 22 males); gud, Gudal 1993 ($n = 20$ females, 20 males); ins, Inskip Lagoon 1986 ($n = 21$ females, 28 males); loo, Loon Creek 1986 ($n = 21$ females, 35 males); lut, Lutea 1981 ($n = 14$ females, 36 males); may, Mayer 1982, 1997, and 2003 ($n = 115$ females, 73 males); mer, Mercer 2007 ($n = 16$ females, 24 males); mid, Middle 1979 ($n = 12$ females, 23 males); ots, Otter South 1986 ($n = 21$ females, 18 males); qcm, Queen Charlotte Marina 1992 ($n = 13$ females, 37 males); ric, Richter 1979 ($n = 5$ females, 25 males); rsp, Roadside Pond 1995, 2010, and 2016 ($n = 37$ females, 68 males); sti, Stiu 1986 ($n = 15$ females, 35 males); van, Van 1976 ($n = 16$ females, 18 males); Yak, Yakoun River 1986 ($n = 9$ females, 34 males). Made using QGIS version 3.4 (QGIS Development Team 2009). Colour version online.



conducted did not involve repeat measures, a GLM or linear model was used. All analysis was done in R version 3.4.3 with the use of the lme4 package (R Core Team 2013; Bates et al. 2015).

Results

There is extensive variation in LP pores and their neuromasts within and among individuals. Number of pores per plate ranged from 0 to 5 (mean = 1.89), with similar mean pore counts among all plates ($\chi^2_{[4]} = 8.49$, $p = 0.08$; Fig. 3). In contrast, number of neuromasts per plate ranged from 1 to 8, with LP4 having a disproportionately large number of neuromasts relative to the more posterior plates ($\chi^2_{[4]} = 260.44$, $p < 0.001$). Population means of pore numbers and neuromast numbers per pore are highly correlated (LP4: $r = 0.95$; LP5: $r = 0.97$; LP6: $r = 0.97$; LP7: $r = 0.97$; LP8: $r = 0.98$), and as such, we restrict our subsequent results to neuromasts.

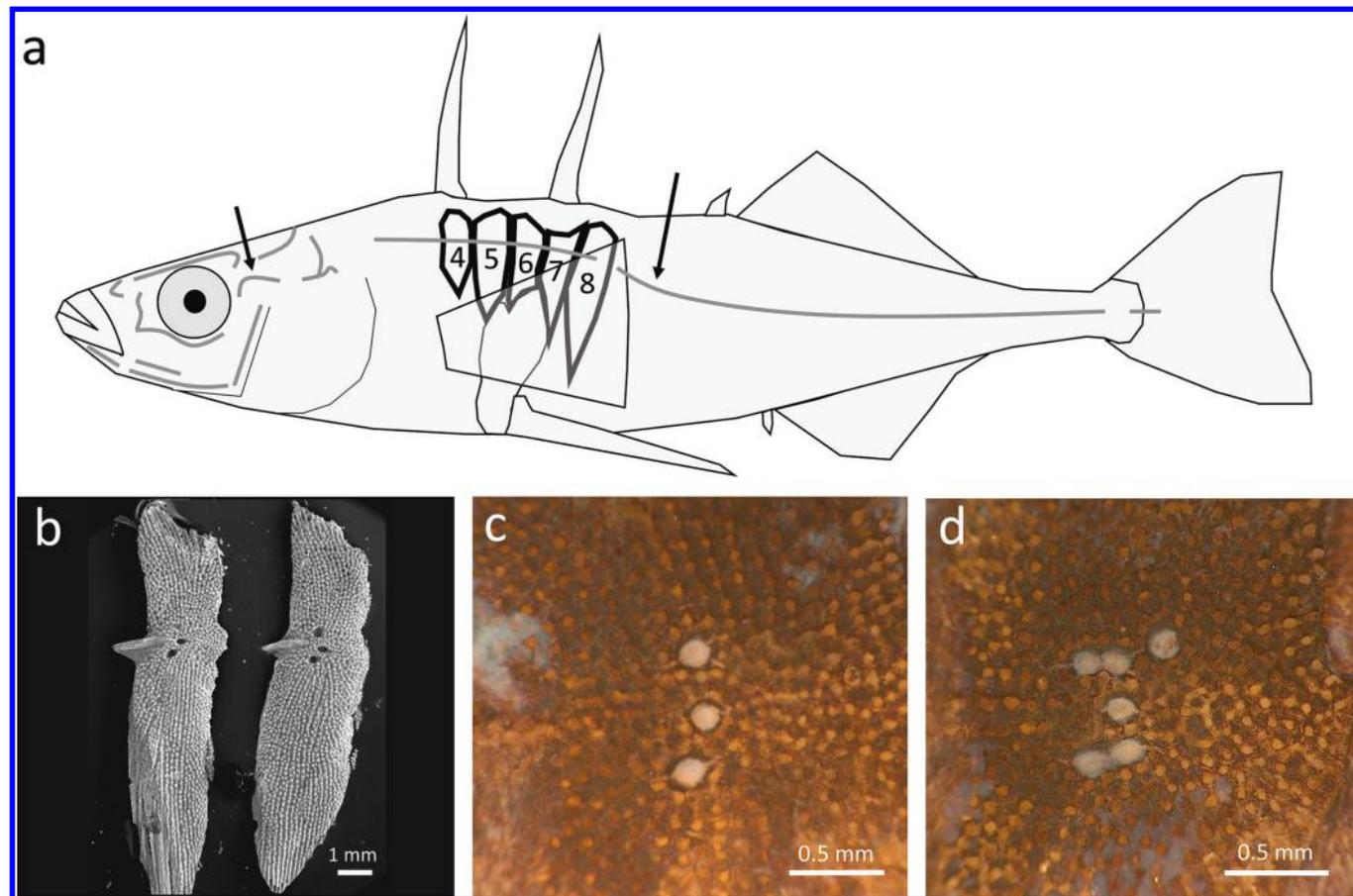
Populations differed in the occurrence of neuromasts on the individual lateral plates. Mean number of neuromasts within each of the five buttressing plates ranged from 0.3 to 4.0 ($F_{[25,1719]} = 300.4$, $p < 0.001$; Fig. 4). Neuromast counts per plate did not differ

significantly across the three major habitats (marine, lake, and stream: $\chi^2_{[2]} = 1.16$, $p = 0.56$) or with lake area ($\chi^2_{[1]} = 0.64$, $p = 0.42$). However, all of the clear-water habitats, including marine, lake, and stream localities, have elevated neuromast counts compared with stained lakes and stained streams ($\chi^2_{[2]} = 15.14$, $p < 0.01$), with two exceptions (Mayer Lake and South Otter Lake) that are both stained and have elevated neuromast counts per plate.

Number of neuromasts per plate exhibits a sigmoidal relationship to the mean number of plates for the population with the asymptote occurring at about 7 or 8 plates ($\chi^2_{[1]} = 822.21$, $p < 0.001$; Fig. 5). Fully plated populations had neuromast densities similar to populations with a mean of 7 or 8 lateral plates ($F_{[1,22]} = 2.83$, $p = 0.11$). The neuromast relationship is plate-specific because the greatest increase in the neuromasts per plate occurs on the fourth plate ($\chi^2_{[4]} = 260.44$, $p < 0.001$).

Sexual dimorphism is often present in the number of neuromasts. Of the 26 populations, 22 populations had males with a greater mean number of neuromasts per plate than that shown by females. All populations that are individually significant for sex-

Fig. 2. Depiction of neuromasts on the buttressing plates of threespine stickleback (*Gasterosteus aculeatus*). (a) Sketch of lateral-line system of threespine stickleback with buttressing region of lateral plates emphasized. Arrows indicate regions of superficial neuromasts as denoted in Wark and Peichel 2010. (b) Scanning electron micrograph of the seventh and eighth left lateral plates, with two and three neuromast pores, respectively. (c) Light micrograph of neuromasts on the fifth left lateral plate, with three neuromasts innervating three separate pores. (d) Light micrograph of neuromasts on the fourth left lateral plate, with two oblong pores containing two neuromasts each and two pores containing single neuromasts. Colour version online.



ual dimorphism exhibit a male bias ($\chi^2_{[1]} = 53.9$, $p < 0.001$; Fig. 6). Although the population differences in dimorphism were variable, we did not identify any consistent pattern to the extent of dimorphism in neuromasts among the major habitats (marine, streams, lakes: $\chi^2_{[2]} = 3.57$, $p = 0.17$) or between stained and clear-water lakes ($\chi^2_{[2]} = 0.76$, $p = 0.68$).

The transplant populations both exhibit significant shifts from the source populations. Mayer Lake threespine stickleback, a source population, had about 3.2 neuromasts per plate and showed conserved neuromast counts over two decades (~7 generations). Following the transplant of 100 adult fish from Mayer Lake to Roadside Pond in 1992, neuromast counts remained similar within the first two generations of colonists; yet by the 9th generation, the number of neuromasts decreased by a mean of 11% (from 3.0 to 2.7) and remained reduced in the 12th generation ($\chi^2_{[1]} = 1.61$, $p = 0.20$; Tukey test, 2nd to 9th generation: $p < 0.001$; Tukey test, 2nd to 12th generation: $p < 0.001$; Fig. 7). These differences were plate-specific because relative to the trends in Mayer Lake, the transplant population had the greatest decrease on LP8 (25.4%) followed by LP4 (10.6%) and LP6 and LP7 (7.3%), with the least change on LP5 (4.0%) (LP4: $F_{[1,101]} = 15.28$, $p < 0.001$; LP5: $F_{[1,101]} = 6.24$, $p < 0.05$; LP6: $F_{[1,101]} = 0.57$, $p = 0.45$; LP7: $F_{[1,101]} = 4.22$, $p < 0.05$; LP8: $F_{[1,88]} = 6.93$, $p < 0.05$).

Drizzle Lake threespine stickleback, the source population for Drizzle Pond, had a mean of 0.87 neuromasts per plate (LP4–LP8) that varied over the 30-year sampling period (0.77–1.07) ($F_{[6,455]} =$

3.42, $p < 0.01$). Post hoc tests indicate that April 1981 is elevated relative to all other dates. The Drizzle Pond transplant population, established from 16 adults in 1997 (T.E. Reimchen, unpublished data), shows an approximately doubling of the mean neuromast counts (mean = 0.87 to 1.55) by the 3rd generation and which remained similar to the 8th generation (range = 1.52–1.57; $\chi^2_{[1]} = 22.15$, $p < 0.001$; Fig. 8). The increase occurred for each lateral plate but was accentuated on the most anterior plates (LP4: 238%, $\chi^2_{[1]} = 19.7$, $p < 0.001$; LP5: 101%, $\chi^2_{[1]} = 11.9$, $p < 0.001$; LP6: 73%, $\chi^2_{[1]} = 16.4$, $p < 0.001$; LP7: 26%, $\chi^2_{[1]} = 14.3$, $p < 0.001$; LP8: 27%, $\chi^2_{[1]} = 4.8$, $p < 0.03$). We also observed that there was a 5% increase in the number of plates after eight generations relative to the source population ($t_{[655]} = 3.2$, $p < 0.001$).

Discussion

Using threespine stickleback with diverse morphologies from the Haida Gwaii archipelago, coastal British Columbia, we examined the distribution and abundance of superficial neuromasts and associated pores on the five bony plates that buttress the dorsal and pelvic spines. Although mean pore counts were similar across LP positions for any population, there was a large range in the number of pores on any given plate (0–5) and a large range of neuromasts within these pores (0–8). In populations with few lateral plates (<5), there tend to be similar number of neuromasts per plate on each of the five plates. Yet in populations with

Fig. 3. Mean (± 2 SE) number of pores and neuromasts for the five buttressing plates (L4–L8) of Haida Gwaii samples of threespine stickleback (*Gasterosteus aculeatus*). White circles indicate the number of pores and grey circles indicate the number of neuromasts. Pore count did not significantly vary among plates (L4pore to L8pore: $\chi^2_{[4]} = 8.49$, $p = 0.08$), whereas neuromast count did (L4neuro to L8neuro: $\chi^2_{[4]} = 260.44$, $p < 0.001$).

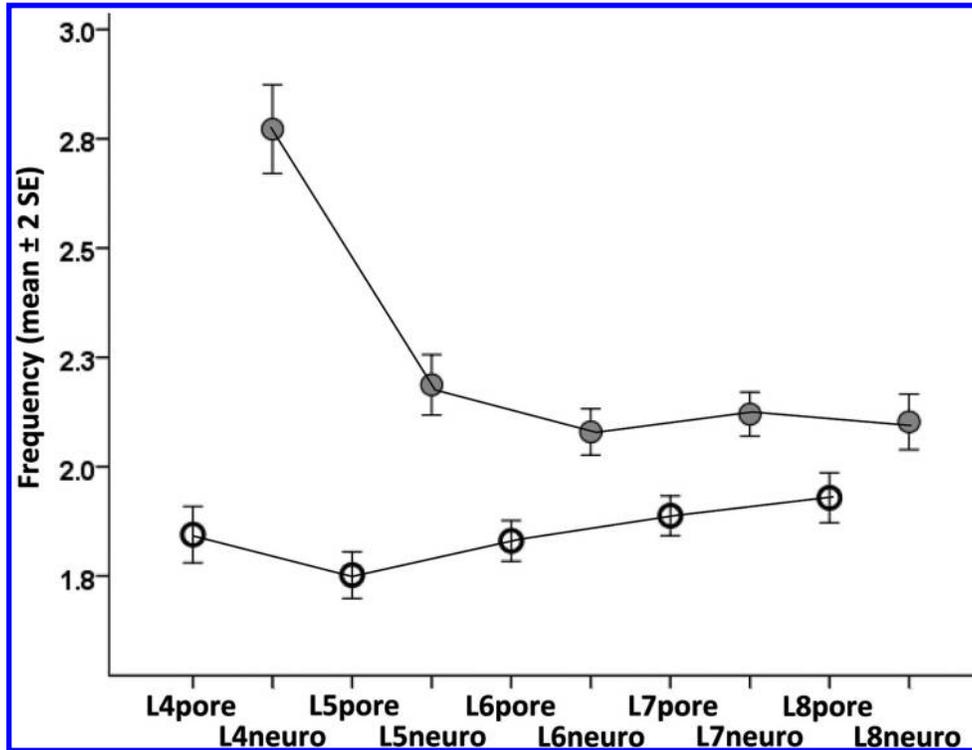


Fig. 4. Mean number of neuromasts per left lateral plate for all localities of Haida Gwaii populations (refer to Fig. 1) of threespine stickleback (*Gasterosteus aculeatus*). General habitat indicated above each bar as M (marine), S (stream), and L (lake). Bars indicate aquatic spectrum, with white bars representing clear-water habitats and grey bars representing stained habitats. Transplant populations were excluded. There was significant variation among localities ($F_{[25,1719]} = 300.4$, $p < 0.001$), which correlated with water clarity ($\chi^2_{[2]} = 15.14$, $p < 0.01$), but not habitat type ($\chi^2_{[2]} = 1.16$, $p = 0.56$) or lake area ($\chi^2_{[1]} = 0.64$, $p = 0.42$).

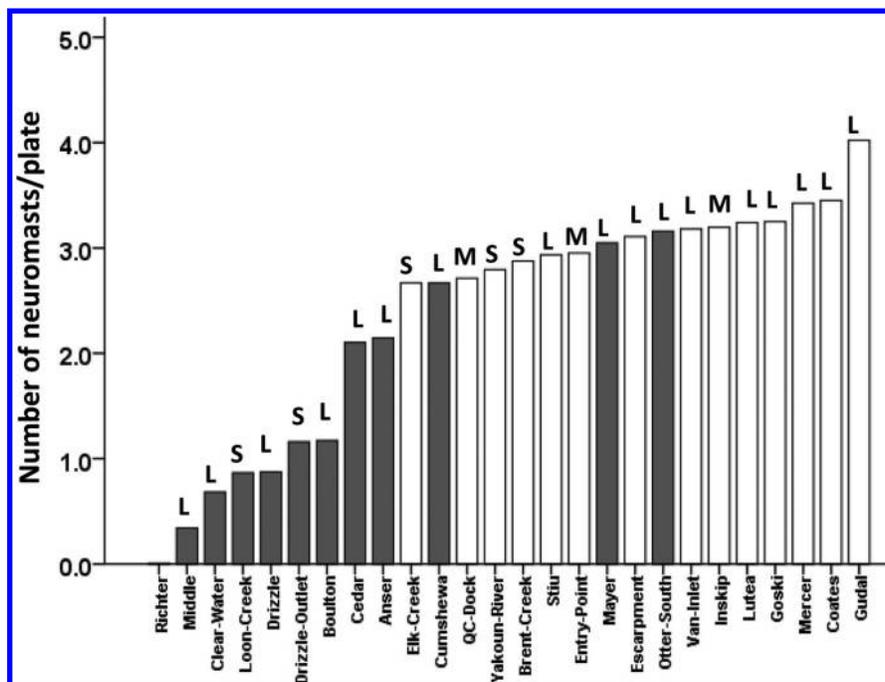
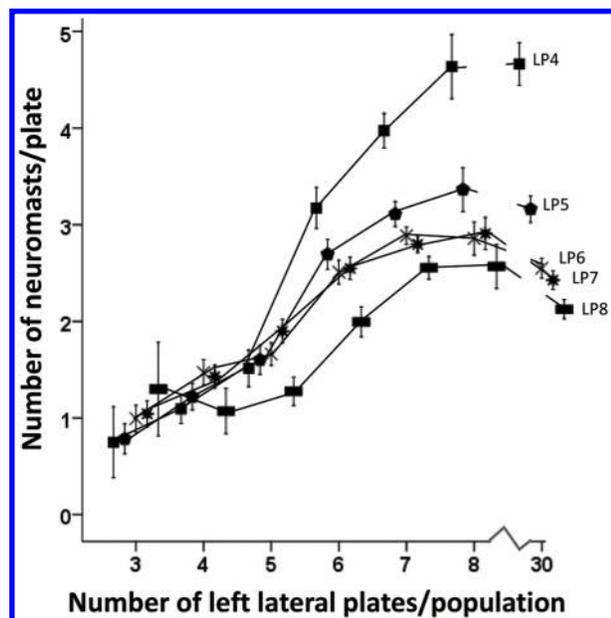


Fig. 5. Mean (\pm SE) number of neuromasts on each left lateral plate (LP) in relation to population mean number of lateral plates for Haida Gwaii populations of threespine stickleback (*Gasterosteus aculeatus*). There was a significant increase in neuromasts per plate with an increase in lateral plates ($\chi^2_{[1]} = 822.21, p < 0.001$), with anterior plates showing a greater degree of variation ($\chi^2_{[4]} = 260.44, p < 0.001$). There was no significant difference between fish with 7–8 plates and fully plated fish ($F_{[1,22]} = 2.83, p = 0.11$). No populations occur with a mean between 8 and 30 plates.



increased number of plates (>5), number of neuromasts increased on each plate, but the greatest increase occurred on LP4, which is the smallest and most anterior of the buttressing plates (see Fig. 2a). This trend of higher anterior neuromast counts on the anterior plates in these natural populations is similar to neuromast development on transgenic stickleback (Mills et al. 2014).

Previous studies of cranial and trunk neuromasts in the threespine stickleback from marine, stream, and lake habitats in southern British Columbia indicate substantial variability among different habitats (Wark and Peichel 2010). Stream-resident stickleback exhibited higher neuromast counts than anadromous stickleback on all regions of the lateral line, including those on the buttressing plates. As well, benthic ecotypes had higher neuromast counts than sympatric limnetic ecotypes, whereas stream and lake populations did not differ (see also Jiang et al. 2017). Our results from Haida Gwaii, in northern British Columbia, are generally similar to that of Wark and Peichel (2010) because we also found large differences in number of neuromasts per plate among populations, but we did not detect consistent differences between marine and freshwater populations or between stream and lake populations. We did, however, find that aquatic spectrum was a significant correlate to neuromast expression, with clear-water habitats exhibiting higher neuromast counts per plate. Lake size was not a predictor of neuromasts; yet, this factor is an important factor of morphological differentiation among stickleback populations (Nosil and Reimchen 2005; Reimchen et al. 2013).

We predicted that stickleback in the dark waters of dystrophic lakes would have reduced reliance on visual senses; thus, they would have increased number of neuromasts per plates relative to stickleback from clear-water habitats. It is well established that the lateral line plays an important role in predator–prey interaction, especially when visual detection is limited (Janssen and Corcoran 1993; Kanter and Coombs 2003; Junges et al. 2010). Our

results are inconsistent with this prediction. Rather than being higher or at least similar to clear-water habitats, stickleback from the majority of stained lakes had reduced neuromast counts on the plates. Examination of stickleback across habitats ranging from full clarity to deeply stained suggest no major differences in absolute predation levels. However, all of the clear-water habitats in the archipelago have predatory fish, whereas stained habitats, often with reduced presence of predatory fish, have increased occurrence of avian piscivores, such as loons (genus *Gavia* J.R. Forster, 1788), mergansers (genera *Mergus* Linnaeus, 1758 and *Lophodytes* Reichenbach, 1853), and grebes (genus *Podiceps* Latham, 1787) (Reimchen and Douglas 1984; Reimchen 1988, 1994). It is interesting to note that Mayer Lake, one of the two stained localities with stickleback with high neuromast counts per plate, also has exceptional high densities of predatory fish that are the dominant predators on stickleback (Moodie 1972). We do not yet understand why reduced prevalence of predatory fish or increased prevalence of avian piscivory should favour a reduction in neuromasts per plate in stickleback in dystrophic habitats, but the results are opposite to our original prediction.

Lateral-line expression is positively associated with schooling behaviour in fishes, as it facilitates increased sensory information on adjacent fish (McHenry et al. 2009; Faucher et al. 2010; York et al. 2016). In coral reefs, various fish taxa that are schooling during daylight disperse during nocturnal periods (Helfman 1986). Marine pollock (*Pollachius virens* (Linnaeus, 1758)) and Atlantic mackerel (*Scomber scombrus* Linnaeus, 1758) are more likely to school during daylight than during crepuscular and nocturnal periods (Smith et al. 1993). Marine and freshwater threespine stickleback also school during daylight (Wootton 1976; Greenwood et al. 2013), but the extent of schooling during darkness has not been investigated. In stained dystrophic lakes, downwelling light can attenuate within several metres of the surface, and other than individuals immediately near the surface, stickleback are effectively in a crepuscular or a nocturnal light regime. It is reasonable inference that schooling of stickleback in dystrophic lakes could be of more limited value compared with diurnal activity. If so, then this could partially account for the reduced neuromast expression in these habitats.

Number of neuromasts per lateral plate on stickleback, at an individual level and a population level, increases asymptotically with number of lateral plates (Wark and Peichel 2010; current study). Part of this is associated with *Eda* because this gene influences lateral-plate expression and neuromast expression (Wark et al. 2012; Mills et al. 2014). Furthermore, *Eda* also is associated with schooling behaviour in stickleback (Mills et al. 2014; Mekdara et al. 2018). Such pleiotropic effects are probably ecologically relevant given the association between lateral plates, predation landscape, and spectral regime in the evolution of Haida Gwaii threespine stickleback (Reimchen et al. 2013).

We tested for sexual dimorphism in neuromast counts per plate and observed male-biased counts in the majority of localities. Although there was extensive population variability, the bias occurred in multiple habitats including streams, stained lakes, and clear-water lakes. That it also occurred in the three marine populations suggests that this is the ancestral state and has been retained to a greater or lesser extent following post-glacial colonization of these freshwater habitats (Deagle et al. 2012). Such a dimorphism is more likely to persist if there is niche differentiation between the sexes. In the majority of stickleback populations, males, which tend to be smaller than females, tend to have more lateral plates, more fin rays, larger relative jaw size, and larger relative head size and are more likely to be found in benthic and littoral habitats (Reimchen et al. 2016). Such niche differentiation between the sexes could readily facilitate divergence in neuromast expression on the lateral plates because the sexes would be subject to distinctive biophysical conditions.

Fig. 6. Sexual dimorphism for mean neuromast counts per left plate (male minus female) for Haida Gwaii populations (refer to Fig. 1) of threespine stickleback (*Gasterosteus aculeatus*). General habitat indicated above each bar as M (marine), S (stream), and L (lake). Bars indicate aquatic spectrum, with white bars representing clear-water habitats and grey bars representing stained habitats. Transplant populations were excluded. Significant comparisons (unpaired *t* tests) indicated with an asterisk (*). There was significant sexual dimorphism in neuromast count ($\chi^2_{[1]} = 53.9, p < 0.001$), which does not correlate with habitat type ($\chi^2_{[2]} = 3.57, p = 0.17$) or water clarity ($\chi^2_{[2]} = 0.76, p = 0.68$).

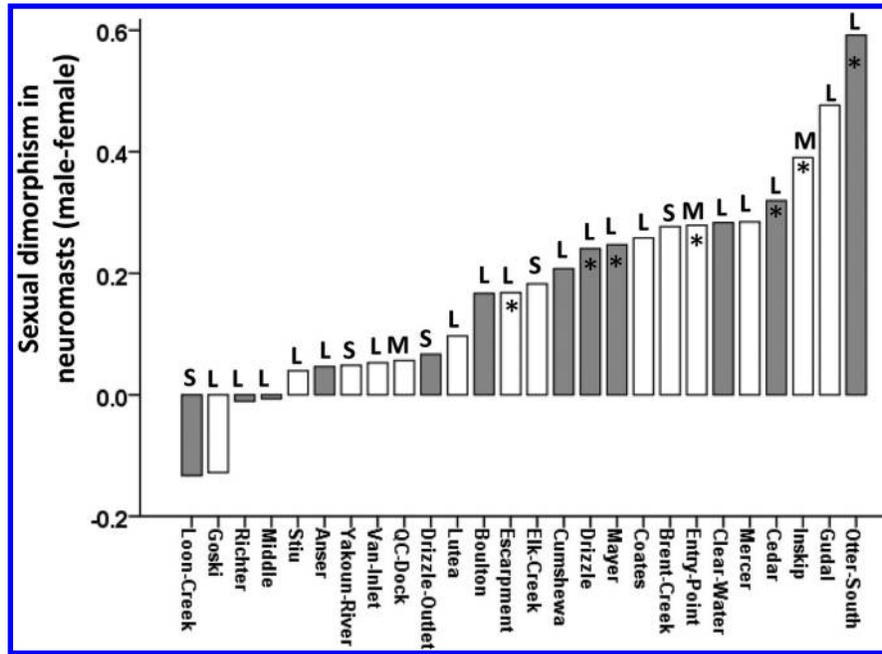
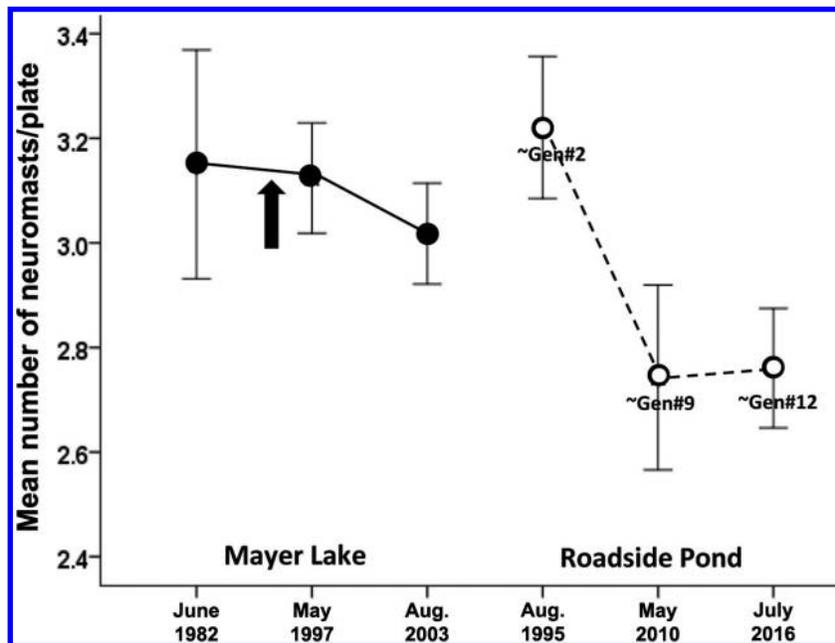


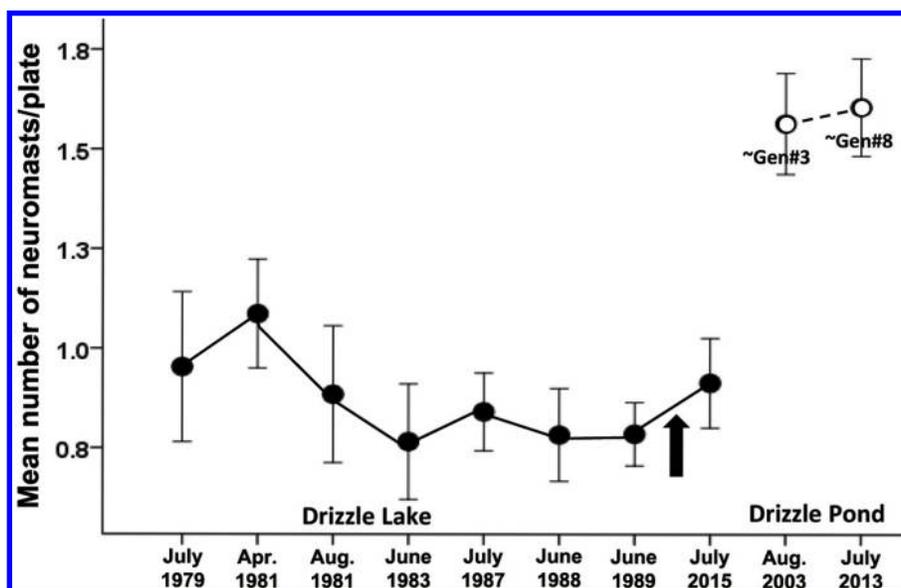
Fig. 7. Mean (\pm SE) number of neuromasts per left lateral plate of threespine stickleback (*Gasterosteus aculeatus*) for the Mayer Lake–Roadside Pond transplant experiment. Sampling date shown on the x axis. Generation number given for the three Roadside Pond samples. Arrow indicates date of transplant (1992). There was no significant difference in neuromast count between Mayer Lake and Roadside Pond ($\chi^2_{[1]} = 1.61, p = 0.20$).



To evaluate the potential for change in neuromast expression following colonization to highly divergent ecological conditions, we examined multiple generations of stickleback prior to and following their transplants to ecologically opposite habitats. We expected there to be few if any changes in the neuromasts over the short time frame, but our two separate transplant populations demonstrate that neuromast counts per plate diverge from the

source populations by 10% to 80% within a decade. The Mayer Lake–Roadside Pond experiment involved a shift of limnetic stickleback from a large dystrophic lake (stained, low conductivity) with high levels of mortality from predatory vertebrates, particularly salmonids (Moodie 1972), to a shallow small eutrophic pond (unstained, higher conductivity) with no predatory fish and no diving birds. Eight generations of the colonists yielded reduction

Fig. 8. Mean (\pm SE) number of neuromasts per left lateral plate of threespine stickleback (*Gasterosteus aculeatus*) for the Drizzle Lake–Drizzle Pond transplant experiment. Sampling date shown on the x axis. Generation number given for the two Drizzle Pond samples. Arrow indicates date of transplant (1997). There was a significant difference in neuromast count between Drizzle Lake and Drizzle Pond ($\chi^2_{[1]} = 22.15$, $p < 0.001$).



in frequency of lateral plates on the 2nd and 8th positions, as well as multiple changes in spines and trophic characters in the predicted direction based on the altered selective regime (Leaver and Reimchen 2012). In the current study, we observed that number of neuromasts per plate in this transplant population remained high for at least two generations and this was followed by a significant reduction in subsequent generations. That the loss of neuromasts per plate was greatest on LP4 and LP8, the most anterior and posterior of the buttressing plates, indicates body segment specificity to neuromast reduction rather than domain-general responses. Although the reduction in neuromast counts is what would be expected given the absence of predatory fish, the shift from stained to clear water and low conductivity to high conductivity should have resulted in an increase in neuromasts based on the occurrence of higher neuromast numbers in stickleback from clear-water habitats. The functional explanation for this reduction is not known, but the trends are consistent with the previously identified developmental integration between neuromasts, plate expression, and plate position (Wark and Peichel 2010; Wark et al. 2012). In the sequential order of loss of buttressing plates among populations with progressively fewer plates (Reimchen 1983), as well as the Mayer Lake–Roadside Pond transplant (Leaver and Reimchen 2012), LP4 and LP8 are the first plates to exhibit a reduction in size and number of neuromasts per plate, perhaps additional evidence for the role of *Eda* in the covariation between lateral plate and neuromast expression. Heritability of neuromast reduction is further supported by recent genomic analysis of this transplant experiment, as the *Eda* gene is a significant outlier for genetic divergence between Roadside Pond and Mayer Lake that indicate strong selection at this locus (Marques et al. 2018).

Although the changes in the Roadside Pond experiment were consistent with one of our original predictions (i.e., less predatory fish, more neuromasts per plate), the results for the second transplant were not. The Drizzle Lake–Drizzle Pond experiment also involved a shift of a limnetic stickleback in a large dystrophic lake (stained, low conductivity), where the fish incur major mortality from avian piscivores (primarily loons) and salmonids (cutthroat trout, *Oncorhynchus clarkii* (Richardson, 1836)) (Reimchen 1994), to a shallow small eutrophic pond (unstained, higher conductivity) with no predatory fish and no diving birds. Despite the high levels of staining and predator-related mortality in Drizzle Lake, the

stickleback have a low number of neuromasts per plate; however, following transplant to Drizzle Pond, the neuromast count increased on all plates, up to 230% in the most anterior structural plate (LP4). Although predatory fish and large diving birds are absent in this pond, there are occasional Great Blue Herons (*Ardea herodias* Linnaeus, 1758) and Belted Kingfishers (*Megaceryle alcyon* (Linnaeus, 1758)) (T.E. Reimchen, unpublished observations). This potentially increases the benefit of schooling in the unstained waters, both of which might favour increased neuromast expression. That the rate of change was so rapid suggests developmental plasticity yet, because the number of bony plates also increased, is consistent with the proximal role of *Eda* in the covarying expression between plates and neuromasts (Wark and Peichel 2010). Whatever the functional interpretations to the responses in this transplant study, the data further support evidence of rapid shifts in this sensory modality, suggesting that the differentiation that we observed among allopatric populations could potentially have developed early following colonization of freshwater habitats.

In summary, threespine stickleback exhibit differences in neuromast expression on the buttressing plates within and among individuals, among localities, and between the sexes. Differences among localities in predation landscape and aquatic spectrum are important correlates in neuromast expression, but our transplant experiments yield contrasting trends with respect to ecological predictors. It is clear that the rate of change in neuromast expression per plate can be rapid, within several generations. The explicit functionality of these changes to the sensory modality of the fish merits further attention.

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