

Sex matters for defence and trophic traits of threespine stickleback

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ABSTRACT

Background: The extent and importance of sexual dimorphism in non-sexually selected traits has received considerable attention in studies of birds and reptiles but similar studies in fishes are limited.

Aim: Quantify the extent and direction of sexual dimorphism in both defence and trophic traits of adult threespine stickleback (*Gasterosteus aculeatus*).

Methods: We amassed data from research done on 103 ecologically pristine stream and lake localities on the Haida Gwaii archipelago, western Canada. Using these data, we tested many hypotheses of ecological correlation with variability in the amount of dimorphism. We used a nested ANOVA with principal components and individual size-corrected morphometric traits as the dependent variables, with sex (male, female), water clarity (stained, clear) and water body (stream, small lake, large lake) as fixed factors, and locality as a random factor.

Results: Adult body length (SL) is mainly female-biased but this is accentuated in large clear water lakes. Dorsal and anal fin ray numbers are male-biased, the dimorphism being greater in clear water than stained lakes. The number of bony lateral plates is male-biased in most habitats except for large clear water lakes, which are female-biased. Relative size of the pelvis (spine length, ventral plate length) is female-biased in most habitats. Relative head length, head depth, jaw length, and eye diameter are male-biased in most populations. Dual isotopic signatures of muscle tissues of adult male stickleback in 33 populations show a higher trophic level ($\delta^{15}\text{N}$) but no consistent differences in $\delta^{13}\text{C}$. In a single locality where multiple size classes were located, males show a steeper ontogenetic slope of $\delta^{15}\text{N}$ against SL than do females, resulting in their signatures being depleted relative to sub-adult females but enriched relative to adult females. Stickleback from two localities sampled over several decades exhibit substantial temporal variability in the extent of the isotopic sexual dimorphism.

Conclusions: Sexual dimorphism in stickleback is widespread in multiple morphometric traits not directly associated with sexual selection. Population differences in the extent and direction of dimorphism are related to niche separation across predator and trophic gradients within localities and reflect general diversifying selection within populations.

Keywords: adaptive variation, diversifying selection, *Gasterosteus*, Haida Gwaii, niche width, sexual dimorphism.

INTRODUCTION

There is considerable evidence for habitat or niche segregation between the sexes among a diversity of taxa (Selander, 1966; for a summary, see Wearmouth and Sims, 2008). Perhaps best studied are divergent feeding structures between the sexes, generally inferred to result from intersexual competition (Van Valen, 1965; Shine, 1991; Grant and Grant, 2003; Nebel and Thompson, 2011). As an extension from trophic differentiation, the sexes might also be expected to differ in the broader selective landscape, perhaps through different exposures to predators and refuge, either of which could lead to further dimorphism between the sexes. For example, sexual size dimorphism is common in many ungulates in which small females and young seek habitats with lowered predation risk with a resultant change in multiple ecological parameters (Wearmouth and Sims, 2008). *Anolis* lizards are exceptionally sexually dimorphic, which is associated with habitat segregation between the sexes (Butler *et al.*, 2007). Many of the highly conspicuous sexually dimorphic butterflies, also originating with sexual selection, may be greatly accentuated by niche differentiation between the sexes (Allen *et al.*, 2011).

Quantifying habitat or niche partitioning by the sexes is often challenging and partitioning is inferred indirectly from morphological traits differentiating the sexes. Over the last two decades, stable isotope signatures of tissues have become progressively more widely used as a direct quantification of niche space, for these signatures provide a time-integrated proxy of trophic level and spatial axes (Fry, 2006). Application of these new techniques to the assessment of sexual niche space remains limited and largely focused on avian taxa (e.g. Hobson, 1995; Mancini *et al.*, 2013).

Fishes, the most diverse of the vertebrates, also exhibit extensive sexual dimorphism, particularly in nuptial displays and body size. While sexual selection is considered the dominant process structuring this dimorphism (Kodric-Brown, 1990), habitat segregation might also be important as it is in other vertebrates (Wearmouth and Sims, 2008; Mucientes *et al.*, 2009). Threespine stickleback (*Gasterosteus aculeatus*), well studied for their morphological, behavioural, and genetic differentiation among populations (reviewed in Bell and Foster, 1994), also show sexual dimorphism that is variable among populations. Bright nuptial colours developing on males during the reproductive seasons (reviewed in Wootton, 1976) are reduced or absent in habitats where red-shifted aquatic spectra mask the nuptial signal (Reimchen, 1989; Boughman, 2001). In addition to these seasonally restricted nuptial displays, some skeletal traits also show evidence for sexual dimorphism that persist throughout the life history. For example, the numbers of bony lateral plates are slightly higher in males than in females but this varies geographically (Moodie and Reimchen, 1976; Reimchen *et al.*, 1985; McPhail, 1992; Fernandez *et al.*, 2000). Relative length of pelvic spines can be female-biased (Reimchen, 1980; Reimchen *et al.*, 1985; Kitano *et al.*, 2007; Aguirre *et al.*, 2008) or male-biased (Reimchen and Nosil, 2005). Head size and gape are occasionally female-biased (Olafsdottir *et al.*, 2007) but much more commonly male-biased (Aneer, 1973; McPhail, 1992; Caldecutt and Adams, 1998; Kristjánsson *et al.*, 2002; Reimchen and Nosil, 2005; Kitano *et al.*, 2007; Spoljaric and Reimchen, 2008; Aguirre and Akinpelu, 2010; Cooper *et al.*, 2011). Length of gill rakers is male-biased in Vancouver Island populations while number of gill rakers varies from male-biased to female-biased (Bolnick and Lau, 2008). The adaptive significance of this sexual dimorphism is at least partially associated with niche differentiation between the sexes (Reimchen, 1980; Reimchen and Nosil, 2002; Bolnick and Lau, 2008; Cooper *et al.*, 2011), some of which is present in ancestral anadromous populations (Aguirre *et al.*, 2008; Spoljaric and Reimchen, 2008).

The high morphological diversity in threespine stickleback identified in 25 lakes and streams during initial sampling of Haida Gwaii, western Canada (Moodie and Reimchen, 1976)

facilitated an archipelago-wide survey that yielded about 100 insular populations. This allowed assessment of defence morphology (Reimchen, 1994; Reimchen *et al.*, 2013), fluctuating asymmetry (Bergstrom and Reimchen, 2003), genomics of lake–stream species pairs (Deagle *et al.*, 2012), and phylogeography of the populations throughout the archipelago (Deagle *et al.*, 2013). Analyses of geometric body shape between the sexes in a subset of these populations (Spoljaric and Reimchen, 2008) showed male biases in head size, gape, anal fin length, and posterior positioning of the dorsal spines and pelvic girdle, but also geographical variability with the greatest dimorphism in marine and large lake populations and minimal dimorphism in shallow stained ponds. Here, we investigate the extent of sexual dimorphism in freshwater populations from throughout the archipelago using multiple morphological structures associated with defence (size of pelvic girdle and pelvic spines, number and position of bony lateral plates), trophic niche (head size, jaw length, snout length, eye diameter, number of gill rakers, stable isotopes), and swimming (adult body size, number of dorsal and anal fin rays). These populations provide a useful focus for such a study of sexual dimorphism, as they occupy over a small geographical area an exceptional diversity of pristine habitats, including large oligotrophic clear mountain lakes through to small dystrophic bog ponds, and where the high morphological diversity of the stickleback can be functionally related to ecological conditions, mainly lake volume and aquatic spectra, which predict associated differences in predation landscape (Reimchen, 1994; Reimchen *et al.*, 2013). Based on genome-wide single nucleotide markers, these populations represent multiple independent colonization events from marine ancestry (Deagle *et al.*, 2013). We consider the following questions and predictions:

1. Which traits are sexually dimorphic? Based on previous studies of stickleback, we predict dimorphisms in adult body size (female bias), relative head size (male bias), number of lateral plates (male bias), relative size of the pelvic spines (female bias), and number of dorsal and anal fin rays (male bias).
2. Is there geographical variability in the dimorphism, and if so, is it associated with habitat? We predict that the extent of sexual dimorphism will be greater in localities where stickleback is the only fish species present or in large lakes where there is greater spatial separation of littoral and limnetic habitats (Nosil and Reimchen, 2005; Spoljaric and Reimchen, 2008).
3. Using a subset of the populations, are there stable isotope differences in the muscle tissue between the sexes, and if so, are these correlated with trophic morphology? Based on the larger gape and littoral habitat of adult males, we would predict they would have higher $\delta^{15}\text{N}$ signatures than females.

METHODS

Study area

Samples of stickleback used in this study were collected by one of the authors (T.E.R.) during multiple geographical surveys of the Haida Gwaii archipelago between 1976 and 2010. Collecting methods (0.5 cm mesh minnow traps), general habitat description (stream, pond, lake), physical attributes (lake volume, water clarity, calcium levels), and predation regime (fish, birds, macroinvertebrates) are summarized elsewhere (Reimchen, 1994; Reimchen *et al.*, 2013). Samples were preserved in 10% formalin and later transferred to 95% alcohol.

Morphological traits

Dimorphism in defence structures was computed from a previously assembled morphometric database (see summary for males in Reimchen *et al.*, 2013). Sample size averaged 35 individuals, usually with similar proportions of males and females, and we excluded all samples where $N < 7$ for either sex. For adult standard length (SL), number and position of lateral plates and pelvic girdle components, all individuals were measured for 98 populations. For meristic traits, including gill raker number as well as dorsal fin rays (DRAY) and anal fin rays (ARAY), we had data for 70 populations and scored between 7 and 10 individuals for each sex (dependent on availability). Several populations contained fish with small keel plates on the caudal peduncle but we did not include these in our analyses of total number of lateral plates or plate asymmetry.

Among low-plated fish, which comprised the majority of populations, there is variability in the body positions that the plates occupy independent of the total number of plates. For example, stickleback with a total of three lateral plates typically show these at positions 5, 6, and 7 beneath the first and second dorsal spines but occasionally these three plates are shifted anteriorly to positions 4, 5, and 6 (see Reimchen, 1983) (Fig. 1). As plate positions influence stability of the spines, we tested whether there were any differences between the sexes in the two most common sequences for each plate count. This was initially done on the pooled data (all populations) and then on a single population where large samples were available.

We compared the sexes for the extent of bilateral asymmetry (right side minus left side) in number of lateral plates (LPNASY), in position of lateral plates (LPPASY), and in number of forks on the ascending process of the pelvis (APFASY). For these traits, we used a minimum sample size of 10 for each sex. Methods are described in Bergstrom and Reimchen (2000).

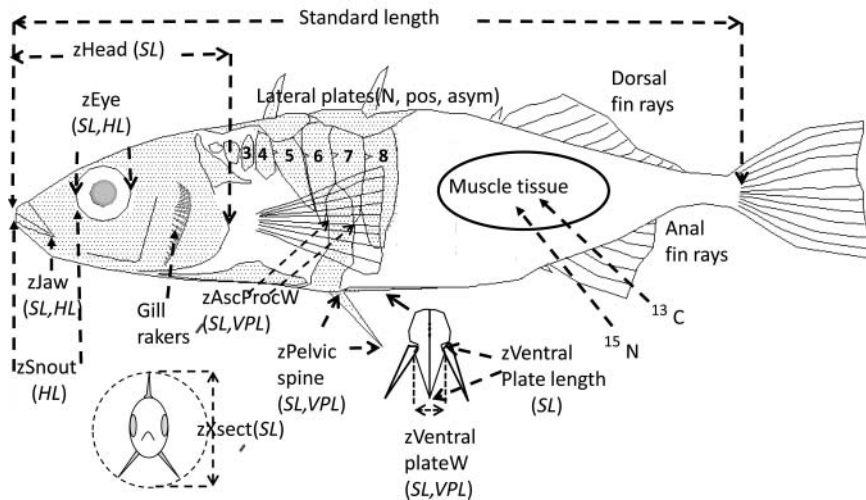


Fig. 1. Morphometric traits used to assess sexual dimorphism in freshwater populations of threespine stickleback from Haida Gwaii, British Columbia. Data for metric traits are based on residuals (z) from either standard length (SL) or head length (HL).

On 2341 digital images of these fish from 75 populations photographed during a previous study on body shape (Spoljaric and Reimchen, 2008), we measured jaw length, eye diameter, head length (distance from tip of jaw to posterior edge of operculum), and profile of head (depth of the head at the posterior edge of the eye and depth of the head at the posterior edge of the operculum) (see Fig. 1).

Stable isotope signatures

For inter-population analyses of stable isotopes, we used adult stickleback from 33 localities (22 lakes, 11 streams) chosen to encompass a geographically broad region of the archipelago and the full range of morphological diversity in adult body size (30–100 mm SL) and defence apparatus (unarmoured to full plate expression and robust spines). We processed 20 individuals from each locality (10 for each sex when available) for a total of 622 individuals. We were not able to use the same individuals as those from the morphometric analyses.

To test for temporal repeatability of isotopic signatures between the sexes, we examined a longer time series from two localities, Drizzle Lake, a 114 ha deep dystrophic lake with giant black stickleback and Boulton Lake, a 14 ha shallow lake with unarmoured stickleback. Samples processed for isotopic analyses were as follows: Drizzle Lake (October 1981, $N = 114$; September 1982, $N = 206$; May 1983, $N = 11$; June 2011, $N = 154$; June 2012, $N = 200$) and Boulton Lake (May 1978, $N = 41$; October 1978, $N = 41$; March 1981, $N = 43$; August 1981, $N = 35$; September 1983, $N = 34$; June 2010, $N = 24$; June 2012, $N = 129$; June 2013, $N = 153$).

From each fish, a strip of trunk musculature was extracted, dried (8–12 days at 60°C), powdered and packaged (1.0 mg) for isotope ratio mass spectrometry analysis at the Stable Isotope Facility at the University of California at Davis. Standard notations of delta values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) relative to Pee Dee Belemnite for carbon and atmospheric nitrogen are used. As the tissues had been in alcohol for two decades, we did not perform any lipid extraction.

Statistical analyses

We computed both univariate and multivariate indices of morphology to assess the extent of sexual dimorphism. For adult SL, we used the means of the top ten for each sex. For head length, pelvic spine length, ventral plate length, ventral plate width, and ascending process width, we extracted residuals from the bivariate associations with SL separately for each population. For jaw length and eye diameter, we also extracted residuals relative to head length separately for each population. A principal component analysis (PCA) for defence traits (PC1def) was performed using SL, number of left lateral plates (LLP), and residuals (against SL or HL) of pelvic spine length (zPSLSL), ventral plate length (zVPLSL), ventral plate width (zVPWSL), ascending process width (zAPWSL), and cross-sectional diameter (zXSECTSL). A PCA for trophic traits (PC1troph) was performed using SL, and residuals of head length (zHDSL), jaw length (zJAWHD), eye diameter (zEYEHD), head depth behind the eye (zHD1HD), and head depth at the posterior edge of the operculum (zHD2HD). The number of populations was lower for defence PC than for trophic PC (63 vs. 80), as some populations lacked parts of the pelvic girdle such that full measurements could not be obtained. We computed nested ANOVAs, using a

morphometric trait as the dependent variable, sex (male, female), clarity (stained, clear) and water body (stream, small lake, large lake) as fixed factors, and locality as a random factor. For all traits, we calculated a Mann-Whitney *U*-test on independent samples for males and females in each population. Mann-Whitney tests are conservative when there are ties in the data, making any significant results more robust. We also performed a discriminant analysis separately for each population using sex as the grouping variable and five univariate traits exhibiting sexual dimorphism as predictor variables to assess the extent of correct assignment of the sexes in the classification matrix. To evaluate the potential influence of other fish species on the sexual dimorphism, we classified localities in three competition categories (0 = no other fish species; 1 = one other species; and 2 = two or more other species), and for each trait performed a nested ANOVA with sex, competition (0, 1, 2), and locality as random factors. We also calculated a correlation coefficient of competition categories against all the individual traits. For both of these tests, we restricted our comparisons to small stained lakes, as these were the only localities that had each of the competition categories.

Graphical results were displayed using two methods. We categorized populations into six habitat categories (stained vs. clear spectra for creeks, small lakes, and large lakes) and plotted for each trait the aggregated means for each sex based on the means for each population in the habitat category. We also plotted the dimorphism for each trait of the residuals (male–female) for all populations ranked from most female-biased to most male-biased. For SL, we used percentage differences between the sexes for the 10 largest males and 10 largest females in each sample.

For isotopic data, residuals of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ against SL were calculated for each population and the sexes compared using paired *t*-tests and Mann-Whitney (MW). The dual isotope dimorphism for each population was calculated by measuring the Euclidean distance between males and females, where:

$$\text{Euclidean distance} = \text{sqrt} [(\delta^{13}\text{C male} - \delta^{13}\text{C female})^2 + (\delta^{15}\text{N male} - \delta^{15}\text{N female})^2].$$

RESULTS

Adult standard length (top 10)

In the majority of populations, adult SL was greater in females than in males (Fig. 2, inset), and of the 57 populations with statistically significant paired comparisons (MW: $P < 0.05$), 51 were female-biased and 6 male-biased. Nested ANOVA for SL and major habitat variables showed a highly significant effect for sex ($F_{1,1719} = 330.1$, $P < 0.001$), higher dimorphism in waters of higher clarity (sex*clarity: $F_{1,1719} = 18.1$, $P < 0.001$), and higher dimorphism in large lakes compared with small lakes and streams (sex*water body: $F_{1,1717} = 4.5$, $P < 0.02$) (Fig. 2). Differing from the overall trend, 13 populations had a male bias, six of which were statistically significant ($P < 0.05$) and these were all from streams and small lakes. These also exhibited a smaller adult body size (SL: $\bar{x} = 56.5$ mm, $N = 13$) relative to populations with the most female-biased dimorphism (SL: $\bar{x} = 70.0$ mm, $N = 13$) ($t_{24} = 3.9$, $P < 0.01$).

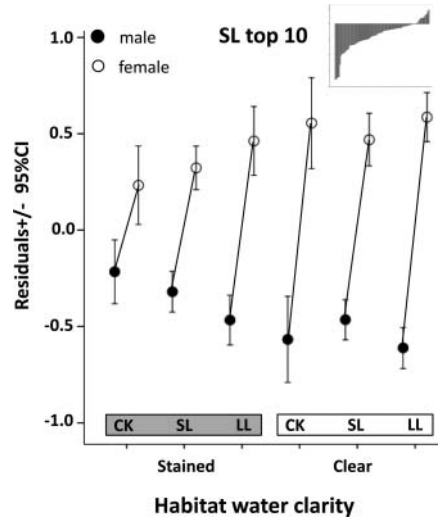


Fig. 2. Mean standard length of the 10 largest adult stickleback of each sex averaged for the six major habitats: creeks (CK), small lakes (SL), and large lakes (LL) for two spectral categories (stained, clear). Inset shows mean SL differences (M – F) for all localities. Above mid-horizontal line: male bias; below horizontal: female bias.

Number of fin rays

For both dorsal and anal rays, there was a marginal excess of populations with a male bias (Fig. 3A,B, insets), of which 18 populations showed significant (MW: $P < 0.05$) differences between the sexes, four with male-biased dorsal rays, 13 with male-biased anal rays, and a single population with female-biased anal rays. The extent of the male bias in fin rays occurred in the majority of habitat types apart from reduced differences in dorsal rays for stained streams (Fig. 3A,B). Nested ANOVA, which showed a strong effect for sex (dorsal rays: $F_{1,1329} = 7.2$, $P < 0.01$; anal rays: $F_{1,1329} = 43.3$, $P < 0.01$), indicated no consistent habitat correlates to population differences in the dimorphism (sex*clarity, sex*water body: both $P > 0.7$).

Principal component for defence

PC1def explained 30% of the variance in defence traits, with the highest positive loadings for residuals of ascending process width of the pelvis against SL (0.91) and ventral plate length of the pelvis against SL (0.88). Within-population comparisons (Fig. 4, inset) showed significant (MW: $P < 0.05$) female bias in 19 populations and male bias in eight populations. These population differences in sexual dimorphism are associated with ecological conditions. In stained waters PC1def did not differ between the sexes, whereas in clear waters PC1def was substantially female-biased in streams, small lakes, and large lakes (Fig. 4). Nested ANOVA showed a highly significant effect for sex ($F_{1,5168} = 25.3$, $P < 0.001$), for sex*clarity ($F_{1,5168} = 26.4$, $P < 0.001$), and a marginal effect for sex*clarity*water body ($F_{2,5168} = 4.3$, $P < 0.02$).

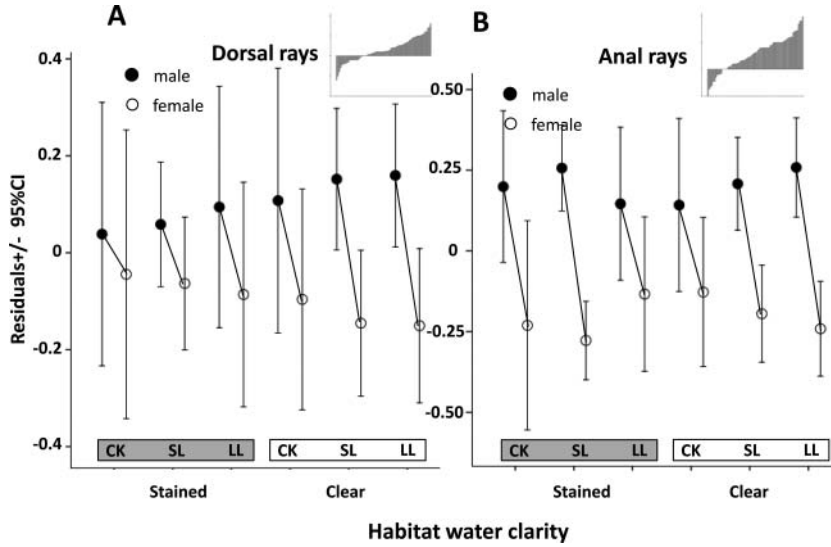


Fig. 3. Mean number of fin rays (A, dorsal rays; B, anal rays) in male and female stickleback for the six major habitats: creeks (CK), small lakes (SL), and large lakes (LL) for two spectral categories (stained, clear). Insets show mean ray differences (M – F) for all localities. Above mid-horizontal line: male bias; below horizontal: female bias.

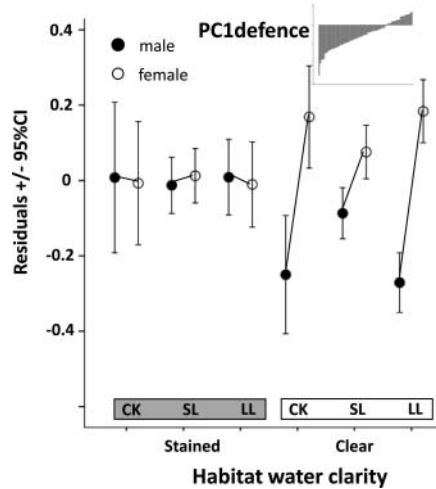


Fig. 4. First principal component (PC1) of defence structures in male and female stickleback for the six major habitats: creeks (CK), small lakes (SL), and large lakes (LL) for two spectral categories (stained, clear). Highest loading for width of ascending process and length of ventral plate. Inset shows PC1 differences (M – F) for all localities. Above mid-horizontal line: male bias; below horizontal: female bias.

Number of bony lateral plates

Of the 95 populations monomorphic for the low-plated morph, the majority were male-biased (Fig. 5, inset), and of the 16 populations with significant differences (MW: $P < 0.05$) between males and females, all were male-biased. Among the remaining populations that were polymorphic for plate morph, two localities showed a female excess (MW: $P < 0.01$) of the complete morph. Grouping of major habitats showed accentuated male bias in stained habitats and reduced differences in clear water habitats (sex: $F_{1,6159} = 38.5$, $P < 0.001$; sex*clarity: $F_{1,6156} = 13.9$, $P < 0.001$) (Fig. 5). There were no consistent differences in the dimorphism for water body (streams, small lakes, and large lakes: $P > 0.5$).

Position of bony lateral plates

There was sexual dimorphism in the frequencies of the lateral plate positions among fish with the same number of plates (Table 1). In analyses of the full data set (all individuals), among fish with a total of 2, 3, 5 or 6 plates, females exhibited a higher frequency of the more anterior sequences than did males. Fish with either 4 or 7 plates had very few individuals with a second sequence and differences between the sexes were not detected for these phenotypes. Among fish with a total of 8 lateral plates, the pattern was reversed, as the anterior sequence was significantly more common in males than in females.

However, these trends in the sexual dimorphism of sequence positions could be an artifact of combining all the population samples. Consequently, we also tested whether these trends were present in the Drizzle Lake population where total plate number ranged from 2 to 7 and where large samples were available. These data (Table 1) showed a similar trend for the anterior sequences being more prevalent in females than in males for fish having a total of 3 or 5 plates.

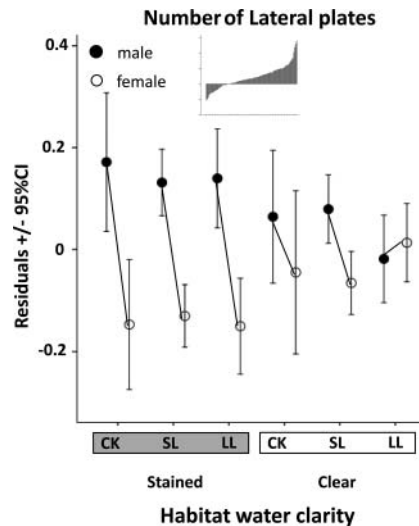


Fig. 5. Mean number of lateral plates in male and female stickleback for the six major habitats: creeks (CK), small lakes (SL), and large lakes (LL) for two spectral categories (stained, clear). Inset shows lateral plate differences ($M - F$) for all localities. Above mid-horizontal line: male bias; below horizontal: female bias.

Table 1. Anterior lateral plate positions between the sexes for the complete data set (all populations pooled) and for Drizzle Lake alone

Total no. of plates	Plate positions	All populations			Drizzle Lake		
		Males	Females	χ^2 [#]	Males	Females	χ^2
1	6	15	11	1.2			
	7	12	3				
2	56	17	28	6.5**	20	25	0.6
	67	32	18		34	35	
3	456	11	36	16.6***	38	64	7.0**
	567	141	112		251	235	
4	4567	241	296	0.5	1118	1166	0.4
	5678	13	12		106	99	
5	34567	389	513	3.6*	873	1063	17.5***
	45678	68	63		647	580	
6	234567	280	344	3.65*	133	128	1
	345678	300	296		688	579	
7	1234567	4	10	0.1	14	12	0.9
	2345678	461	476		116	95	
8	12345678	73	62	11.6***			
	23456789	20	49				

Note: Shown are the position phenotypes for the eight most common lateral plate counts. For plate positions, see Fig. 1. Columns for males and females show sample sizes. [#]Chi-squared test or Fisher's exact test when cells < 5. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Residuals of pelvic spine length on SL

For relative pelvic spine lengths, the number of populations with female bias exceeded those with male bias (Fig. 6A, inset) and of those with statistically significant paired comparisons (MW: $P < 0.05$), 10 were female-biased and five male-biased. The female-biased dimorphism was much greater in stained habitats and reduced or absent in clear water habitats (Fig. 6A). Nested ANOVA with locality as a random factor showed a significant effect for sex ($F_{1,5326} = 4.9$, $P < 0.03$) and for sex*clarity ($F_{2,5326} = 4.5$, $P < 0.05$); there was no effect for sex*water body ($F = 0.7$, $P > 0.4$).

Residuals of ventral plate length on SL

Relative lengths of the ventral plate of the pelvis were female biased in most populations (91%) (Fig. 6B, inset) and of 49 populations with significant differences (MW: $P < 0.05$), 46 were female-biased and three male-biased. Grouping for habitat showed accentuated dimorphism in clear water habitats and in creeks (sex: $F_{1,5252} = 299.0$, $P < 0.001$; sex*clarity: $F_{1,5252} = 7.8$, $P < 0.01$; sex*water body: $F_{2,5252} = 11.3$, $P < 0.001$; Fig. 6B).

Bilateral asymmetry of defence apparatus (right side – left side)

We compared the frequencies of fish with plate position asymmetries (LPPASY) between the sexes in populations that contained low or partial morphs. Among 57 populations with these data, 32 had a greater frequency of asymmetric females and 20 had a greater

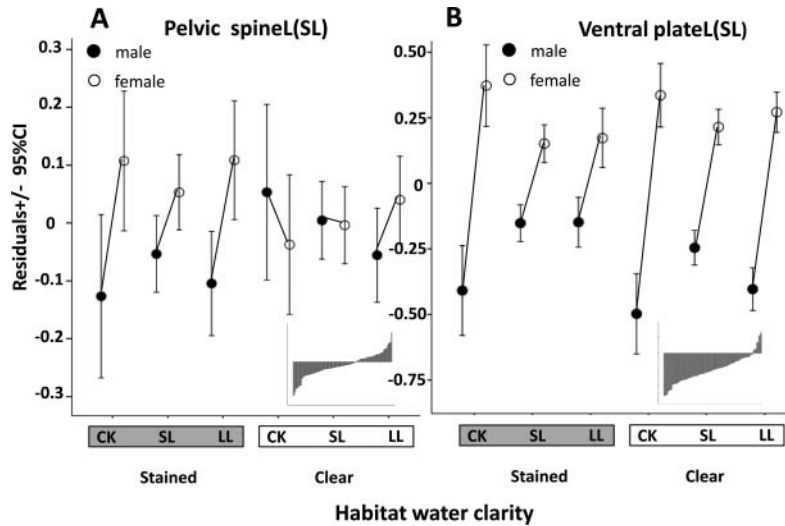


Fig. 6. Mean pelvic girdle size for male and female stickleback for the six major habitats: creeks (CK), small lakes (SL), and large lakes (LL) for two spectral categories (stained, clear). (A) Residuals of pelvic spine length against SL, (B) residuals of ventral plate length against SL (see Fig. 1). Insets show mean residual pelvic girdle differences (M – F) for all localities. Above mid-horizontal line: male bias; below horizontal: female bias.

frequency of asymmetric males but only one was statistically significant, and that with a female bias (MW: $P < 0.05$). When mean plate position asymmetry (LPPASYM) was compared between the sexes instead of frequency, results were similar. Stream populations exhibited significantly greater male left-side bias relative to females than in lake populations ($F = 11.4$, d.f. = 1, $P < 0.001$).

We also compared the frequencies of ascending process fork number asymmetries (APFASYF) between the sexes. Of the 48 populations that exhibited some difference between the sexes, 31 had more asymmetric males than females (a significant difference in one lake, MW: $P < 0.05$), and 17 had more asymmetric females than males ($\chi^2 = 4.08$, $P < 0.05$). Ascending process fork number is generally right-side-biased across the archipelago (Bergstrom and Reimchen, 2002). Females were slightly more right-side-biased than males overall among populations but the difference was non-significant (paired $t_{63} = 1.614$, $P = 0.112$).

Principal component for trophic traits

PC1troph accounted for 30% of the variance in head shape with the highest loadings for zHD1HL (0.83) and zHD2HL (0.68). Among the 69 populations, most were male-biased (Fig. 7, inset), of which the dimorphism was marginally accentuated in stained habitats (Fig. 7) (nested ANOVA: sex: $F_{1,1586} = 95.3$, $P < 0.001$; sex*clarity: $F_{1,1586} = 7.1$, $P < 0.01$).

Residuals of jaw length on SL and on head length

The majority of populations show a male-biased jaw length for both SL and head length (HL) comparisons (Fig. 8A,B insets). Within-population comparisons yielded significance

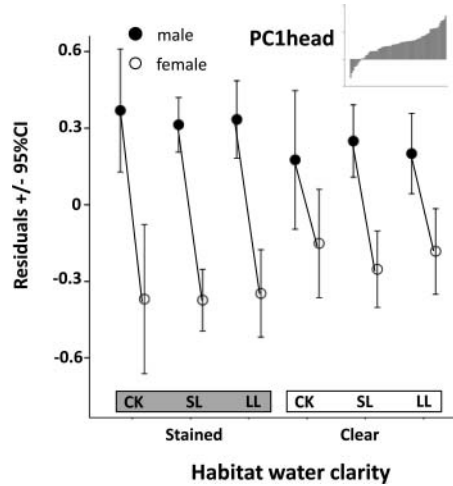


Fig. 7. First principal component (PC1) of head structures in male and female stickleback for the six major habitats: creeks (CK), small lakes (SL), and large lakes (LL) for two spectral categories (stained, clear). Highest loading on PC1 is residuals of head depths against head length. Inset shows mean residual PC1 differences (M – F) for all localities. Above mid-horizontal line: male bias; below horizontal: female bias.

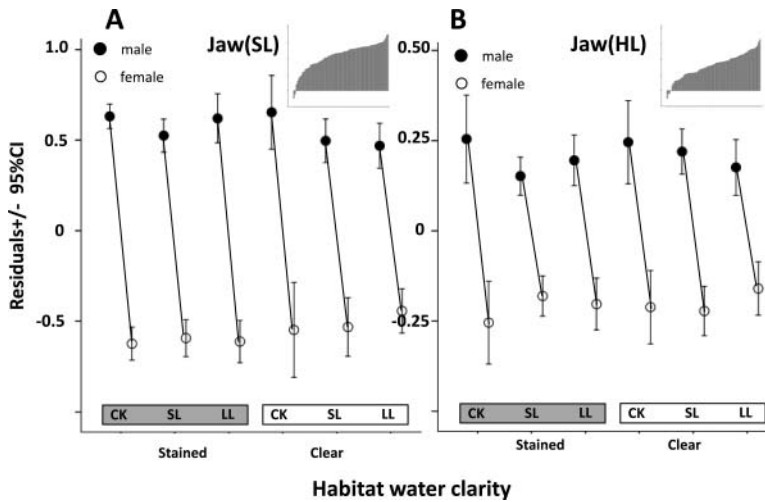


Fig. 8. Mean residual jaw lengths for male and female stickleback for the six major habitats: creeks (CK), small lakes (SL), and large lakes (LL) for two spectral categories (stained, clear). (A) Residuals of jaw length against SL, (B) residuals of jaw length against head length. Inset shows mean residual jaw differences (M – F) for all localities. Above mid-horizontal line: male bias; below horizontal: female bias.

(MW: $P < 0.05$) for 61 and 35 populations respectively, all with a male bias. For both SL and HL comparisons, the extent of bias was similar among the major habitats (sex: $F_{1,1723} = 670.2$, $P < 0.001$; sex*clarity: $F_{1,1723} = 2.2$, $P = 0.14$; sex*water body: $F_{2,1723} = 2.5$, $P = 0.08$) (Fig. 8A,B).

Residuals of eye diameter on SL and on HL

Eye diameter relative to SL was male-biased in most populations (Fig. 9A, inset), among which 32 within-population comparisons were significant (MW: $P < 0.05$), all with a male bias, the difference being greater in stained than in clear water habitats (nested ANOVA: sex: $F_{1,1723} = 240.1$, $P < 0.001$; sex*clarity: $F = 5.7$, $P < 0.02$; sex*water body: $F = 2.5$, $P = 0.08$) (Fig. 9A). Eye diameter relative to HL showed little consistent dimorphism (Fig. 9B, inset), although of the significant intra-population comparisons (MW: $P < 0.05$), five had a male bias and a single population a female bias. The male bias was marginally higher in stained habitats (nested ANOVA: sex: $F_{1,1723} = 8.8$, $P < 0.01$; sex*clarity: $F = 4.6$, $P < 0.04$; sex*water body: $F = 1.9$, $P = 0.16$).

Number of gill rakers

Gill rakers were male-biased in 39 of 66 populations ($t_{62} = 2.7$, $P = 0.007$) (no figure provided) but only three of these showed significant differences (MW: $P < 0.05$) between the sexes (all male bias). The extent and direction of bias did not differ statistically among stream and lake habitats (nested ANOVA: all $P > 0.05$).

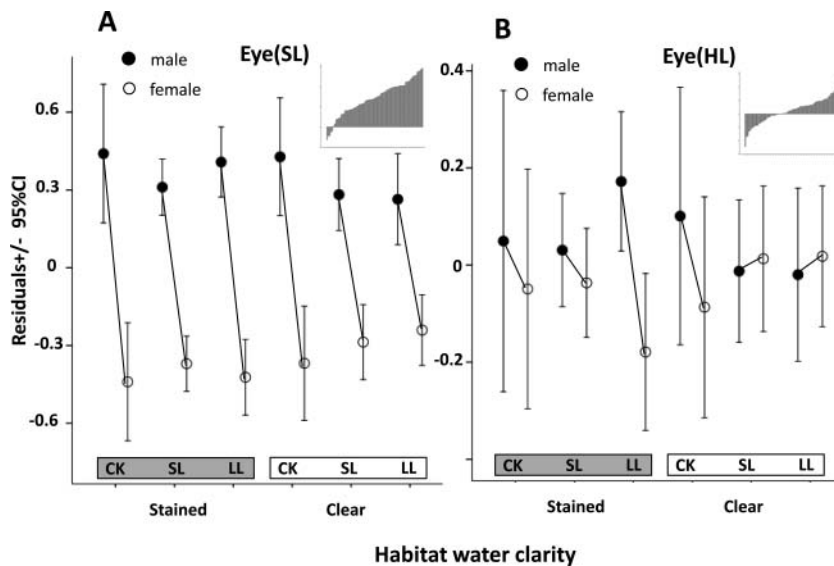


Fig. 9. Mean residual eye diameter for male and female stickleback for the six major habitats: creeks (CK), small lakes (SL), and large lakes (LL) for two spectral categories (stained, clear). (A) Residuals of eye diameter against SL, (B) residuals of eye diameter against head length. Insets show mean residual eye differences (M – F) for all localities. Above mid-horizontal line: male bias; below horizontal: female bias.

The classification matrix extracted from a discriminant analysis using the five most informative dimorphic traits (z_{JAWHD} , z_{EYESL} , z_{HDSL} , z_{VPLATLSL} , LLP) showed that among 68 populations, an average of 88% (range 44–100%) and 90% (range 50–100%) for male and female respectively were correctly classified according to gender. There was 100% correct classification in 31 (46%) and 36 (53%) populations for males and females respectively.

Stable isotopes

$\delta^{15}\text{N}$ ranged from 6‰ to 16‰ among the 30 populations. Twenty of the populations were isotopically enriched in males (higher trophic level), producing an overall male bias (paired $t_{28} = 3.46$, $P < 0.003$) (Fig. 10A, inset). The six populations with significant within-locality differences between the sexes (MW: $P < 0.05$) were all male-biased. The extent of male bias was not statistically associated with habitat type (Fig. 10A).

$\delta^{13}\text{C}$ ranged from -47 ‰ to -15 ‰ among the 30 populations. There was a similar distribution of populations with male versus female enrichment ($N = 17$ and 13 respectively), of which four within-lake comparisons were significant (MW: $P < 0.05$), two with female and two with male enrichment (Fig. 10B, inset). Overall, there was a weak but significant male enrichment (paired $t_{28} = 2.6$, $P < 0.02$) and a marginal effect of habitat (Fig. 10B), as six of the seven large lakes in the survey had higher enrichment (0.2–1.74) in males (Fisher's exact test: $P = 0.08$).

We also examined the dual isotope Euclidean distance between the sexes as a proxy for trophic differences. This ranged from 0.08‰, comprising minimal dimorphism, through to a maximum of 1.28‰. There were no differences between streams, small lakes, and

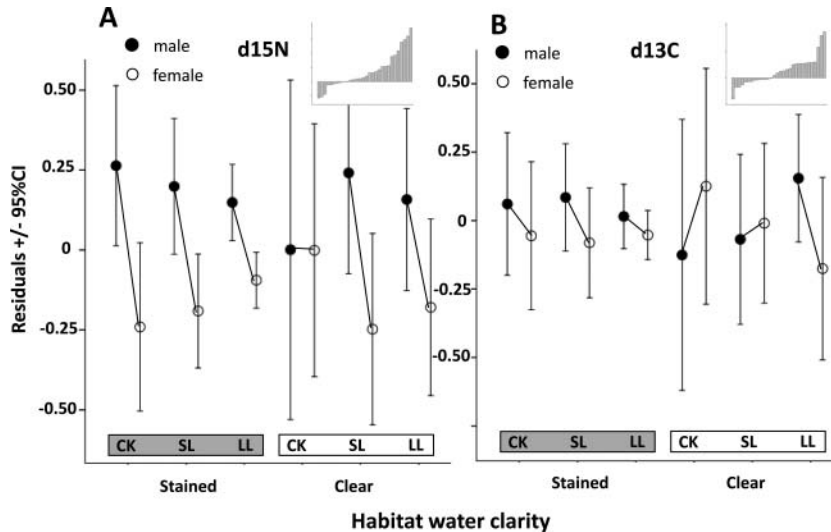


Fig. 10. Mean residual isotopic signatures (A, $\delta^{15}\text{N}$; B, $\delta^{13}\text{C}$) against SL for male and female stickleback for the six major habitats: creeks (CK), small lakes (SL), and large lakes (LL) for two spectral categories (stained, clear). Insets show isotopic differences ($M - F$) for all localities. Above mid-horizontal line: male bias; below horizontal: female bias.

large lakes ($F_{2,20} = 0.4$, $P = 0.67$) but a statistically significant greater dimorphism in clear versus stained lakes ($F_{1,16} = 6.0$, $P < 0.03$). The five large clear water lakes in this data set each had high Euclidean distances relative to stained lakes (no figure provided).

Temporal trends in isotopic signatures for the sexes were examined from two ecologically differentiated localities where multiple year sampling had been undertaken. At Drizzle Lake, adult males were enriched in four of the five years while among sub-adults, females were enriched in three of five years (Fig. 11). ANCOVA using $\delta^{15}\text{N}$ as the dependent variable, sex and year as fixed factors, and SL as a covariate produced a significant three-way interaction (sex*year*SL: $F_{8,968} = 13.9$, $P < 0.001$). Consequently, we separated our data by year and undertook additional ANCOVA to test for possible dimorphism in the regression slopes of $\delta^{15}\text{N}$ against SL. Slopes were statistically homogeneous in 1983 (sex*SL: $F_{1,107} = 0.1$, $P = 0.74$) and 2011 (sex*SL: $F_{1,353} = 2.0$, $P = 0.16$) but were significantly male-biased in 1981 (sex*SL: $F_{1,110} = 3.8$, $P = 0.05$), 1982 (sex*SL: $F_{1,202} = 8.1$, $P < 0.01$), and 2012 (sex*SL: $F_{1,196} = 16.9$, $P < 0.001$). The higher slope in males resulted in $\delta^{15}\text{N}$ depletion among sub-adults but enrichment among adults relative to females. Comparable ANCOVA for $\delta^{13}\text{C}$ showed no difference for the main effect (sex: $F_{1,968} = 0.04$, $P = 0.84$) and no significant two-way (all $P > 0.2$) or three-way interactions ($P = 0.16$).

At Boulton Lake, where collections of adults had been made over a span of 35 years, for $\delta^{15}\text{N}$ there was a significant effect of sex ($F_{1,484} = 9.2$, $P < 0.01$), with males having higher $\delta^{15}\text{N}$ signatures relative to females, although the individual sample effects were significant only in the summers of 1978, 1981, and 2012 (Fig. 12A). For $\delta^{13}\text{C}$, there was no overall effect of sex ($P > 0.4$) but the within-sample signatures were significantly male-biased in summer and autumn of 1978 and summer of 2012 and female-biased in spring 1981 and summer 2010 (sex*YrMo: $F_{7,485} = 4.3$, $P < 0.001$) (Fig. 12B).

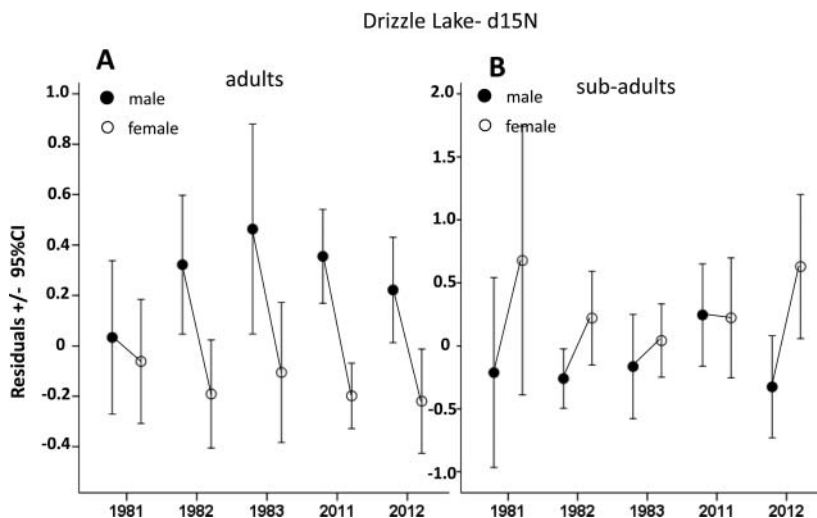


Fig. 11. Mean residual isotopic signatures for male and female Drizzle Lake stickleback sampled across years. (A) Adult size classes: 70–90 mm SL, (B) sub-adult size classes: 50–60 mm SL.

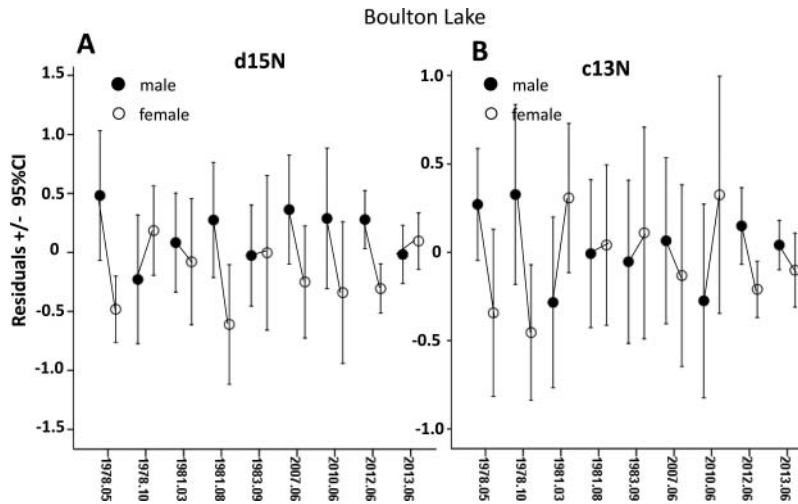


Fig. 12. Mean residual isotopic signatures (A, $\delta^{15}\text{N}$; B, $\delta^{13}\text{C}$) against SL for male and female Boulton Lake stickleback sampled across years.

Competition

Examination of potential interspecific competitive effects on sexual dimorphism for each trait showed no significant effects for any of the morphometric or isotopic traits (ANOVA: all $P > 0.2$). Correlations between the extent of dimorphism for each of 25 traits and the number of competitor species yielded one statistically significant result ($r = 0.41$, d.f. = 31, $P < 0.03$) for a single trait (zPSLSL) and the direction was positive rather than negative.

DISCUSSION

Unlike most epigamic traits that develop near the onset of reproduction, we focused our study on a broad diversity of defence and trophic traits of threespine stickleback that develop in early ontogeny to assess whether there was sexual dimorphism. All morphometric and isotopic traits, apart from several measures of pelvic asymmetry, exhibited sexual dimorphism but the extent and, occasionally, directionality was variable among populations. Because these populations represent at least 50 independent colonizations from the marine ancestral stickleback (Deagle *et al.*, 2012) and comprise an exceptional range of morphological diversity (Reimchen *et al.*, 2013), we feel these data will probably be representative of stickleback elsewhere in their circumboreal distribution.

Adult body length was female-biased in the majority of populations, consistent with that observed in other regions of the circumboreal distribution of stickleback (Kitano *et al.*, 2007; Herczeg *et al.*, 2010). This trend is evident in many fishes and can result from higher growth rates of females relative to males and the associated benefits of increased fecundity (Baker, 1994; Moyle and Cech, 2003). The dimorphism could also emerge from reduced growth rate in males due to their nest construction, territoriality, and higher associated metabolic costs (Henderson *et al.*, 2003; Rennie *et al.*, 2008). Another process that will accentuate body size dimorphism is life span. Female stickleback tend to show greater longevity than males, particularly where adult body size in the population is exceptionally large (DeFraipont *et al.*, 1992; Herczeg *et al.*, 2010;

Gambling and Reimchen, 2012; DeFaveri *et al.*, 2014). Extending from analyses of reproductive allocation in fishes, Roff (1983) predicted that species with territoriality and male-dominated parental care would have a male-biased body size dimorphism as a consequence of the benefits to large size in agonistic interactions. The occasional instances of male-biased body size in some stained streams and ponds in our study would be consistent with this prediction, yet the more common pattern of female-biased dimorphism is inconsistent.

Dorsal and anal fin ray numbers were male-biased in the majority of populations, a trend previously reported for limnetic and benthic species pairs of stickleback (McPhail, 1992) and for fin lengths based on body shape landmarks in the Haida Gwaii populations (Spoljaric and Reimchen, 2008). The function of this dimorphism is not known but may in part relate to the divergent reproductive behaviours of the sexes. Dorsal and anal fins in stickleback are positioned between the centre of body mass of the fish and the caudal fin (see Fig. 1) and this region influences stability and thrust (Aleev, 1977; Drucker and Lauder, 2001). Consequently, the larger fins in males could be particularly important during nesting activities such as hovering, fanning, gluing, vigilance, and burst acceleration. There were no consistent habitat correlates to population differences in the extent of male-biased fin rays.

Defences against predators tend to be distinctive for each species and reflect different life histories and ecological conditions (Endler, 1991). The extent of sexual dimorphism in defences has received limited attention in the literature, possibly because the focus on sexual selection has emphasized the trade-off between the benefit of conspicuous nuptial displays and increased predation risk or other costs (Kodric-Brown, 1990; Forsman, 1991; Reznick, 1996). Highly sexually dimorphic pigmentation patterns of numerous lepidopterans are generally due to sexual selection, yet some or possibly many of the dimorphisms in this group appear to be associated with ecological adaptations to the different niche space of the two sexes (Allen *et al.*, 2011). The horns of many ungulates are often restricted to males, the consequence of sexual selection, yet when present on both sexes, the horns are used for defence against large carnivores (Packer, 1983; Stankowich, 2011). Dorsal and pelvic spines of stickleback are important for display by males (Symons, 1966; Wilz, 1970; Huntingford, 1976) and for predator defence (Hoogland *et al.*, 1957; Moodie, 1972b), and one might expect the relative length of spines to be similar or larger in males. While there are populations without obvious dimorphism in the spines (McPhail, 1992), a more general trend is for relatively larger spines in females (Reimchen *et al.*, 1985; Kristjánsson *et al.*, 2002; Kitano *et al.*, 2007; Aguirre *et al.*, 2008). We found that pelvic spines and supporting ventral pelvic plate were marginally female-biased in most localities and this effect was accentuated in stained habitats and reduced in clear water habitats. The most divergent expression of this dimorphism occurs in one of the small bog lakes where most males exhibited a highly reduced pelvis and loss of spines relative to females (Reimchen, 1980). The bony lateral plates are an integral component to the defence apparatus of threespine stickleback, as they buttress the dorsal and pelvic spines and protect the trunk from epidermal injury (Reimchen, 1983, 2000). Early studies of stickleback at 25 lakes and streams on Haida Gwaii (Moodie, 1972a; Moodie and Reimchen, 1976; Reimchen *et al.*, 1985) showed slightly higher lateral plate counts in males than in females in most populations. Our current data, from some 100 populations, confirm the earlier trends but also show that the male-biased plate expression is accentuated in stained habitats and is reduced or absent in large clear water lakes. We suspect these population differences in sexual dimorphism and occasional reversal in the spines and the lateral plates are associated with differences in the amount of spatial overlap of the sexes in their use of littoral, limnetic, and benthic habitats with the resulting changes in the predation regime and trophic options (Reimchen *et al.*, 2013).

We observed an unusual difference between the sexes in the positional sequences of lateral plates that are involved in the buttressing of the dorsal and pelvic spines. Although both sexes have the same dominant sequence, females are more likely than males to have secondary sequences that are shifted anteriorly by a single position. This effect was observed for the overall data set as well as within a population where large samples were available. We do not know the significance of this anterior shift in females, although it might increase buttressing of the dorsal spines, as females have a slight anterior positioning of the spines relative to males (Spoljaric and Reimchen, 2008). Alternatively, the shift might influence swimming through a slight anterior movement in the centre of mass or an increase in the mid-trunk flexibility (Bergstrom, 2002).

We compared levels of asymmetry between the sexes. Two different predictions have been made regarding sexual dimorphism in asymmetry of non-sexually selected traits. First, the larger sex may have elevated asymmetry due either to reduced developmental stability resulting from directional selection for body size or nutritional stress during periods of rapid growth (Clutton-Brock *et al.*, 1985; Sheldon *et al.*, 1998). While this may be the case (Karvonen *et al.*, 2003; Pokhrel *et al.*, 2013), it is not always detected (Bortolotti and Gabrielson, 1995) and in some cases the smaller sex has elevated asymmetry (Söderman *et al.*, 2007). Second, males may have elevated asymmetry due to physiological stress of antagonistic steroid hormones (Folstad and Karter, 1992; Sheldon *et al.*, 1998). We found no strong overall sexual dimorphism in morphological asymmetries of non-sexually selected traits, although males tended to have asymmetric ascending processes more often than females. While females are larger, and may suffer greater developmental instability as a result, males do all the parental care, thus possibly experiencing more nutritional and temperature stress, and also potentially more steroidal hormonal stress. As a result, both sexes could be expressing comparable levels of asymmetry, albeit for different reasons. In addition, any existing dimorphism in asymmetry may have been too subtle to detect with our modest sample sizes. In studies from one of the Haida Gwaii populations where much larger samples were available, there are spatial and temporal differences in the extent of sexual dimorphism (Reimchen and Nosil, 2001a, 2001b, 2001c, 2004; Reimchen *et al.*, 2008).

Male stickleback have larger heads than females, a dimorphism that is geographically widespread (Aneer, 1973; McPhail, 1992; Reimchen and Nosil, 2005; Kitano *et al.*, 2007; Aguirre and Akinpelu, 2010; Kitano *et al.*, 2012; De Kermoyan, 2013; this study), including those in marine habitats (Aguirre *et al.*, 2008; Spoljaric and Reimchen, 2008; McGee and Wainwright, 2013). There could be multiple factors structuring this dimorphism. Larger heads provide greater buccal volume and suction velocity (Caldecutt and Adams, 1998), which, in combination with the larger jaws, will facilitate the capture of macrobenthos (Manzer, 1976; Wootton, 1976; Reimchen, 1980; Gill and Hart, 1994) or handling of vegetation for nest construction (reviewed in Wootton, 1976). Female-biased head size in the marine clingfish (*Diademichthys lineatus*) is associated with divergence in diet between the sexes (Magurran and Garcia, 2000). Larger heads also involve larger eyes, which, in most vertebrates, are associated with a greater photon-absorbing area of the retina and increased capacity for crepuscular or nocturnal activity (Schmitz and Motani, 2010). Although the relative activity of the sexes in stickleback during dusk and darkness has yet to be quantified, this might be particularly important for adult male stickleback during territorial defence, nest fanning, and tending throughout the day/night cycle. That the relative eye diameter was most dimorphic in large dystrophic stained lakes, all of which have low light levels (Reimchen, 1989), is consistent with some role of spectral regime in selection on eye dimorphism. Larger heads could also be a consequence of selection for increased brain size, possibly functionally associated

with the behavioural complexity of the male's reproductive life history relative to females (Kotrschal *et al.*, 2012).

The number and length of gill rakers in fish can be useful predictors of particle sizes in the diet (Helfman *et al.*, 1997). Analyses of species pairs of stickleback show a consistent trend for fewer gill rakers in the benthic forms, including those from parapatric lake–stream habitats (Moodie, 1972a; Reimchen *et al.*, 1985; Kaeuffer *et al.*, 2012) and limnetic–benthic habitats (Lavin and McPhail, 1986; McPhail, 1994). Given the sexual dimorphism in head size, which includes differentiation in the same traits seen in benthic–limnetic forms, one would also predict fewer gill rakers in males than females. Yet, geographical data on raker number between the sexes either show no dimorphism (Gross and Anderson, 1984; McPhail, 1992; Kaeuffer *et al.*, 2012; Millet *et al.*, 2013), a marginal male-biased dimorphism in central British Columbia (Reimchen and Nosil, 2005), opposite to what was predicted, or a combination of both male-biased and female-biased populations on Vancouver Island (Bolnick and Lau, 2008). Our large survey of gill raker number for the sexes across ecologically diverse stream and lake habitats on the Haida Gwaii archipelago shows no obvious dimorphism apart from occasional populations that exhibit a significantly higher raker count in males, similar to the Banks-Estevan archipelago in central British Columbia (Reimchen and Nosil, 2005). It is possible that the general low incidence of sexually dimorphic raker counts reflects the major habitat overlap of the sexes early in ontogeny where, by nature of their smaller gape, the diet of the sexes is more constrained. This is partially supported by our isotopic data (see below).

Assessment of nitrogen and carbon stable isotope signatures of tissues provides insight to animal diet (Fry, 2006). Enrichment of $\delta^{15}\text{N}$ reflects a higher trophic position, while enriched $\delta^{13}\text{C}$ in aquatic systems is associated with greater use of littoral rather than limnetic habitats (Post, 2002; McIntyre *et al.*, 2006). Even within each of these habitats, isotopic signatures differ among zooplankton taxa (Lepak *et al.*, 2006) and among macrobenthos (Grey *et al.*, 2004). Unlike analyses of stomach contents, isotopic data on tissues is a time-integrated signature, which, for muscle tissues in fish, can be 4–6 months (Bearhop *et al.*, 2004). This can reveal small and persistent dietary differences between individuals not identifiable with short-term stomach analyses (for a summary, see Wearmouth and Sims, 2008), yet it can equally mask short-term differences in diet due to the longer integration of the signals (Fry, 2006). Isotopic data comparing the sexes have received only limited attention but yield novel evidence for niche differentiation in pinnipeds (Tucker *et al.*, 2007), songbirds (Mazerolle and Hobson, 2007), temperate but not tropical seabirds (Mancini *et al.*, 2013; Michalik *et al.*, 2013), and juvenile caiman (Marques *et al.*, 2013). Bolnick and Paull (2009) observed significant differences in carbon and nitrogen signatures between male and female stickleback at a small eutrophic lake on southern British Columbia. Our assessment of 30 stickleback populations across ecologically diverse habitats showed variability in both the direction and extent of isotopic differences between the sexes. For $\delta^{15}\text{N}$, we detected a male-biased enrichment in the majority of samples from all of the habitats (creeks, small lakes, and large lakes), including the replicated samples within several localities. This is consistent with general isotopic studies in fishes that individuals with larger gape generally can forage on larger and higher trophic level prey (McIntyre *et al.*, 2006). One might anticipate that this isotopic enrichment in male stickleback would be domain general and occur across different size classes within a population, yet at the single locality where we compared size classes, the ontogenetic slopes of $\delta^{15}\text{N}$ against SL adjusted for a common mean were more positive in males than in females, suggesting $\delta^{15}\text{N}$ depletion in sub-adult males and $\delta^{15}\text{N}$ enrichment in adult males relative to females. This larger ontogenetic dietary shift in males than in females could emerge when pre-reproductive

males shift from an offshore profundal/water column niche, where females are also found, to an inshore littoral/benthic niche (Reimchen, 1980, 1990). The general similarity in $\delta^{13}\text{C}$ signatures between the sexes in most localities is consistent with seasonal, possibly winter, overlap of their foraging habitats. Although $\delta^{13}\text{C}$ enrichment is generally known to characterize increased littoral sources of carbon (Post, 2002), this can be obscured by the relative contribution of autochthonous versus allochthonous carbon sources that structures productivity in dystrophic versus oligotrophic habitats (Carpenter and Pace, 1997). This might account for the atypical isotopic data that we observed in one of the dystrophic streams and small dystrophic lakes where $\delta^{13}\text{C}$ was enriched in females.

Since isotopic data on muscle tissues is time-integrated, multi-year samples can give insight to potential longer-term trends and changes in the diet (Dalerum and Angerbjörn, 2005). We observed relatively consistent $\delta^{15}\text{N}$ enrichment in males spanning a 30 year period at Drizzle Lake, yet at Boulton Lake the sexual dimorphism was variable both seasonally and yearly (from $\delta^{15}\text{N}$ enriched through to $\delta^{15}\text{N}$ depleted) over a similar period. These isotopic data are interesting because they supplement previous studies at this locality showing that the sexes partition the lake habitat, with adult males in littoral and adult females in limnetic habitats. Yet there are corresponding yearly differences in diet and relative parasitism of the sexes reflective of whole-lake shifts in the relative movement among years (Reimchen, 1980, 1997; Reimchen and Nosil, 2001a).

Extending from the niche-breadth hypothesis (Van Valen, 1965), we evaluated whether the presence or absence of competitor species in the stream and lake habitats was associated with population differences in the extent of sexual dimorphism. Increased dimorphism would be predicted in the absence of interspecific competition, as this can allow ecological release and a resulting expansion of niche space (Bolnick and Doebeli, 2003; Dayan and Simberloff, 2005; Nosil and Reimchen, 2005; Hsu *et al.*, 2014; Martin *et al.*, 2015). However, there has been substantial criticism of this hypothesis based on the lack of any relationships between the extent of sexual dimorphism and species richness in island communities (Meiri *et al.*, 2014). Previous studies of body shape on a subset of the stickleback populations used in the present study showed a reduction in the extent of dimorphism in ponds relative to large lakes, which was interpreted as a general reduction in niche space (Spoljaric and Reimchen, 2008). While pond habitats have reduced diversity of species, there was no explicit comparison of these localities with and without competitors. In the current study, we compared stickleback populations in small lakes with and without other fish species, predicting increased dimorphism in the absence of interspecific competitors. Of 25 traits, we observed a single significant correlation (dimorphism in residual pelvic spine length) but it was positive rather than negative, opposite to our prediction and we would infer that there is no support for the expected increased dimorphism in the absence of competitors, consistent with Meiri *et al.* (2014). Although this appears to weaken support for the niche-breadth hypothesis, our comparisons of habitats with and without competitors may be much too coarse. The majority of habitats with only stickleback are small shallow ponds with little niche breadth such that ecological differentiation between the sexes is limited. The locality that is a major exception to this is Boulton Lake, which is large enough to include horizontal (inshore–offshore) and vertical (limnetic–benthic) gradients, but stickleback is the only fish species in the lake. Here, stickleback are highly sexually dimorphic in defence and trophic traits as well as life histories, including relative parasitism, exposure to predators, and spatial distribution (Reimchen, 1980, 1997; Reimchen and Nosil, 2001a, 2004). In other words, where there is direct evidence for increased ecological opportunity, intraspecific competition can facilitate differentiation

between the sexes, one of the basic predictions in the niche-width hypothesis (Van Valen, 1965; Bolnick and Doebeli, 2003; Nosil and Reimchen, 2005).

Where 'sexual' selection occurs, 'natural' selection can drive further phenotypic differences between the sexes when they occupy different niche space and are exposed to different selective pressures. Sexual segregation in habitat is widespread in both terrestrial and marine taxa, including teleosts and elasmobranchs, and is frequently associated with size dimorphism (reviewed in Wearmouth and Sims, 2008). Even in a sexually monomorphic species such as the minnow, the sexes have different habitat preferences during non-reproductive periods (Griffiths *et al.*, 2014). Our cumulative data indicate widespread sexual dimorphism in body size, fin ray number, defence and trophic traits. The dimorphism occurs across a broad diversity of pristine habitats, from stained, slow-moving bog streams to large oligotrophic clear water mountain lakes. Some of the traits such as the jaw and the ventral plate of the pelvis are consistently dimorphic in all habitats, while other traits such as number of lateral plates, length of pelvic spines, and isotopic signatures vary in the extent and direction of the dimorphism. Whether these trends are more widespread throughout the circumpolar distribution of threespine stickleback is unclear, as the sexes have frequently not been differentiated in morphological and genetic studies (e.g. Hagen and Gilbertson, 1972; Gross, 1977; Lavin and McPhail, 1985, 1986, 1987; Francis *et al.*, 1986; Bourgeois *et al.*, 1994; Klepaker, 1995; Bell *et al.*, 2004; Hermida *et al.*, 2005; Kitano *et al.*, 2008; Berner *et al.*, 2010; Willacker *et al.*, 2010; Webster *et al.*, 2011; Kaeuffeur *et al.*, 2012; Lescak *et al.*, 2013; Lucek *et al.*, 2013, 2014; Ravinet *et al.*, 2013; Voje *et al.*, 2013). The recent discovery of a sex-determining linkage group in stickleback (Peichel *et al.*, 2004) facilitates the integration of the explicit genetic expression of multiple dimorphic characters with ecological functionality.

We infer that the extent of sexual dimorphism in defence and trophic traits in threespine stickleback is an adaptive response to divergent life histories between the sexes and the ecological landscape opportunities that vary within and among habitats. While this study is one of the first to examine intraspecific variability in sexual dimorphism in freshwater fish, our conclusions are largely consistent with recent meta-analyses showing widespread habitat sexual segregation in vertebrates (Wearmouth and Sims, 2008) and are important in a species that has become a 'supermodel' for evaluating evolutionary processes.

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