

Natural selection and the adaptive radiation of Haida Gwaii stickleback

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ABSTRACT

Questions: What are the selective landscapes structuring inter-population variability on the Haida Gwaii archipelago? How much morphological variability is functional given the common potential for stochastic founder effects when new habitats are colonized?

Organism and sites: Threespine stickleback (*Gasterosteus aculeatus*) from 56 isolated watersheds with separate marine ancestry encompassing 102 freshwater localities ranging from large oligotrophic mountain lakes to darkly stained bog lakes and ponds on the Haida Gwaii archipelago, western Canada.

Field data: Biophysical attributes of the habitat (lake volume, depth, percent light transmission at 400 nm, calcium availability, pH, distance to marine waters, predation regime including puncturing, compression, and grappling species), morphological traits of the fish (body size, body depth, lateral plate number and position, dorsal spine length, pelvic girdle size, ascending process height, cross-sectional diameter, basal and lateral plate structural overlap), and geographical distance between localities. Based on previous surveys in this archipelago, we predicted the greatest expression of post-capture defences in lakes with high water clarity and the greatest reduction in small stained ponds, as this characterizes regular shifts in the predation landscape.

Analyses: Principal components, univariate linear models, and Akaike Information Criterion (AIC) analyses.

Results: Populations range from 30 to 90 mm in adult male standard length, from full-plated to naked, and from full-spined to un-spined. Again and again, populations converge towards functional multivariate or univariate phenotypes predicted by the biophysical attributes of each habitat, primarily lake volume, aquatic spectra, and predation regime. Across a broad diversity of lake volumes, body size is larger in stained lakes and we hypothesize that this is a defence adaptation for increased burst velocity and rapid access to the aphotic zones near the water surface. Defence armour is well developed in clear lakes, independent of lake volume, and in large stained lakes. Completely plated populations are only found in clear habitats. Reduction in posterior plates is associated with accentuated armour development in the anterior trunk in large lakes where predatory vertebrates are common. Anterior armour, including lateral plates and spines, is progressively reduced towards an unarmoured condition in shallow, stained ponds.

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Conclusion: Threespine stickleback from pristine lakes and ponds on the Haida Gwaii archipelago demonstrate remarkable morphological differentiation among populations associated with habitat differences that is largely consistent with changes in predation regime, potentially mediated through a shift from post-capture to pursuit adaptations. Thus, even if founder effects occur when new populations are colonized, natural selection is a major determinant of morphological evolution. Our study emphasizes the efficacy of selective landscapes in this adaptive radiation and provides an opportunity for informed integration of emerging genome-wide data of these populations.

Keywords: body size, Canadian Galapagos, dystrophic lakes, morphological variability, predator-prey defences, Queen Charlotte Islands, stickleback.

INTRODUCTION

A number of spectacular adaptive radiations have been described in great detail, including those of Darwin's finches (Grant and Grant, 2008), *Anolis* lizards (Losos, 2009), Hawaiian silverswords (Witter and Carr, 1988; Baldwin and Sanderson, 1998), and cichlid fishes (Meyer, 1993; Seehausen, 2006; Salzburger, 2009; Wagner *et al.*, 2012). Since the early stages of colonization are generally lost to history, the relative importance of stochastic and deterministic processes to differentiation in these adaptive radiations is empirically unknown and frequently contentious (Losos *et al.*, 1998; Taylor and McPhail, 2000; Kolbe *et al.*, 2012). A broader generalization on the relative interplay of these processes would be possible if a widely distributed and morphologically conserved common ancestor repeatedly and independently colonized a diversity of habitats that were highly variable in biophysical parameters. Lack of predictability between phenotype and habitat would emphasize historical constraints, which could characterize much differentiation within a species (Gould, 1984), while replicated differentiation that was predictable based on habitat would emphasize deterministic models of morphological radiation.

In contrast to common adaptive radiation models in which a small group of colonists diversify in a vacant archipelago, as occurred with finches in the Galapagos (Grant and Grant, 2008), the marine threespine stickleback has independently colonized thousands of freshwater habitats (analogous to 'islands') throughout coastal zones of North America, Europe, and Asia (Wootton, 1976; Bell and Foster, 1994). These multiple colonizations provide the potential for both stochastic founder effects and deterministic natural selection to drive evolution and have resulted in morphological diversification in adult body size, body armour, trophic structures, behaviour, and life histories (for a review, see Wootton, 1984). The lateral plates that cover the trunk of the stickleback function as a defence against puncturing predators such as fish, and as buttressing support for dorsal and pelvic spines during predator manipulation (Reimchen, 1983). Reductions in defence traits are widespread in freshwater habitats and may reflect combinations of phenotypic plasticity (Frommen *et al.*, 2011) or genetic adaptations to cope with reduced salinity (Heuts, 1947; Giles, 1983; Bourgeois *et al.*, 1994), reduced buoyancy (Myhre and Klepaker, 2009), reduced predation intensity (Gross, 1978; Bourgeois *et al.*, 1994), or shifts in pursuit versus post-capture adaptations to predators (Reimchen, 1992, 2000).

Haida Gwaii (aka Queen Charlotte Islands), located about 100 km off the continental west coast of Canada, is one of the most remote archipelagos in temperate latitudes and harbours numerous freshwater stickleback populations. Initial surveys of 27 lakes on this archipelago showed major morphological differentiation in threespine stickleback among lakes exceeding that found elsewhere throughout the circumboreal distribution of

the species (Moodie and Reimchen, 1976; Reimchen *et al.*, 1985). More detailed investigation at several of these localities with highly divergent stickleback populations showed evidence for adaptive differentiation of defensive traits (Moodie, 1972a, 1972b; Reimchen, 1980, 1994, 1995; Reimchen and Nosil, 2002). Geological evidence strongly suggests that the islands were fully glaciated during the Pleistocene (Sutherland-Brown, 1968), although there is recent archaeological, palynological, oceanographic as well as genetic evidence of a large ice-free refugium that may have persisted on the continental shelf separating the archipelago from the mainland (Fladmark, 1979; Josenhans *et al.*, 1995; Byun *et al.*, 1997; Reimchen and Byun, 2005).

The current study greatly expands the original surveys to encompass the majority of lakes and ponds throughout the archipelago ranging from small shallow dystrophic ponds (<1 ha) to large and deep oligotrophic lakes (>500 ha). Of these 700+ ecologically intact localities, most lack fish due to high gradient streams that are impassable to upstream migration. However, 102 intact ponds and lakes contain resident stickleback and these are distributed among 56 different primary watersheds with separate drainages to open marine waters. Recent studies on many of these populations have addressed population variation in nuptial colour (Reimchen, 1989), fluctuating asymmetry (Bergstrom and Reimchen, 2000, 2002), and body shape (Spoljaric and Reimchen, 2007, 2008, 2011). Genetic investigations indicate colonization of the archipelago by two mitochondrial lineages (O'Reilly *et al.*, 1993; Orti *et al.*, 1994; Deagle *et al.*, 1996), while current genomic surveys of these populations using a 1536 SNP (single nucleotide polymorphism) array are consistent with assumptions of independent origin for most primary watersheds (Deagle *et al.*, 2012, 2013).

We provide here the first archipelago-wide evaluation of adult body size and defence morphology of stickleback in relation to biophysical parameters, including lake volume, aquatic spectra, distance to marine waters, pH, calcium availability, predation regime, and geography using replicated natural experiments in diversification. As well as allowing us to identify the best predictors of differentiation and test specific hypotheses such as the competing roles of calcium availability, buoyancy, and predation assemblages in relative armour expression, these data provide a powerful test of the relative strength of deterministic processes in morphological diversification and complement recent conceptual and experimental perspectives on the mechanisms of evolution (Wade and Kalisz, 1990; Kolbe *et al.*, 2012).

MATERIALS AND METHODS

Sampling localities and habitat

Fish were collected by T.E.R. on the Haida Gwaii archipelago during expeditions primarily from 1977 to 1989. Of the 56 primary watersheds, 43 contained a single lake and 13 multiple lakes ($n = 2-16$ lakes per watershed). In this paper, we refer to 'primary watershed' when the river mouth is separated from other watersheds by marine waters. Stickleback (mainly adults) were captured (usually May and June) using standard Gee-minnow traps. These were baited with old cheddar cheese, which provided a solid matrix with slow-release of aromatic oils to which the fish are attracted (T.E. Reimchen, personal observation).

Multiple biophysical variables were estimated for all lakes during fish sampling, including lake volume, water clarity, and distance to the ocean along the watershed outlet stream. For a subset of lakes ($n = 50$), pH and ion concentrations (13 elements) were determined but we restrict our analyses to calcium as it has received the greatest attention in the stickleback literature. Water clarity was estimated by two methods: one categorical and one continuous.

At each locality, clarity was subjectively categorized as clear or stained based on detection of a light substrate at a depth of 1 m. Visibility was a function of dissolved substances, as suspended particles were infrequent. At 69 localities, a 20 mL water sample was obtained from the littoral zone, stored in an opaque container, and subsequently measured for percent light transmission at 400 nm (T400) on a bench spectrophotometer (Reimchen, 1989). These continuous data were categorized into 'clear' (T400 > 74%) and 'stained' (T400 < 74%), with equal proportions of the two and generally equivalent to the dichotomous subjective field classification of clear versus stained. Aquatic spectra (percent transmission at 400 nm) were generally similar within but differed among the physiogeographic regions (ANOVA: $F = 38.47$, d.f. = 2, $P < 0.001$). Montane ($n = 28$ lakes) and plateau ($n = 21$ lakes) regions both had waters of high clarity (88.4% and 84.7%, respectively) compared with the stained waters of the lowlands (65.5%, $n = 53$ lakes). Although we used primarily categorical data for aquatic spectra analyses, we also used percent transmission as a covariate and computed a derived transmission in 33 localities based on the average transmission for the remaining lakes in the watershed. All continuous data were log-transformed.

Predation regime

Predator data were collected at all localities, including presence/absence data on the major species associated with lakes in the archipelago, such as fish (cutthroat trout, *Oncorhynchus clarki*; rainbow trout, *O. mykiss*; coho salmon, *O. kisutch*; Dolly Varden, *Salvelinus malma*; prickly sculpin, *Cottus asper*; coastrange sculpin, *C. aleuticus*), birds (loons, *Gavia immer* and *G. stellate*; grebes, *Podiceps grisegena* and *P. auritus*; mergansers, *Mergus serrator*, *M. merganser*, and *Lophodytes cucullatus*; cormorant, *Phalacrocorax auritus*; kingfisher, *Ceryle alcyon*; heron, *Ardea herodias*), mammals (river otter, *Lutra canadensis*), and macroinvertebrates (odonates, *Aeshna* spp.; diving beetles, *Dytiscus* spp.). Predatory fish were assessed from gill-nets, rod and line fishing or minnow traps, while avian piscivores were visually identified and counted. Macroinvertebrates were often present in the minnow traps when salmonids and cottids were rare in the lake. Relationships between lake habitat and predation regime have been described elsewhere (Reimchen, 1994). For present purposes, three general predation regimes were identified: A – rainbow trout, B – cutthroat trout and bird, and C – macroinvertebrate and bird, and these largely correspond to physical attributes of the lakes, including lake size and aquatic spectra. Rainbow trout are found primarily in small and large clear water lakes of the montane regions that typically have low densities of avian piscivores. Cutthroat trout are found primarily in medium to large stained lakes on the plateau and lowland regions and these tend to have abundant avian piscivores. Small stained lakes and ponds without predator fish are found primarily in the lowlands and these have macroinvertebrates and small-bodied avian piscivores. At present, there is no empirical field evidence that these localities differ in relative predation levels, although they do differ in terms of predator assemblage.

Morphology

We measured all fish with dial calipers and because of the widespread sexual dimorphism (Reimchen, 1980; Reimchen and Nosil, 2004, 2006; Spoljaric and Reimchen, 2008), only males were used in this study for a total sample size of 3186. Standard length (SL) was measured on each fish as well as a suite of defensive traits (Fig. 1), including lateral plate number on the left side

(LLP), position of each lateral plate (LPx) following the classification of Reimchen (1983), length of left pelvic spine (LPS), length of first dorsal spine (LDS), width of first dorsal spine (WDS), cross-sectional diameter with spines erect (XSD), width and length of the ventral plate (VPW, VPL), width and height of the left ascending process of the pelvis (APW, APH), and length of the basal support at the base of the second dorsal spine (BP2). We also scored the amount of overlap of the basal plate on LP6 (no contact = 0, touching = 1, slight overlap = 2, and major overlap = 3), as in Reimchen (1983). The left ascending process had been cut on the fish from seven populations, and so for these we measured ascending process height on the right side. All morphometric traits were log-transformed. All metric traits increased in size linearly with standard length (all $P < 0.001$, bivariate correlation) and were size-standardized (to the overall mean SL) using equation (1):

$$\log y'_{ij} = \log y_{ij} - \beta(\log x_i - \log x), \quad (1)$$

where y'_{ij} is the adjusted value of trait j for individual i , y_{ij} is the unadjusted value, β is the unstandardized regression slope, x_i is the standard length of individual i , and x is the overall mean standard length (SL = 52.31 mm) of all individuals in the study (Reimchen *et al.*, 1985). The slopes of metric trait size versus standard length differed among localities (all $P < 0.001$, trait \times SL interaction, ANCOVA) and therefore all standardizations were based on locality-specific slopes. Lateral plate number did not change with standard length in a consistent way among localities and so was not size-standardized. For population comparisons of standard length, we calculated the average for the 10 largest adult fish in each sample. We do not include data for lateral plate positions as these were highly conserved with respect to the relationship with plate number in the majority of populations (Reimchen, 1983).

Principal component analyses were run on all fish using LLP and the following log-transformed, size-adjusted defensive traits: LPS, LDS, WDS, XSD, VPW, VPL, APW, APH, BP2, as well as log-transformed SL. Because some populations with reduced defences lacked morphological structures for measurement, the full suite of defensive traits was limited to about two-thirds of the total number of fish ($N = 2336$), so only these fish were included in the principal component analyses.

Trait–environment correlations

We investigated associations among biophysical lake attributes and principal component scores derived from the collective morphological measurements, adult body size, lateral plate number, plate position, spine length, and cross-sectional diameter. Using the mean data for each lake, we ran multiple regressions on our dependent continuous morphological variables (excluding plate position) using the complete dataset including lake volume (log), spectra, and outlet stream length (log) as independent variables. We also ran multiple regressions on the subset of lakes that included calcium concentration (log) and pH (log) as independent variables. Therefore, for each continuous dependent variable, we ran two regressions: one on the complete dataset of 102 lakes, excluding calcium and pH from the model ('all'), and one on the subset of 41 lakes that included both calcium data and pH in the model ('subset'). Co-linearity was checked among predictor variables for every multiple regression, and was moderate to negligible with all tolerance levels greater than 0.24. Co-linearity was most evident for pH and calcium, as both of these were correlated with lake volume and water spectra. To account for this, we ran additional separate correlations

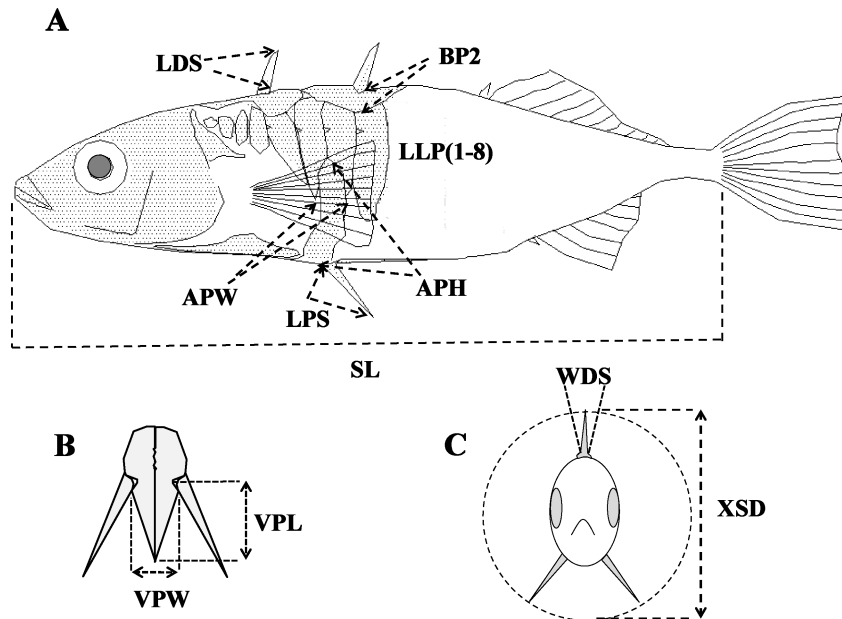


Fig. 1. Threespine stickleback morphology and the traits used in this study. (A) Whole left side of the body showing standard length (SL), length of first dorsal spine (LDS), length of second basal plate (BP2), number of lateral plates (LLP) with plates 1 through 8 shown, height and width of the ascending process (APH, APW), and length of the left pelvic spine (LPS). (B) Ventral view of pelvic girdle (anterior towards top) showing ventral plate length and width (VPL, VPW). (C) Anterior view of cross-sectional diameter with spines erect (XSD) and width of first dorsal spine (WDS).

between morphological traits with both pH and calcium within each of four categories of lakes: clear and large, clear and small, stained and large, and stained and small.

For the complete dataset we ran Akaike Information Criterion (AIC) models to identify which of the multiple regressions resulted in the best model. Delta AICs (Δ AIC) were calculated for each model for each morphological trait, with the best model (having the smallest AIC value) equal to a Δ AIC of 0. The AIC values of this best model were subtracted from each remaining AIC value for other models involving that trait in order to calculate Δ AIC. If Δ AIC values of the remaining models were <2 , they were considered equivalent to the best model. Δ AIC values of 3–9 had considerably less support, and those ≥ 10 had very little support (Burnham and Anderson, 2002).

To further investigate potential interaction terms among our biophysical variables and predation regime category, we ran ANCOVAs for each continuous morphological trait with lake volume as a covariate, and either categorical water clarity or predation regime category (both defined above) as factors. Here we were interested primarily in significant interaction terms, as well as the main effects of predation regime. Comparisons of main effects among the three predation regime categories were made with LSD *post-hoc* tests.

Mantel tests

We tested if differences in morphology between lake pairs were associated with their geographical distance from each other and their differences in biophysical variables. This was done using simple and partial Mantel randomization tests, as these tests account for potential non-independence of matrix elements (Manly, 1997). We measured geographical distance between all pairs of lakes as the distance between the mouths of their outlet streams along the marine perimeter of the archipelago (a proxy for potential dispersal and gene flow among watersheds). Map resolution was not sufficient to reliably estimate stream distances among lakes within watersheds, so pairs of lakes in the same watershed were given a distance value of zero.

For each continuous morphological trait, we first ran a simple Mantel test to determine if there was a correlation between morphological distance and geographical distance among lake pairs. Then we ran a partial Mantel test that included a lake physical variable as a covariate and controlled for geographical distance (which was also included as a covariate in the analysis). This was repeated for volume, water clarity, and so on. We did this to determine whether differences in morphology were still correlated with differences in lake characteristics (i.e. volume, water clarity, etc.) after geographical distance was accounted for.

Statistical procedures

Akaike Information Criterion (AIC) analyses and Mantel tests using 1000 randomizations were done in R 2.6.2 (R Development Core Team, 2008) and all other statistical analyses were done in SPSS versions 13.0 and 17.

RESULTS

Multivariate analyses

Principal component analyses were run on all data using log-transformed, size-adjusted defensive traits as well as log-transformed standard length (SL). Two PCs resulted: the first accounted for 62.6% of the variance and was a general defence robustness PC with high loadings for all defensive traits, while the second accounted for 11.8% of the variance and was due almost exclusively to a large loading score for body size. Among the defence traits, length of the pelvic spine and ventral plate as well as height and width of the ascending process showed the highest loadings (Table 1).

For graphical display, we partitioned PC1 into five categories, ranging from low scores (few lateral plates, small spines, reduced pelvis, and no overlap of the basal plates) to high scores (robust defences with increased number of lateral plates, large spines, wide pelvic girdle, and large basal plates), and including a similar number of localities in each, demonstrates geographical pattern in distribution of the five defence categories (Fig. 2). Populations with greater defence occurred throughout the archipelago but with increased representation in the montane and plateau watersheds. There were seven low-plated populations with a defence PC exceeding the maximum PC values observed in the five completely plated populations. Weak defences were not observed in the montane zone, were uncommon in the plateau region, but were very common in the lowlands. This major reduction occurred in 11 different primary watersheds with outlet streams widely separated

Table 1. Principal component loadings for defensive traits and body size (SL) of stickleback from Haida Gwaii

Trait	PC1	PC2
LLP	0.77	-0.23
LPS	0.91	0.06
LDS	0.84	0.20
WDS	0.64	0.38
XSD	0.89	0.20
VPW	0.83	-0.26
VPL	0.91	-0.11
APW	0.91	-0.10
APH	0.85	0.00
BP2	0.84	0.01
SL	-0.09	0.91

Note: LLP = left lateral plate number, LPS = length of left pelvic spine, LDS and WDS = length and width of the first dorsal spine respectively, XSD = cross-sectional diameter with spines erect, VPW and VPL = width and length of the ventral plate respectively, APW and APH = width and height of the ascending process, BP2 = length of the second basal plate, SL = standard length. See Fig. 1.

by marine waters. Thus, PC1 varied among geographical regions ($F = 25.41$, d.f. = 2, $P < 0.001$).

Multiple regression of defence PC against biophysical variables combined with AIC criteria confirmed that the best model to account for geographical variation in defence PC included spectra and volume, with or without stream distance (Table 2). Using the complete lake database, defence PC was significantly positively correlated with spectra (partial $r = 0.35$, $P < 0.001$) and volume (partial $r = 0.40$, $P < 0.001$; Table 3). Of the 15 lakes with the highest scores, 14 were from clear water habitats, 11 of which occurred in different primary watersheds. Of the 15 lakes with the lowest scores, 14 were from stained systems, seven of which were in different primary watersheds (Fisher exact test, $P < 0.001$). A marginal but non-significant interaction (ANCOVA: volume \times spectra interaction, $F = 3.12$, d.f. = 1, $P = 0.08$) was observed between volume and water spectra category (clear vs. stained), indicating a slightly greater increase in PC1 defence scores as lake volume increased in stained lakes rather than in clear lakes and a larger difference in PC1 between stained and clear lakes that were small (Fig. 3a). When geographical distance between lakes was controlled for, differences in defence PC between lake pairs were still strongly positively correlated with differences in lake volume (partial Mantel test, $r = 0.18$, $P = 0.001$) and water spectra (partial Mantel test, $r = 0.20$, $P < 0.001$).

In the subset database, which also contained chemical data, there were no significant correlations between PC1 and either calcium concentration or pH (Table 3); nor were there significant correlations with pH or calcium within any of the four water clarity–lake volume

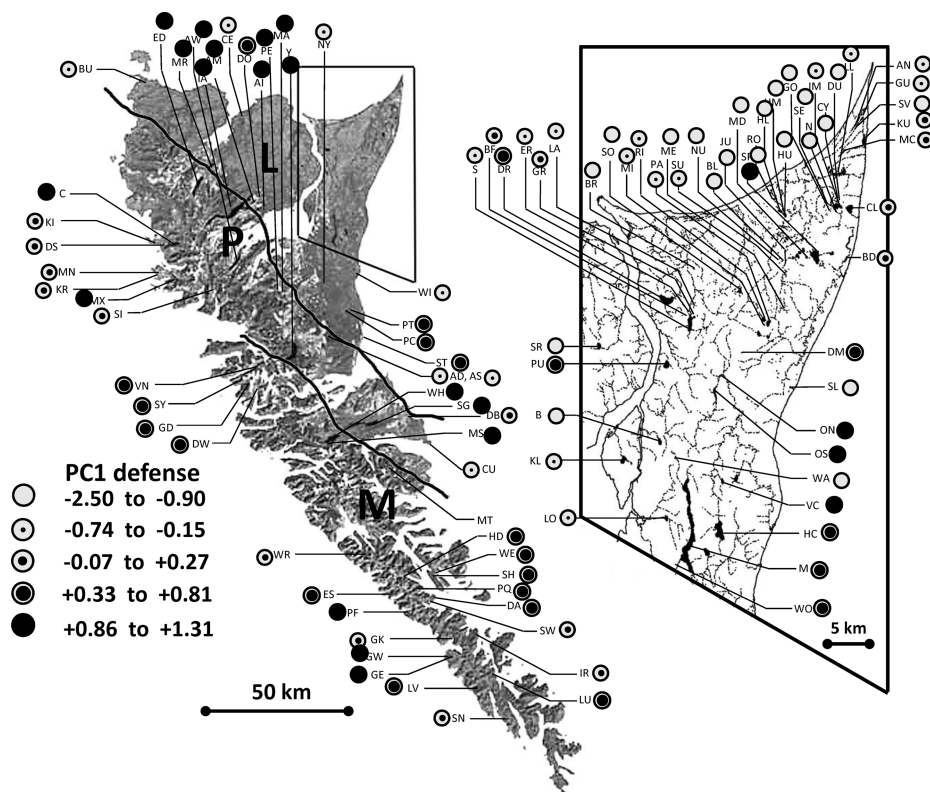


Fig. 2. Distribution of PC1 scores in Haida Gwaii ponds and lakes. Large bold letters designate major geographical regions of the archipelago: L = lowlands; P = plateau; M = mountains (Sutherland-Brown, 1968). Lake acronyms, names, and sample sizes as follows: AI = Ain (12); AM = Amber (24); AD = Anderson North (48); AS = Anderson South (34); AN = Anser (48); AW = Awun (27); BF = Big Fish Pond (19); BL = Blowdown (10); BD = Blue Danube Pond (12); B = Boulton (62); BR = Branta (58); BU = Bruin (19); CE = Cedar (19); CL = Clearwater (42); C = Coates (31); CU = Cumshewa (29); CY = Cygnet (33); DM = Dam (14); DA = Darwin (40); DW = Dawson (26); DB = Debris (6); DS = Desolate (33); DO = Downtree (9); DR = Drizzle (108); DU = Duck (6); ED = Eden (53); ER = Eriophorum Pond (11); ES = Escarpment (38); GK = Goski (37); GO = Gosling (14); GE = Gowgaia East (49); GW = Gowgaia West (33); GR = Gross Pond (23); GU = Grus (48); GD = Gudal (35); HL = Harelda Lower (42); HM = Harelda Middle (20); HU = Harelda Upper (17); HC = Hickey (18); HD = Hidden (4); IA = Ian (9); IM = Imber (23); IR = Irridens (36); JU = Juno (22); KI = Kiokathli (62); KR = Krajina (67); KU = Kumara (22); KL = Kumdis (7); LA = Laurel Pond (18); LO = Loon (8); LV = Lower Victoria (37); LL = Lumme (34); LU = Lutea (53); MA = Marie (20); MR = Marion (10); MT = Mathers (41); M = Mayer (112); MN = Menyanthes (65); MX = Mercer (23); ME = Mesa Pond (5); MC = Mica (22); MI = Middle Pond (19); MD = Midge (27); MS = Mosquito (28); N = Naked (3); NY = New Years (37); NU = Nuphar (24); ON = Otter North (44); OS = Otter South (44); PA = Parkes (17); PE = Peter (20); PC = Pontoon Center Pond (26); PT = Pontoon Tlell Pond (34); PQ = Poque (47); PF = Puffin (40); PU = Pure (54); RI = Richter (13); RO = Rouge (54); SI = Seal Inlet (30); SE = Serendipity Pond (73); SV = Silver (10); SG = Skidegate (8); S = Skonun (14); SL = Slim (35); SH = Smith (14); SN = Snub (24); SO = Solstice (44); SP = Spence (18); SR = Spraint (36); ST = Stellata (23); SY = Stiu (45); SU = Stump (11); SW = Sundew (49); VC = Vaccinium (14); VN = Van Inlet (48); WA = Watt (12); WE = Wegner (5); WH = White Swan (37); WI = Wiggins (40); WO = Woodpile (62); WR = Wright (49); Y = Yakoun (32).

Table 2. R^2 and delta Akaike Information Criterion (Δ AIC) values for different regression models used to explain morphological variation in stickleback as a function of lake characteristics

Model	Defence PC		Plate number		Spine length		Body size		Cross-sectional diameter	
	R^2	Δ AIC	R^2	Δ AIC	R^2	Δ AIC	R^2	Δ AIC	R^2	Δ AIC
S, V, D	0.38**	1.66	0.44**	0	0.18**	1.89	0.23**	0.26	0.35**	0
S, V	0.38**	0	0.36**	12.12	0.11*	8.62	0.13*	10.89	0.30**	5.71
S, D	0.27**	16.83	0.38**	8.66	0.09 [#]	10.76	0.12*	10.95	0.21**	17.54
V, D	0.29**	12.61	0.38**	8.42	0.18**	0	0.21**	0	0.30**	5.46
S	0.26**	15.46	0.30**	18.15	0.01	17.86	0.01	21.72	0.14**	23.33
V	0.29**	11.15	0.22**	29.75	0.09*	8.59	0.07*	15.52	0.27**	7.02
D	0.01	43.60	0.21**	31.62	0.07*	10.70	0.12**	9.17	0.01	37.10

Note: R^2 values are for the full regression model; see Table 3 for partial R^2 . S = percent spectral transmission at 400 nm, V = lake volume, and D = length of the outlet stream between the lake and the estuary. Results in **bold** represent the best regression model for that trait as indicated by Δ AIC. Pelvic spine length is size-adjusted and independent of plate number, and body size is the standard length of the 10 largest fish in the sample. Plate number, pelvic spine length, volume, and distance are \log_{10} -transformed to improve normality.

[#] $P < 0.05$; * $P < 0.01$; ** $P < 0.001$.

Table 3. Partial r -values from multiple regressions in Table 2

	Complete dataset			Partial dataset				
	Spectra	Volume	Stream distance	Spectra	Volume	Stream distance	Calcium	pH
Defence PC	0.35**	0.40**	0.06	0.02	0.43*	0.04	0.24	0.25
Plate number	0.31*	0.31*	-0.36**	-0.14	0.37[#]	-0.45*	0.24	0.41[#]
Pelvic spine length	-0.04	0.32*	0.28*	0.02	0.34[#]	0.27	0.15	-0.06
Body size	-0.13	0.34**	0.34*	-0.11	0.51*	0.40[†]	0.03	0.11
Cross-sectional diameter	0.27*	0.43**	0.28*	0.03	0.45*	0.27	0.24	0.15

Note: Regressions for partial dataset omit missing values pairwise (Quinn and Keough, 2002). See Table 2 for further explanations of traits.

[#] $P < 0.05$; * $P < 0.01$; ** $P < 0.001$.

categories. Several outliers to hypothesized associations with calcium are informative. Stickleback from the oligotrophic Coates Lake on western Graham have very well developed armour, yet this lake has exceptionally low lake calcium concentrations. Those from the small dystrophic Slim Lake, on the east side of Graham Island, are completely naked, have the lowest PC1 defence scores, and occur in one of the most stained of all lakes, yet the lake has higher calcium concentrations than Coates Lake. Thus, PC1 defence scores appear relatively uncoupled from calcium concentrations.

We examined the relationship between defence PC1 and predation categories. There were significant differences in overall defensive morphology among lakes that differed in their predation regime (ANCOVA: predation main effect, $F = 29.06$, d.f. = 2, $P < 0.001$; Fig. 4a). Stickleback in invertebrate/bird lakes had significantly lower PC1 scores than those

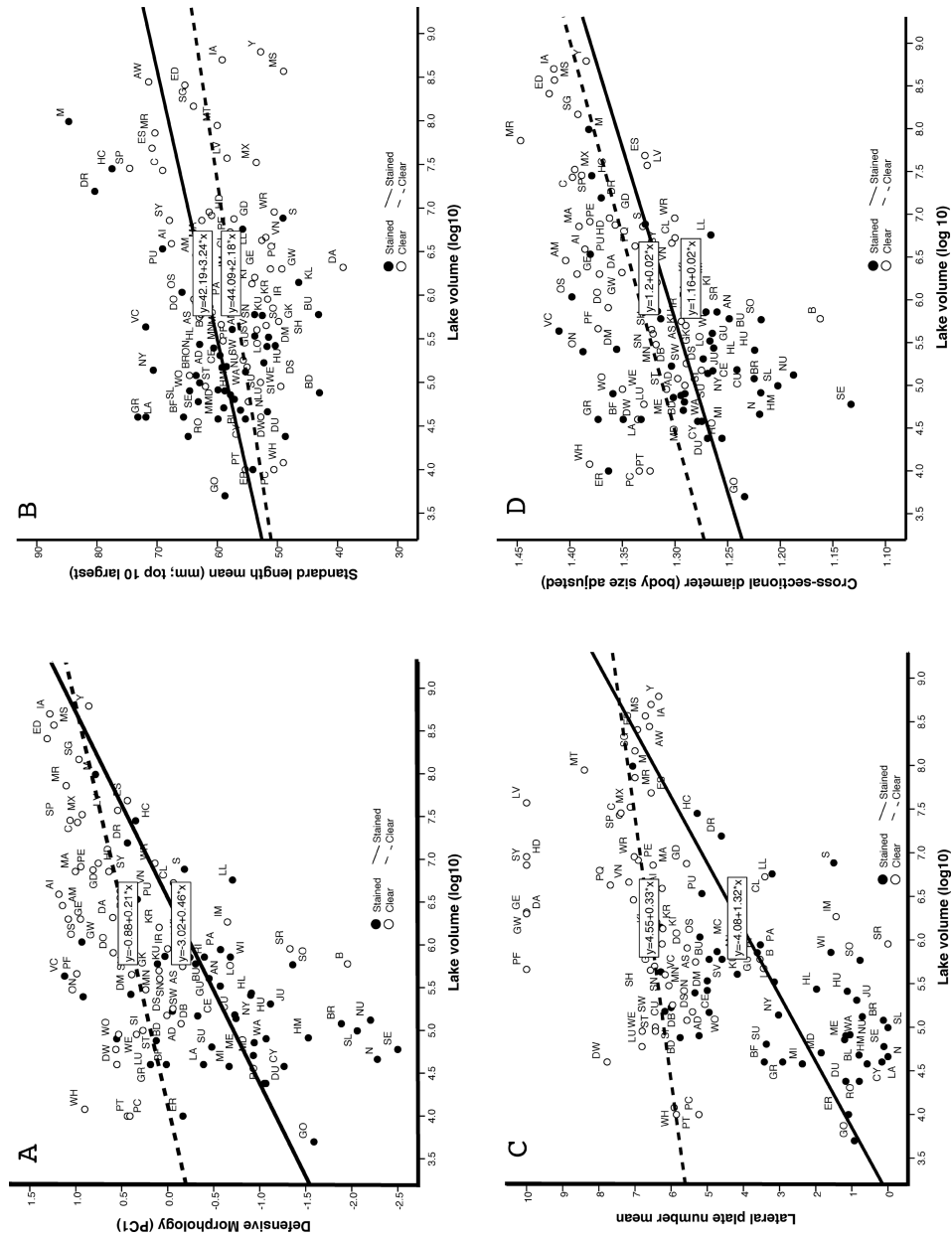


Fig. 3. Relationship between stickleback morphometrics and lake volume in relation to aquatic spectra in Haida Gwaii. (A) Defence PC1, (B) standard length, (C) number of lateral plates, (D) cross-sectional diameter. See Fig. 2 for lake names and locations.

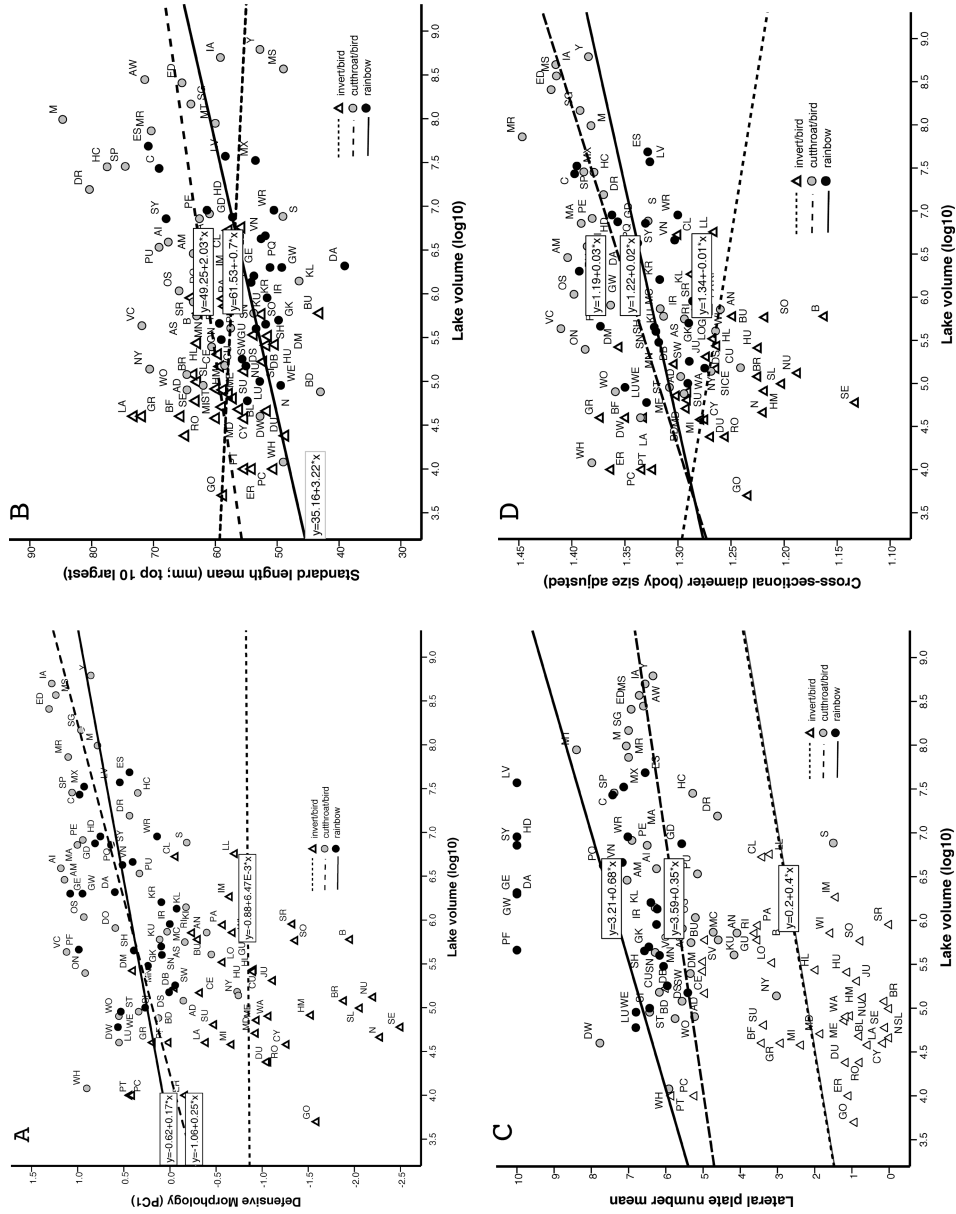


Fig. 4. Relationship between stickleback morphometrics, lake volume, and predation regime in Haida Gwaii lakes and ponds. (A) Defence PC1, (B) standard length, (C) number of lateral plates, (D) cross-sectional diameter. See Fig. 2 for lake names and locations.

in either rainbow or cutthroat systems (LSD: both $P < 0.001$), but there was no significant difference between the latter two (LSD: $P = 0.93$). The relationship between PC1 and lake volume was consistent among the predation categories (predation \times volume interaction: $F = 1.13$, d.f. = 2, $P = 0.33$), and volume continued to have an overall positive relationship with PC1 overall ($F = 8.25$, d.f. = 1, $P = 0.004$).

Univariate analyses

We used univariate analyses to investigate associations between lake biophysical variables and a subset of individual morphological traits that have received particular attention in previous work. Specifically, these traits were chosen due to demonstrations of their ecofunctional importance and included total body size, lateral plate number, dorsal and pelvic spines, and cross-sectional diameter.

Standard length (10 largest)

Adult male body size averaged 59 mm among lake populations (range 39–85 mm SL). This was similar to the average size, but with a greater range, for the five anadromous populations from the region (mean = 54 mm, range 46–69 mm). The smallest adults (29 mm) occurred at a montane lake (Darwin) of Moresby Island, while large adults (70–90 mm) occurred in 11 widely distributed primary watersheds on Graham and Moresby Islands. The largest adult male (Mayer) had 28 times the mass of the smallest adult (8.6 g vs. 0.3 g).

We examined relationships between male SL and lake biophysical variables. Among the multiple regressions, the three-way interaction of spectra, volume, and stream length exhibited the highest correlation and produced an AIC value similar to the top model that had only volume and stream distance (Table 2). Body size was positively correlated with lake volume (partial $r = 0.34$, $P < 0.001$) and stream distance (partial $r = 0.34$, $P < 0.01$) but not significantly correlated with spectra, calcium, or pH (all $P > 0.05$; Table 3). In addition, there were no significant correlations with pH or calcium within any of the four water clarity–lake volume categories. When geographical distance between lakes was controlled for, differences in body size between lake pairs were still positively correlated with differences in lake volume (partial Mantel test, $r = 0.13$, $P = 0.011$), but only approached significant correlations with stream length (partial Mantel test, $r = 0.07$, $P = 0.07$).

Body size showed a significant increase with lake volume (ANCOVA, $F = 12.52$, d.f. = 1, $P = 0.001$) and was significantly larger on average in stained than clear lakes (ANCOVA, $F = 6.25$, d.f. = 1, $P = 0.014$), especially among the largest lakes (Fig. 3b). Across the full range of lake sizes, although the localities with largest stickleback were always from stained conditions, the partial r -value for body size against water clarity (spectra) from the multiple regression was not significant (Table 3).

We examined the relationship between body size and predation categories. There were significant differences in body size among lakes that differed in their predation regime (ANCOVA, $F = 6.50$, d.f. = 2, $P < 0.01$; Fig. 4b). Stickleback in rainbow trout lakes were significantly smaller than those in invertebrate/bird lakes (LSD: $P < 0.05$) and cutthroat trout lakes ($P < 0.001$), but there was no significant difference between the latter two categories (LSD: $P = 0.282$). Although there was some heterogeneity in the relationship between body size and lake volume among these predation categories (Fig. 4b), the difference was non-significant (ANCOVA: predation \times volume interaction, $F = 1.53$,

d.f. = 2, $P = 0.22$) and when this interaction was removed, body size increased significantly with lake volume (ANCOVA, $F = 5.27$, d.f. = 1, $P < 0.05$).

Lateral plate number

Among the 102 lake populations, lateral plate number ranged from zero to 35 with an overall average of 5.8 plates per side. The geographical distribution of plate counts shows distinctive trends among physiogeographic regions ($F_{2,99} = 45.0$, $P < 0.001$; Fig. 5). Six populations from five different primary watersheds had predominantly completely plated phenotypes and occurred in montane regions of western Graham Island and Moresby Island. In addition, eight populations contained variable occurrence of intermediate or 'partial' plate phenotypes (mean counts >7) and these are also largely restricted to montane regions. The dominant phenotypes ranging from five through seven plates occurred throughout the archipelago but were more prevalent in the plateau region. Populations with major reduction in plates (<5) were restricted to the lowlands on the

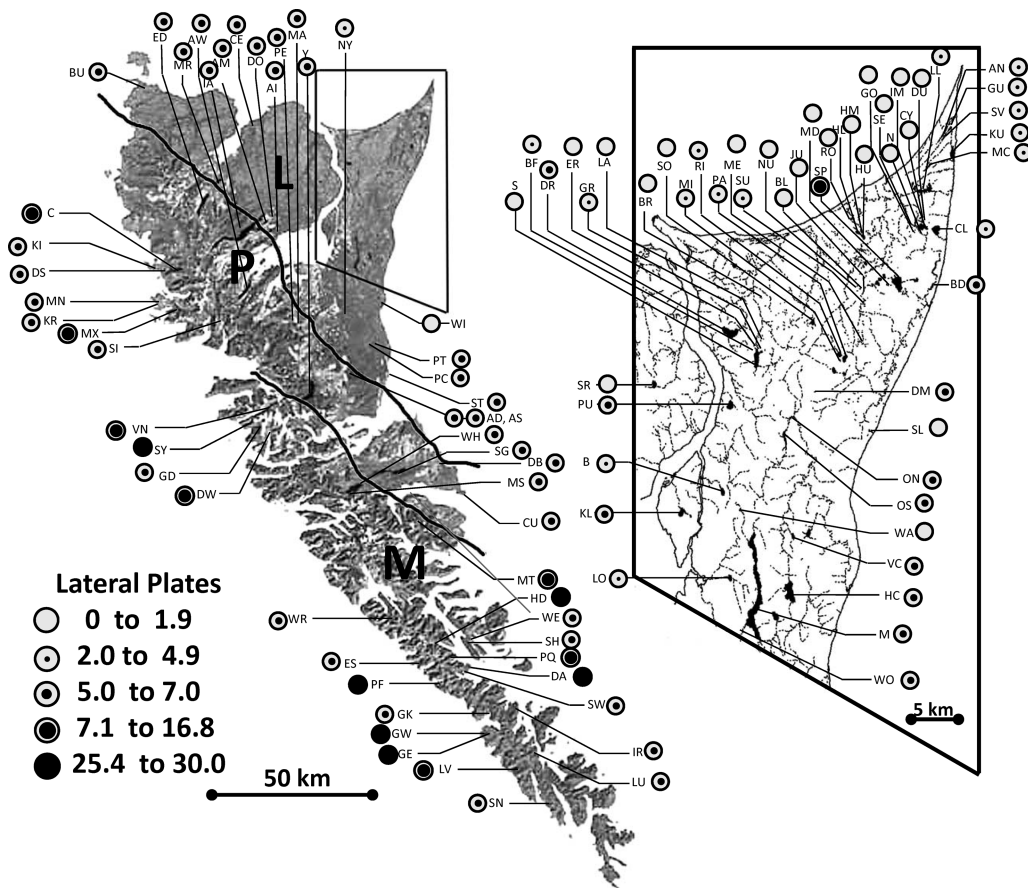


Fig. 5. Distribution of lateral plate phenotypes in Haida Gwaii lakes and ponds. Large bold letters designate major geographical regions of the archipelago: L = lowlands; P = plateau; M = mountains (Sutherland-Brown, 1968). See Fig. 2 for lake names and sample sizes.

northeast corner of Graham Island. Naked stickleback (all plates missing) occurred in 27 lakes from 10 different primary watersheds, and in three of these lakes (Spraint, Slim, Naked) all fish captured were naked.

Multiple regression and AIC analyses showed that the best model to explain lateral plate number variation included spectra, volume, and stream distance (Table 2). Using the complete lake database, plate number was significantly positively correlated with spectra (partial $r = 0.31$, $P < 0.01$) and volume (partial $r = 0.31$, $P < 0.01$), and negatively correlated with stream distance (partial $r = -0.36$, $P < 0.001$; Table 3). In the subset database there were no significant correlations with calcium concentration, but there was a significant positive correlation with pH (partial $r = 0.41$, $P < 0.05$; Table 3). The direction of this correlation was consistent among all four water clarity–lake volume categories, but only approached significance within the large clear lakes ($P = 0.09$). There were no significant correlations with calcium within any of the four categories. When geographical distance between lakes was controlled for, differences in lateral plate number between lake pairs were still positively correlated with differences in water clarity (partial Mantel test, $r = 0.21$, $P < 0.001$) and stream length (partial Mantel test, $r = 0.19$, $P < 0.001$), but only approached significance with differences in lake volume (partial Mantel test, $r = 0.08$, $P = 0.083$).

All of the populations with more than seven plates occurred in clear water lakes. We examined this interaction using a categorical measure of water spectra and observed a statistically significant interaction (ANCOVA: clarity \times volume interaction term, $F = 8.94$, d.f. = 1, $P = 0.004$) in which lateral plate number increased significantly with lake volume in stained water ($R^2 = 0.27$, d.f. = 1, $P < 0.001$) but not in clear water ($R^2 = 0.02$, d.f. = 1, $P = 0.37$; Fig. 3c).

Lateral plate number differed among the three predation regimes. Rainbow trout lakes had the highest average plate counts (mean = 11.3), followed by cutthroat trout/bird lakes (mean = 5.9) and invertebrate/bird lakes (mean = 2.2) (ANCOVA, $F = 44.21$, d.f. = 2, $P < 0.001$). All three means were significantly different from the other two (LSD *post-hoc* tests; all $P < 0.001$, Fig 4c). The relationship between plate number and lake volume was consistent among the predation categories (ANCOVA: predation \times volume interaction, $F = 0.72$, d.f. = 2, $P = 0.49$), and when this interaction term was removed, volume still had only a marginally significant positive effect on plate counts (ANCOVA, $F = 3.86$, d.f. = 1, $P = 0.05$), largely consistent with the regression analyses including the interaction.

Dorsal and pelvic spines

The majority (91/102) of stickleback populations on the archipelago had the usual complement of three dorsal spines, two pelvic spines, and an anal spine, while 11 populations exhibited departures from the standard complement: a fourth dorsal spine was detected at low frequency (2%) in a single population (Eden), while 10 populations exhibited a reduction in the number of spines, including the first dorsal ($n = 6$), the second dorsal ($n = 5$), the third dorsal ($n = 2$), the left pelvic ($n = 4$), the right pelvic ($n = 6$), and anal spine ($n = 2$). All of the spine-deficient populations occurred in the north-east lowlands. The three populations that contained the highest frequencies of loss (Serendipity, 100% lacking pelvic girdle; Boulton, 80% lacking second dorsal, 70% lacking pelvis; Rouge, 85% lacking third dorsal) were each in different primary watersheds.

Pelvic spine length averaged 7% of adult body length overall but ranged from 5% to 17% among populations. Populations with the largest body sizes had relatively longer spines than smaller-bodied forms, and spine length was also positively correlated with lateral plate

number ($R^2 = 0.48$, $P < 0.001$). We therefore calculated a body size-corrected and plate number-corrected pelvic spine length variable and tested for associations with biophysical variables. Consistent with the multiple regression models, AIC values confirmed that the best model to explain pelvic spine length variation included volume, stream length, and spectra (Table 2). Using the complete lake database, spine length was significantly positively correlated with volume (partial $r = 0.32$, $P < 0.01$) and stream distance (partial $r = 0.28$, $P < 0.01$; Table 3). In the subset database there were no significant correlations of spine length with calcium concentration or pH. In addition, there were no significant correlations with pH or calcium within any of the four water clarity–lake volume categories. When geographical distance between lakes was controlled for, differences in pelvic spine length between lake pairs only approached significant correlations with differences in lake volume (partial Mantel test, $r = 0.06$, $P = 0.09$) and were no longer significantly correlated with differences in stream outlet length (partial Mantel test, $r = 0.02$, $P = 0.29$).

Pelvic spine length increased with lake volume in a similar way in both stained and clear lakes (ANCOVA: volume \times water clarity interaction, $F = 0.05$, d.f. = 1, $P = 0.82$). When this interaction was removed, lake volume still had a significant effect on spine length ($F = 10.11$, d.f. = 1, $P = 0.002$) but there was no effect of water clarity ($F = 0.82$, d.f. = 1, $P = 0.37$). We also examined the relationship between pelvic spine length and predation categories. There was no significant difference in the relationship between spine length and lake volume among the three predation categories (ANCOVA: volume \times predation interaction term, $F = 0.89$, d.f. = 2, $P = 0.41$). When this interaction was removed from the ANCOVA, the effect of predator category on spine length only approached significance ($F = 2.40$, d.f. = 2, $P = 0.096$) while lake volume still had a significant effect ($F = 5.80$, d.f. = 1, $P < 0.018$).

We examined the extent of overlap between the basal support of the dorsal spines and the upper region of the lateral plates, as this influences the stability of the dorsal spine against lateral deflection (see Fig. 1). The amount of overlap was significantly positively correlated with spine length (partial $r = 0.32$) and number of lateral plates (partial $r = 0.62$) ($F = 97.4$, $P < 0.001$).

Cross-sectional diameter

We determined maximum cross-sectional diameter on all fish, as this influences the swallowing success of gape-limited piscivores. Absolute cross-sectional diameter increased with adult body size ($r = 0.72$, $F = 102$, $P < 0.001$) and ranged from 16 to 42 mm among populations, or approximately 21–51% of the standard length of the stickleback. Multiple regression models and AIC values confirmed that the best model to explain cross-sectional diameter variation (body size corrected) included volume, spectra, and stream distance (Table 2). Using the complete lake database, cross-sectional diameter was significantly positively correlated with volume (partial $r = 0.43$, $P < 0.001$), spectra (partial $r = 0.27$, $P < 0.01$), and stream distance (partial $r = 0.28$, $P < 0.01$; Table 3). In the subset database there were no significant correlations between cross-sectional diameter and calcium concentration or pH. However, there was a significant positive correlation with pH in both small and large stained lake categories (both $P < 0.025$), but none in the clear lakes and there were no significant correlations with calcium within any of the four water clarity–lake volume categories. When geographical distance between lakes was controlled for, differences in cross-sectional diameter between lake pairs were still positively correlated with differences in lake volume (partial Mantel test, $r = 0.24$, $P < 0.001$) and water clarity (partial Mantel

test, $r = 0.13$, $P = 0.002$), but were no longer significantly correlated with stream length (partial Mantel test, $r = -0.06$, $P = 0.95$).

Cross-sectional diameter increased with lake volume in a similar way in both stained and clear lakes (ANCOVA: volume \times water clarity interaction, $F = 0.08$, d.f. = 1, $P = 0.78$) (Fig. 3d). When this interaction was removed, lake volume was still significantly positively correlated with cross-sectional diameter (ANCOVA, $F = 21.22$, d.f. = 1, $P < 0.001$) and significantly larger diameters were found in clear lakes (ANCOVA, $F = 6.43$, d.f. = 1, $P = 0.013$). Predation regime affected the correlation between cross-sectional diameter and lake volume (ANCOVA: predation \times volume interaction, $F = 5.23$, d.f. = 2, $P = 0.007$). There was a significant increase in cross-sectional diameter with lake volume in both rainbow trout ($R^2 = 0.18$, d.f. = 1, $P < 0.001$) and cutthroat trout lakes ($R^2 = 0.36$, d.f. = 1, $P = 0.03$), and a slight but non-significant decrease in invertebrate lakes ($R^2 = 0.03$, d.f. = 1, $P = 0.28$) (Fig. 4d). When we restricted this analysis to the smaller lakes that had all three predation regimes (log lake volume < 7), the interaction between lake volume and predation was no longer present (ANCOVA: predation \times volume interaction, $F = 2.38$, d.f. = 2, $P = 0.10$). In these smaller lakes, cross-sectional diameter was significantly affected by predation regime (ANCOVA, $F = 11.71$, d.f. = 2, $P < 0.001$), and was significantly smaller in invertebrate/bird lakes than in either cutthroat/bird lakes or rainbow trout lakes (LSD: both $P < 0.001$) but was not different between the latter two categories (LSD: $P = 0.44$).

DISCUSSION

Our study comprised a comprehensive survey of the majority of intact lakes and ponds on Haida Gwaii, western Canada, one of the most remote archipelagos in the northern hemisphere. Over a relatively small geographical region, these populations contain remarkable morphological diversity in body size and defence traits. Lake size and aquatic spectra are each major predictors of predation regime and of morphological variability in the fish. Across a broad range of lake volumes, adult body size tends to be larger in stained lakes than in clear lakes, particularly so in the largest lakes, while defence armour is greatest in clear lakes and least in small stained lakes (Fig. 6). Most of the trends concerning stickleback morphology and biophysical characteristics of the lakes persist when controlling for geographic distance between population pairs (although sometimes the trends were weakened). In addition, univariate associations of morphological divergence between population pairs and geographic distance were generally weak and non-significant (i.e. all $P > 0.05$), indicative of a general lack of strict isolation-by-distance. Thus, habitat characteristics appear the strongest predictor of morphological variation in this radiation. Potentially up to one-third of the morphological differences in the stickleback between these large lakes and small ponds can develop within decades after initial colonization, some of which may reflect phenotypic plasticity (Leaver and Reimchen, 2012; see also Frommen *et al.*, 2011, for studies of Netherland stickleback; see Bell and Aguirre, 2013, for synthesis), but much of the variability in defences among populations has a large genetic component (Chan *et al.*, 2010). These trends are replicated among separate watersheds where both geological and genetic evidence is most consistent with populations being independently derived from marine colonists in the majority of primary watersheds (Moodie and Reimchen, 1976; Deagle *et al.*, 2012, 2013).

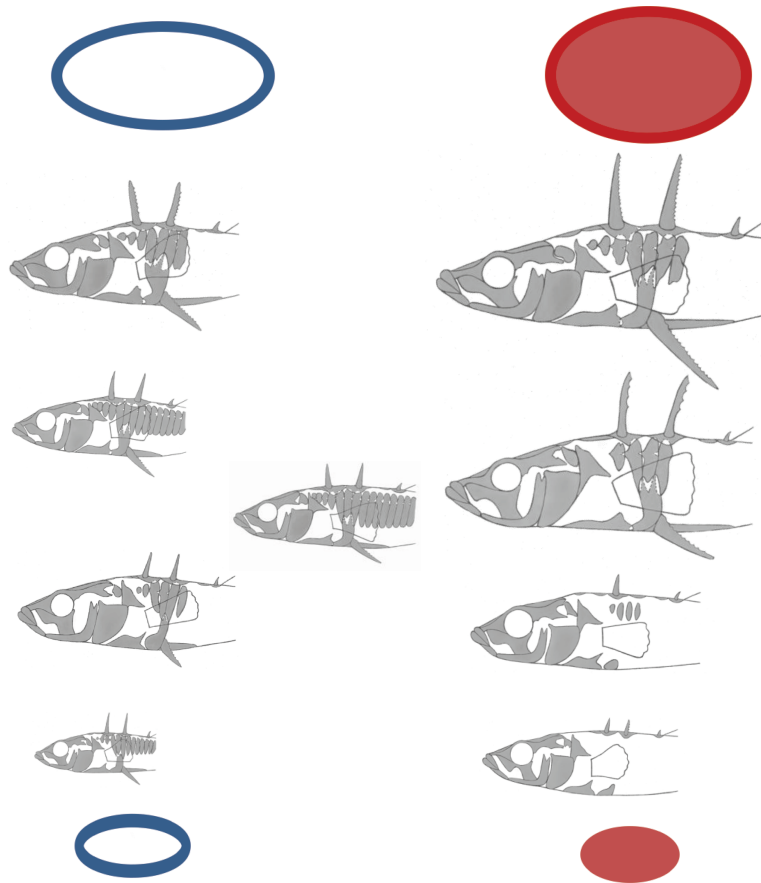


Fig. 6. Representative phenotypes from lakes in Haida Gwaii, western Canada. (Centre) Estuarine phenotype. (Left column) Top to bottom: Eden Lake, Gowgaia Lake, Sundew Lake, Darwin Lake. (Right Column) Top to bottom: Mayer Lake, Drizzle Lake, Boulton Lake, Serendipity Lake. (Ellipses) Left: Large and small clear water (oligotrophic) lakes. Right: Large and small stained (dystrophic) lakes.

Body size variability

Over their circumboreal distribution, including the mainland archipelago adjacent to Haida Gwaii, adult body lengths (SL) of male threespine stickleback typically vary from 40 to 70 mm among populations with occasional instances of individuals as small as 28 mm and as large as 100 mm, both occurring at higher latitudes (Moodie and Reimchen, 1976; Wootton, 1976; Baker, 1994; Reimchen and Nosil, 2006; Maccoll *et al.*, 2013; Ravinet *et al.*, 2013). Such intraspecific geographical variability in adult body size in fishes can reflect phenotypic plasticity from differences in temperature and aquatic productivity (Brett and Groves, 1979; Mori and Takamura, 2004; Shoup *et al.*, 2007; for a review, see Blanckenhorn, 2000). We suspect this is of limited application for variability in Haida Gwaii stickleback as both small- and large-bodied stickleback populations tend to occur in cold lakes of low conductivity and low productivity that dominate the archipelago (Douglas and Reimchen, 1988). Our data on adult body size (29–100 mm SL) span much of the range of

body sizes seen throughout the taxon, yet encompass only about 5% of the latitudinal range of the species. We identified 11 populations among seven primary watersheds in which average adult male size exceeded 70 mm SL. Such large sizes, some identified in preliminary surveys (Moodie and Reimchen, 1976), appear to have few counterparts elsewhere in North America or Europe and include combinations of both increased growth rate and extended longevity (Gambling and Reimchen, 2012). Recent SNP (single nucleotide polymorphism) analyses of three of these ‘giant-bodied’ populations in separate primary watersheds indicate that each of the giants represents parallel and independent differentiation from marine ancestral populations (Deagle *et al.*, 2012).

We found that differences in adult body size of male stickleback were best predicted by three factors: outlet stream length (+), lake volume (+), and water transparency (–). Stream length contributes to the model because of the presence of unusually large-bodied stickleback in central regions of the lowlands. Population differentiation may be facilitated in the headwater populations by reduced gene flow with marine or freshwater populations (Deagle *et al.*, 2013). Lake volume was also an important predictor of increased body size and might be a hydrodynamic adaptation for increased limnetic foraging niches (Moodie, 1972b; Walker, 1997; Spoljaric and Reimchen, 2007). There remains major variability in adult size independent of outlet stream length and lake volume, a significant proportion of which is associated with aquatic spectra, with larger adults occurring in dystrophic conditions rather than clear oligotrophic waters.

Some of the distinguishing attributes of dystrophic lakes are the high levels of tannins and humic acids, which greatly limit light penetration. Such conditions develop where lake levels are maintained by input from ground water that has high concentrations of dissolved organics from long residence time in soils (Carpenter and Pace, 1997). These dissolved substances result in rapid light attenuation producing a steep gradient in downwelling light with ‘nocturnal or aphotic’ conditions occurring ~2 m from the surface (Reimchen, 1989). This alters the spatial geometry of predator–prey interactions relative to those in clear water habitats where a stickleback in the water column can be detected at large distances and accordingly can be large distances from benthic shelter. In stained waters typical for Haida Gwaii, the maximum distance a stickleback swimming at the surface will be from shelter is ~2 m, a distance that converges towards 0 m for a stickleback swimming near 2 m depth. Because burst velocity in fishes, including stickleback, is directly related to body size (Webb, 1982; Law and Blake, 1996; Hendry *et al.*, 2011), we suggest that the large body sizes of stickleback observed in most stained lakes relative to clear water lakes of equivalent size comprises a defence adaptation. Pursuit failures of salmonids and avian piscivores are common in stickleback populations from Haida Gwaii (Moodie, 1972a; Reimchen, 1988, 1991a, 1991b) and such predator failures provide a conceptually broad mechanism for the evolution of larger body size in multiple taxa (for a review, see Vermeij, 1982).

In addition to larger body size in stickleback enhancing pursuit failures, it also enhances manipulation failures after capture by gape-limited predators (Reimchen, 1991a). These failures are largely due to the cross-sectional diameter of the fish that result from erect dorsal and pelvic spines during predator handling. This triangle of erect spines pierces the mouthparts of predators (Hoogland *et al.*, 1957) and increases rejection frequencies by predators as it approaches the maximum prey size predators are capable of swallowing (Reimchen, 1991a). Across the Haida Gwaii archipelago, cross-sectional diameter of the stickleback increases as lakes become clearer and larger, consistent with the prediction that it would enhance post-capture escape, since capture is more likely under these conditions.

Defence morphology variability

Colonization of freshwater habitats in the northern hemisphere by completely plated marine stickleback and widespread reduction of lateral plates comprise one of the most widely documented examples of parallel evolutionary change in defence morphology (Wootton, 1984; Bell and Foster, 1994; Vamosi, 2005; for a review, see Barrett, 2010). Among the populations in our study, we observed five that retained the full expression of lateral plates and 93 that exhibited major loss. The completely plated condition in marine stickleback is highly conserved and extends back to the Miocene (Bell, 1977); consequently, the occasional persistence of the ancestral plate condition in freshwaters may simply represent recent marine colonization or hybridization (Miller and Hubbs, 1969). This may account for two of the populations in our study where complete morphs predominate. Gowgaia Lake (East and West) and Puffin Lake, both on the exposed west coast of Moresby Island, are within several metres of sea level, have seasonal influx of marine waters (Reimchen, 1994) and, for Gowgaia Lake, have stickleback with a body shape and sexual dimorphism similar to those of marine stickleback (Spoljaric and Reimchen, 2007, 2008). The three remaining lakes with complete morphs do not have marine incursion yet the stickleback also appear very similar in shape to marine forms (Spoljaric and Reimchen, 2007). The major plate morphs are controlled at the *Eda* locus, with the allele for the completely plated morph dominating marine populations and the low-plated allele dominating freshwaters (Colosimo *et al.*, 2005), although data from Icelandic stickleback suggest additional complexity to this result (Lucek *et al.*, 2012). As such, the abundance of the complete morph in these three remote lake populations may reflect founder effects such as the lack of the low-plated allele in colonists or possibly subsequent loss of the allele. Recent genetic data for two of these populations (Deagle *et al.*, 2013), however, show both alleles present. Yet, there are distinctive features of these three lakes that suggest ecological functionality to the completely plated condition. Each is in mountainous terrain, has a steep shore profile largely lacking a littoral zone and aquatic macrophytes, each is deep and oligotrophic with high water clarity, and each has rainbow trout as the dominant predatory fish. Such a habitat is structurally most similar to marine waters where the completely plated condition predominates. This extensive armour in marine populations is generally recognized as a post-capture adaptation to puncturing predators that are prevalent in open water marine habitats (Reimchen, 2000), and would account for the persistence of the completely plated populations that we observed in Haida Gwaii mountain lakes. This hypothesis of increased post-capture adaptations in clear water habitats has received experimental support (Leinonen *et al.*, 2012) and may account for recent shifts in plate morph frequencies following increases in water clarity of a large freshwater lake in Washington (Kitano *et al.*, 2008).

The rich historical studies on the geographical distribution of lateral plate morphs (for a review, see Wootton, 1984) suggest that the widespread reduction of posterior plates in freshwater stickleback populations has complex mechanisms that are yet to be adequately resolved (Hagen and Gilbertson, 1972; Gross, 1977, 1978; Barrett *et al.*, 2008; Lucek *et al.*, 2012). Experimental colonizations of marine stickleback into vacant freshwater habitats have shown rapid genetic-based declines in the frequencies of the completely plated morph within several decades (Bell *et al.*, 2004; Colosimo *et al.*, 2005; Le Rouzic *et al.*, 2011). Low conductivity due to a deficiency of calcium and other ions in freshwaters is thought to constrain skeletal development in stickleback and facilitate selection for armour reduction (Heuts, 1947; Giles, 1983; Bell *et al.*, 1993; Bourgeois *et al.*, 1994). Experimental evidence demonstrates increased growth rate of low-plated relative

to complete morphs in low ion concentrations, which would accentuate competitive interactions and potentially susceptibility to predation and provide a general selective mechanism for the widespread transition from full-plated to low-plated conditions (Marchinko and Schluter, 2007; Barrett *et al.*, 2009a, 2009b). Yet geographically broad sampling of stickleback in southern British Columbia yields no relationship between lake conductivity and bony armour expression (Hagen and Gilbertson, 1972), suggestive of a limited role of ion differences in lateral plate expression at this scale in these natural populations. Our limnological data for Haida Gwaii lakes shows that most have calcium concentrations well below the suggested minimal threshold of $12 \text{ mg} \cdot \text{L}^{-1}$ required for normal bone development (Bell *et al.*, 1993), yet our populations below this threshold range from robust defences to a total absence of defences. When we examined multiple biophysical variables including lake volume, water spectra, calcium, and pH, we found no unique (partial r) statistical contribution of calcium concentrations to variability in number of lateral plates or any defence traits and conclude that ion deficiency is not a proximal mechanism for the broad reduction in posterior lateral plates in this archipelago.

Loss of the major posterior lateral plates reduces the mass of stickleback. Myhre and Klepaker (2009) show that stickleback swim bladder volume is larger in freshwater than in marine habitats, is larger in completely plated than in low-plated morphs within freshwaters, and that loss of posterior plates (9–35) largely offsets the reduced buoyancy that occurs in the transition to the low densities of fresh water. This novel hypothesis appears globally robust and assumes that there has been on average a reduction or loss of functionality of posterior plates in freshwaters. If correct, the occasional persistence of the complete morphs in lakes would be predicted in habitats with elevated salinity, as buoyancy would not be compromised. This might account for the unusual prevalence of the completely plated morphs in freshwaters in eastern North America where most populations occur in high conductivity habitats (Hagen and Moodie, 1982). Our data are limited for evaluation of the buoyancy hypothesis, as conductivity is exceptionally low in the majority of freshwater localities but we observed no correlation between conductivity and lateral plate numbers ($r = 0.14$, $P = 0.15$, $N = 89$). Furthermore, there are about 10 low-plated populations that have higher PC defence scores, comprising a more robust pelvis and larger spines, than the maximum observed among the five populations that were completely plated and marine-like in appearance, suggesting that loss of posterior plates allows greater allocation to anterior defences. This is contrary to the predictions of the buoyancy hypothesis.

Loss of posterior plates can modify swimming performance. Combining hydrodynamic theory (Webb, 1982), burst acceleration comparisons between marine and freshwater stickleback (Taylor and McPhail, 1985; Law and Blake, 1996), and experiments on trout foraging failures, Reimchen (1992, 2000) hypothesized that the transition from marine to freshwater life histories should alter the spatial geometry of predator interactions and shift defences from post-capture to pursuit adaptations, such as loss of posterior armour owing to benefits of increased acceleration. This is supported by burst swimming speed experiments and body axis deflection of low-plated and completely plated stickleback (Bergstrom, 2002), as well as recent predation experiments that demonstrated low-plated morphs are favoured where shelter is available (Leinonen *et al.*, 2012). Our geographical survey from throughout the archipelago shows that a loss of posterior lateral plates and a reduction in the non-buttressing plates anterior to the dorsal spines occur in lakes where stickleback are in close proximity to shelter and where predatory fish and avian piscivores are prevalent. The larger adult body sizes of stickleback in stained lakes, relative to clear water lakes of similar

size, is consistent with the increased importance of acceleration as a selective mechanism for armour reduction.

For most of the localities in our study, lake size and spectra were predictors of predation regime and stickleback morphology. Consistent with trends reported in Reimchen (1994), we observed an average of 6.5 plates for 62 lakes for the fish/bird assemblage and an average of 2.2 plates for 40 lakes for the bird/invertebrate assemblage. For the latter, all of which occurred in stained habitats, there was a decrease in defence PC, including a reduction in number of plates, reduction in relative size of dorsal and pelvic spines, and reduction in cross-sectional diameter, with this decrease being accentuated on the small lakes where stickleback can have no lateral plates, no pelvic girdle, and tiny dorsal spines. We cannot adequately account for this reduction, particularly in smaller stained lakes and ponds where avian piscivores are found, as it is known that these predators exert a significant selective pressure favouring robust spines and buttressing plates as a post-capture adaptation (Reimchen, 1980, 1988, 1995). However, loss of armour may also be beneficial in stickleback during predator pursuit if it allows for improved burst swimming speed during evasive responses where shelter is accessible (Nelson, 1969; Bergstrom, 2002). Among avian piscivores, pursuit failures appear to be common and despite their importance for evaluating strength of selection, predator efficiencies are known only for a few taxa (Vermeij, 1982; Reimchen, 1994). We suspect that a considerable part of the reason that defence structures are lost, despite the presence of avian piscivores, is the abundance of odonates and coleopterans present in these ponds, which are known to consume small-bodied stickleback and are postulated to produce selection favouring loss of spines and armour (Reimchen, 1980; Reist, 1980). When we included predation regime (bird/invertebrate, cutthroat trout/bird, rainbow trout) as a fixed factor and volume as a covariate, predation regime (bird/invertebrate) was a major predictor of armour reduction. Several additional lines of evidence are consistent with the role of invertebrates in armour reduction, including the spatial covariation between spine phenotypes and several piscivores within lakes (Reimchen, 1980), seasonal and yearly shifts in spine phenotype frequencies with shifts in relative importance of grappling versus gape-limited predation (Reimchen and Nosil, 2002, 2004), and recent experimental evidence on differential predation using laboratory crosses (Marchinko, 2008). However, the uncommon but geographically widespread occurrence of major pelvic girdle reduction in multiple species within Gasterosteiformes suggests multiple processes facilitating pelvic reduction (see Klepaker *et al.*, 2013).

We suspect that our observations on predation regime in Haida Gwaii lakes are of more general relevance to evolutionary studies of morphological variation in fishes. Classification of habitats into high and low predation categories, based largely on the presence or absence of aquatic ectotherms, which is commonly employed in evolutionary studies of freshwater fish, has yielded insight into morphological, behavioural, and life-history differentiation (Hagen and Gilbertson, 1972; Moodie and Reimchen, 1976; Gross, 1978; Reznick and Endler, 1982; Magurran *et al.*, 1995; Langerhans *et al.*, 2004, 2007). Our studies of intact habitats on Haida Gwaii do not support such a dichotomous classification. Although the absence of predatory fish is often correlated with reduction of lateral plates (for a review, see Wootton, 1984), avian piscivores can be found to a lesser or greater extent in all freshwater habitats of stickleback in this archipelago and throughout the circumboreal distribution of these fish (for a review, see Reimchen, 1994). Flocks of up to 80 Common Loon occur on dystrophic lakes of Haida Gwaii during brief periods in summer but have seldom been seen on oligotrophic lakes in the same region (Reimchen and Douglas, 1980), while other taxa such as grebes are usually uncommon or absent in summer but occur in

autumn and winter (Reimchen and Douglas, 1984). Small dystrophic ponds such as Serendipity and Rouge, which lack predatory fish, can have a summer nesting pair of Red-throated Loon and one or two Belted Kingfisher. As stickleback can be the only fish species in these lakes, they incur substantial mortality from these avian piscivores (Reimchen, 1980; Reimchen and Douglas, 1984). Although often absent or overlooked during field surveys, the signatures of avian piscivores are evident in the behavioural responses of stickleback to overhead threats (Giles and Huntingford, 1984; Godin and Clark, 1997), by the aviscars on the trunk of stickleback that have escaped following capture (Reimchen, 1988), as well as by the parasites of stickleback, such as *Schistocephalus*, which use birds as definitive hosts (Wootton, 1976; Heins and Baker, 2008). This parasite is geographically widespread in Haida Gwaii (Reimchen, 1982; Bergstrom and Reimchen, 2005) and throughout much of the distribution of stickleback (for a review, see Wootton, 1984; Poulin *et al.*, 2011). These endothermic piscivores have exceptionally high daily caloric requirements relative to ectotherms (Nilsson and Nilsson, 1976) and despite their often limited visibility and abundance, may constitute an important source of mortality and selective mechanism on many fish populations that are currently characterized as low-predation landscapes based on the lack of ectothermic piscivores. A general conclusion, then, which likely stems beyond our research, is that studies of predator–prey interactions can be informed by evidence more than simple presence versus absence of a dominant predator, such as the relative abundance of different predators as well as their efficiencies, which might vary across detection, pursuit, capture, and post-capture phases.

CONCLUSIONS AND FUTURE DIRECTIONS

Our research provides an improved context to the general study of adaptive radiation where the numbers of such studies are still few (Schluter, 2000a, 2000b; Grant and Grant, 2002, 2008; Gavrillets and Losos, 2009; Losos, 2009) and there is a recent renaissance in the study of the genomic basis of adaptive radiation (Noor and Feder, 2006; Stinchcombe and Hoekstra, 2008; Nosil and Feder, 2012). The stickleback system has been at the forefront of such work (e.g. Peichel *et al.*, 2001; Colosimo *et al.*, 2005; Chan *et al.*, 2010; Hohenlohe *et al.*, 2010; for reviews, see Schluter *et al.*, 2010; Deagle *et al.*, 2012; Jones *et al.*, 2012a, 2012b). Thus, detailed morphological studies on adaptive radiation such as the one presented here combined with the genome-wide SNP analyses of these populations (Deagle *et al.*, 2013) are important contributions to the integration of ecological and genetic data in functional contexts. Among the 102 populations, we see no clear examples of a decoupling between form and habitat, yet the colonization history of up to 56 separate invasions by marine ancestors (Deagle *et al.*, 2013), probably representing small numbers of individuals in most cases, should facilitate such opportunities if this is an important process in evolutionary differentiation. We see little evidence for the assertion that ‘Some geographic variation is adaptive but much is a non-adaptive (non-functional) product of history’ (Gould, 1984). The cumulative evidence emphasizes the efficacy of form and function and devalues any significant role of stochastic processes to morphological differentiation among these undisturbed populations. Our studies and conclusions of the role of natural selection in the major diversification of stickleback on the Haida Gwaii archipelago are largely consistent with other long-term investigations of the adaptive radiation of Galapagos finches (Grant and Grant, 2002) and Caribbean anoles (Losos, 2009).

ACKNOWLEDGEMENTS

We are grateful for the extensive assistance of Sheila Douglas during field surveys to the majority of these ponds and lakes in the archipelago and are indebted to the late Professor Joseph Nelson who greatly facilitated the early phases of this research programme. We thank Debra Wertman for her detailed drawings of representative stickleback shown in Fig. 6. We also thank Ric Moodie, Michael Bell, Mark Spoljaric, Bruce Deagle, and Steve Leaver for discussion during the study, Helene Collin who provided valuable assistance with the Mantel tests, and Andrew Hendry and Katie Peichel for comments on an earlier draft of the manuscript. This research was supported by an NSERC operating grant to T.E.R. (NRC2354) as well as funding by a European Research Council (ERC) grant to P.N.

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