

## Sexual Dimorphism in the External Morphology of the Threespine Stickleback (*Gasterosteus aculeatus*)

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Information about sexual dimorphism is essential for understanding the ecology, behavior, and life history of a species, as well as for making morphological comparisons between populations. Furthermore, in order to understand the evolution of sexual dimorphism, it is important to know whether sexual dimorphism is genetically determined or the result of phenotypic plasticity. To this end, we have characterized patterns of sexual dimorphism in the threespine stickleback (*Gasterosteus aculeatus*). These fish are widely distributed throughout the temperate Northern Hemisphere, and their behavior, ecology, and evolution have been extensively characterized. We first examined sexual dimorphism in morphometric and meristic characters of wild-caught threespine sticklebacks from multiple populations that demonstrate different life history strategies in order to understand general patterns of sexual dimorphism in the threespine stickleback. Next, we made several crosses by *in vitro* fertilization and raised them in the laboratory to investigate developmental and genetic contributions to sexual dimorphism. Morphological analysis of wild-caught breeding males and females from four North American and six Asian populations revealed that adult males have larger heads and mouths than adult females in all populations. In contrast, adult females were longer in standard length and had longer pelvic girdles than adult males in many populations. Sexual dimorphism in dorsal-spine length was variable among populations. Except for body size, sexual dimorphism in most external morphological traits was similar between wild-caught and lab-reared fish. However, sexual dimorphism was only observed after the fish became reproductively mature. These results suggest that general features of secondary sexual characters are shared across different threespine stickleback populations and that sexual dimorphism in some morphological traits may have a genetic basis.

SEXUAL dimorphism is widespread across the animal kingdom. Males and females usually differ not only in reproductive organs, but also in external structures that are not directly related to reproduction (Darwin, 1874; Andersson, 1994). Sexual dimorphism can result from a variety of factors, including both sexual and natural selection. Different reproductive roles, niche divergence between the sexes, preference of one sex for particular traits of the other sex, and intra-sexual competition can drive sexual differences in external structures (Darwin, 1874; Slatkin, 1984; Shine, 1989; Parker, 1992; Anderson, 1994). Information about sexual dimorphism is required for understanding the ecology, behavior, and life history of a species. In addition, knowledge of sexual dimorphism and its appearance during ontogeny is indispensable when making morphological comparisons between populations.

Threespine sticklebacks (*Gasterosteus aculeatus*) occur in coastal areas of the temperate Northern Hemisphere (Wootton, 1984; Bell and Foster, 1994), and their behavior, ecology, and evolution have been well characterized (Tinbergen, 1951; Wootton, 1976, 1984; Bell and Foster, 1994).

Threespine stickleback populations inhabit diverse habitats and display extensive phenotypic divergence in morphological, behavioral, and physiological traits (Bell and Foster, 1994). Male and female sticklebacks have divergent reproductive roles (Wootton, 1984). Breeding males become aggressive to obtain both breeding territory and gravid females (Wootton, 1984). Males build nests, court females, and perform parental care for offspring, while the primary role of breeding females is egg production (Wootton, 1984). In addition to the divergent reproductive roles of the sexes, several morphological structures, such as body size, are important for male and female mate choice in sticklebacks, suggesting that sexual selection may contribute to the evolution of sexual dimorphism in these morphological cues (Tinbergen, 1951; Wootton, 1984; Rowland, 1994; Schluter, 2001; McKinnon and Rundle, 2002). In addition, several ecological factors are associated with sexual dimorphism of armor structures in Canadian stickleback populations, implicating a role for natural selection in the evolution of sexual dimorphism in sticklebacks (Reimchen, 1980; Reimchen and Nosil, 2001, 2004, 2006).

Although sexual differences in a variety of external structures have been noted in many populations from Japan (Ikeda, 1937; Mori, 1984; Mori and Takamura, 2004), Russia (Potapova, 1972), northwest North America (Schluter and McPhail, 1992; Caldecutt et al., 2001; Reimchen and Nosil, 2006), northeast North America (Blouw and Hagen, 1990; Blais et al., 2004), Iceland (Kristjansson et al., 2002a), and Europe (Bakker and Mundwilder, 1999; Kristjansson et al., 2002b), it is unknown whether sexual dimorphism is genetically determined in sticklebacks. Because the genetic architecture of a trait can either facilitate or constrain the evolution of sexual dimorphism (Lande, 1980; Rice, 1984), it is important to understand the genetic factors that contribute to sexual dimorphism. If sexual dimorphism in sticklebacks has a genetic basis, the recently established genomic tools for threespine sticklebacks (Peichel et al., 2001; Peichel, 2005) make these fish a good model system in which to study the genetic architecture of sexual dimorphism.

In the present investigation, we first analyzed sexual dimorphism in external structures within four North American and six Asian populations of threespine stickleback. Next, we made several crosses by *in vitro* fertilization and reared both male and female sticklebacks in the same environment to gain insight into the developmental and genetic mechanisms underlying sexual dimorphism.

#### MATERIALS AND METHODS

*Wild-caught fish.*—We analyzed ten populations, which were chosen to include a wide range of populations that demonstrate different life history strategies, including anadromous, lake-resident, and stream-resident forms, and also to cover the two basal phylogenetic lineages identified by allozyme analysis of global threespine stickleback populations: the Japan Sea and the Pacific/Atlantic Ocean lineages (Haglund et al., 1992; Ortú et al., 1994; Higuchi and Goto, 1996). There is extensive divergence in courtship behavior between these two lineages (Ishikawa and Mori, 2000), suggesting that sexual selection might produce different patterns of sexual dimorphism in these lineages. Therefore, we included both lineages for analysis. All wild-caught sticklebacks were adults that were collected at mid to late breeding seasons from May through June (Fig. 1; for data on breeding seasons, see Hagen, 1967; Foster, 1988; Mori, 1990; Kume et al., 2005). In 2004, Canadian anadromous and stream-resident forms were collected from the estuary (Little Campbell

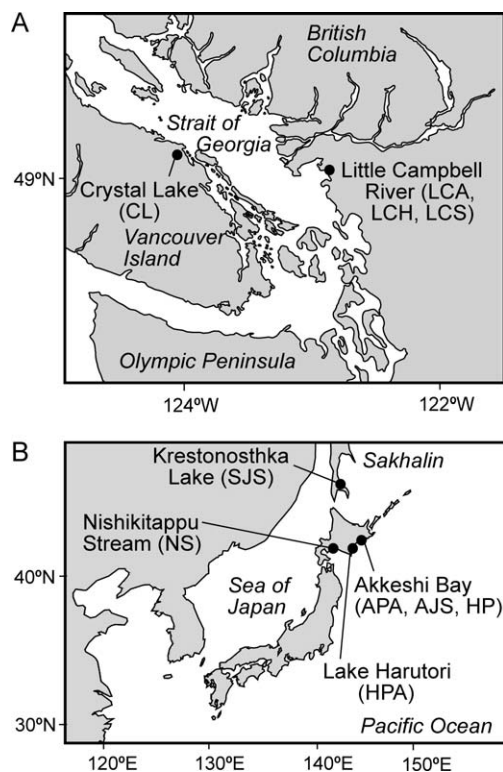


Fig. 1. Map showing collection sites in North America (A) and in Asia (B). Abbreviations are as follows: LCA, Little Campbell River anadromous form; LCS, Little Campbell stream-resident form; LCH, Little Campbell River hybrid form; CL, Crystal Lake population; HPA, Lake Harutori Pacific Ocean anadromous form; APA, Akkeshi Pacific Ocean anadromous form; HP, Hyotan Pond-resident form; NS, Nishikitappu stream-resident form; AJS, Akkeshi Japan Sea population; SJS, Sakhalin Japan Sea population.

anadromous, LCA) and the upstream region (Little Campbell stream, LCS) of the Little Campbell River, respectively. There is reproductive isolation between the LCA and LCS forms except in a previously described hybrid zone of the Little Campbell River, where sticklebacks were also collected in 2004 (Little Campbell hybrid, LCH; Hagen, 1967). Threespine sticklebacks from a Canadian lake were collected in 2004 from Crystal Lake (CL) on Vancouver Island, Canada (Foster, 1988). Anadromous forms of the Japanese Pacific Ocean population were collected from Lake Harutori (Harutori Pacific anadromous, HPA; Mori, 1990) and Akkeshi Bay (Akkeshi Pacific anadromous, APA; Kume et al., 2005) on Hokkaido Island, Japan in 1984 and 2003, respectively. Threespine sticklebacks of the APA population used for geometric morphometrics were collected in 2005 at Akkeshi

Bay. A Japanese lake-resident form and a Japanese stream-resident form were collected from Hyotan pond (HP), Hokkaido, Japan, in 2003 and Nishikitappu stream (NS), Hokkaido, Japan, in 1997, respectively (Arai et al., 2003). Anadromous forms of the Japan Sea lineage were collected from Lake Krestonosthka in Sakhalin Island, Russia, in 2001 (Sakhalin Japan Sea population, SJS) and Akkeshi Bay in Hokkaido Island, Japan, in 2004 (Akkeshi Japan Sea population, AJS), respectively (Kume et al., 2005). For each population, voucher specimens were deposited in the University of Washington fish collection in Seattle (accession numbers UW115835–UW115844).

*Laboratory-reared fish.*—We produced three families of laboratory-reared fish by *in vitro* fertilization (Hagen, 1967). Fish were fed with live *Artemia*, frozen *Mysis*, and frozen Chironomidae. All fish were cultured in bare tanks to prevent them from nesting and reproducing, because we wanted to know whether sexual dimorphism appears before the fish perform reproductive behaviors. In order to compare sexual dimorphism before and after reproductive maturity, we split the progeny from a cross (AxF) between an anadromous APA female and a freshwater HP male into several tanks and cultured them at a density of 30–40 fish/120 L at 16 C with a photoperiod of 16 h light and 8 h dark. Fish in one tank were sacrificed at 12 months before any of them showed apparent signs of reproductive maturity, such as male nuptial color and female eggs. Fish in another tank were kept further for 12 months until males showed nuptial color and females became gravid.

Two crosses (AxA) were established between an anadromous APA female and an anadromous APA male. One cross was kept at a density of 40 fish/120 L at 16 C with a photoperiod of 16 h light and 8 h dark for 12 months and sacrificed before they started breeding. Another AxA cross was divided into two groups and raised under low-density and high-density conditions until they showed apparent signs of reproductive maturity, such as male nuptial color and female eggs. We first split the progeny into four tanks and cultured them for 14 months at a density of 30–40 fish/120 L at 16 C with a photoperiod of 16 h light and 8 h dark. One tank was kept at this condition for another 7 months until they came into reproductive condition. These fish were used as the high-density group. Fish from the other three tanks were transferred to a larger tank at the age of 14 months and cultured at a lower density of 100 fish/517 L at 15 C with a photoperiod of 8 h light and 16 h dark for one

month to stimulate growth. Then, the temperature and light/dark cycle of the tanks was restored to breeding condition: 19 C with a photoperiod of 16 h light and 8 h dark. After three months, they showed signs of reproductive maturity and were sacrificed and used as the low-density group.

*Morphological analysis.*—For both wild-caught and laboratory-reared fish, right pectoral fins were clipped under anesthesia and preserved in ethanol for later DNA analysis, while the rest of the bodies were preserved in formalin for morphological analysis. Sex was determined by either visual examination of the gonads or detection of a male specific polymorphism in the isocitrate dehydrogenase gene by polymerase chain reaction (Peichel et al., 2004). Standard length (SL), head length (HL), body depth (BD), upper-jaw length (JL), snout length (SnL), gape width (GW), eye diameter (ED), first and second dorsal-spine length (1DS and 2DS, respectively), pelvic-spine length (PS), and pelvic-girdle length (PG) were measured from the left side of each fish with a vernier caliper as described previously (Mori, 1984; Peichel et al., 2001). Gill raker number (GRN) and lateral plate number (LPN) were counted under a dissecting microscope from the right sides and both sides, respectively, of alizarin red-stained fish (Peichel et al., 2001). To investigate LPN asymmetry, we subtracted the left LPN from the right LPN for each individual to calculate asymmetry (R–L; Reimchen and Nosil, 2001). We tested whether R–L differed significantly from zero by using a one-sample *t*-test to examine the presence of directional asymmetry in LPN (Reimchen and Nosil, 2001).

To detect sexual dimorphism, SL was normalized by log-transformation (logSL) and compared between the sexes for each population by ANOVA. Since two meristic counts, lateral plate and gill raker numbers, are not correlated with SL in any populations (Spearman rank correlation;  $P > 0.05$ ), these traits were compared between the sexes for each population by the Mann–Whitney U-test. For testing sexual dimorphism of LPN asymmetry, we used a *t*-test to compare R–L between the sexes for each population (Reimchen and Nosil, 2001). For all other traits, we first performed log-transformation and then performed ANCOVA with logSL as a covariate. First, we tested the interaction between logSL and sex in order to examine slope heterogeneity of regression lines between the sexes for each population. When the interaction was not significant ( $P > 0.05$ ), suggesting slope homogeneity, we next excluded the

interaction term from the model and compared the log of the trait by ANCOVA with the logSL as a covariate. If the ANCOVA results were significant, we calculated a body size-corrected trait that was adjusted to a grand mean of logSL in a population and determined which sex has a larger value. When slope homogeneity was rejected ( $P < 0.05$ ), we made no conclusions about the presence or absence of sexual dimorphism. SPSS (SPSS Inc.) and StatView (SAS Institute Inc.) software was used for calculations and statistics. When we performed a multiple comparison, we adjusted the statistical significance of  $\alpha = 0.05$  by sequential Bonferroni correction to avoid a Type I error (Rice, 1989).

For measuring the serration on dorsal spines (Ikeda, 1937; Gross, 1978), a picture of the first dorsal spine of each fish was taken with a digital camera connected to a dissecting microscope (Fig. 2A). The area of the first dorsal spine was calculated with NIH image 1.63 software (<http://rsb.info.nih.gov/nih-image/>). After the serrations were removed by using the eraser tool of Adobe Photoshop 7.0 (Adobe Systems), the area of the smoothed spine was calculated again with NIH image 1.63. The area of the spine serration was calculated by subtracting the area of smoothed spine from the total spine area. A serration index was calculated by dividing the serration area by the total spine area. The serration index was compared between the sexes by the Mann–Whitney U-test. For 15 randomly chosen fish, we repeated the calculation of serration index and confirmed that the first and second measurements are highly correlated with each other (Spearman's rank correlation,  $r_s = 0.925$ ,  $P < 0.001$ ), suggesting that our calculation of serration index is reproducible.

To visualize sexual dimorphism in body shape, we used landmark-based geometric morphometrics (Rohlf and Marcus, 1993; Walker, 1997; Zelditch et al., 2004). Digital pictures of anesthetized fish were taken from the left side of 17 wild-caught APA males and 17 wild-caught APA females. Eight landmarks (landmarks 1–8 in Fig. 3B) cover the body shape and overlap with the landmarks described in Walker (1997), except landmark 7. In addition, we used the eye (landmark 9) and pectoral-fin positions (landmarks 10 and 11), because these positions appeared to be different between males and females. These landmarks were digitized with tpsDig2 software (<http://life.bio.sunysb.edu/morph/index.html>). We used tpsRelw software (<http://life.bio.sunysb.edu/morph/index.html>) to conduct principal component analysis of partial warp scores to calculate relative warp scores (RW) for each individual (Caldecutt and

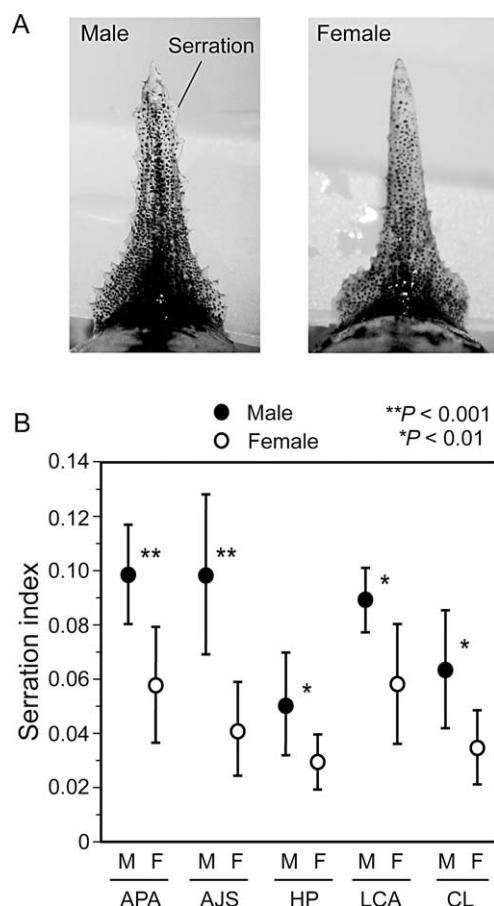


Fig. 2. (A) Representative images of male and female dorsal spines in the Akkeshi Japan Sea population. (B) Means  $\pm$  SD of serration indexes. Fifteen males and 15 females of the Akkeshi Pacific anadromous form (APA), 17 males and 13 females of the Akkeshi Japan Sea population (AJS), 15 males and 15 females of the Hyotan pond-resident form (HP), ten males and ten females of the Little Campbell River anadromous form (LCA), and ten males and ten females of the Crystal Lake-resident form (CL) were analyzed. Abbreviations: M, males; F, females. In all populations, males have a significantly larger serration index than females (Mann–Whitney U-test; \*\*,  $P < 0.001$ , \*,  $P < 0.01$ ).

Adams, 1998; Zelditch et al., 2004). Landmark positions of the fish that have particular RW were visualized with the thin-plate spline method.

## RESULTS

*Sexual dimorphism in wild-caught fish.*—Females were significantly larger than males in SL for eight populations (Table 1; Fig. 4). However, in the other two populations (LCH and CL), males and females did not differ significantly in SL. In

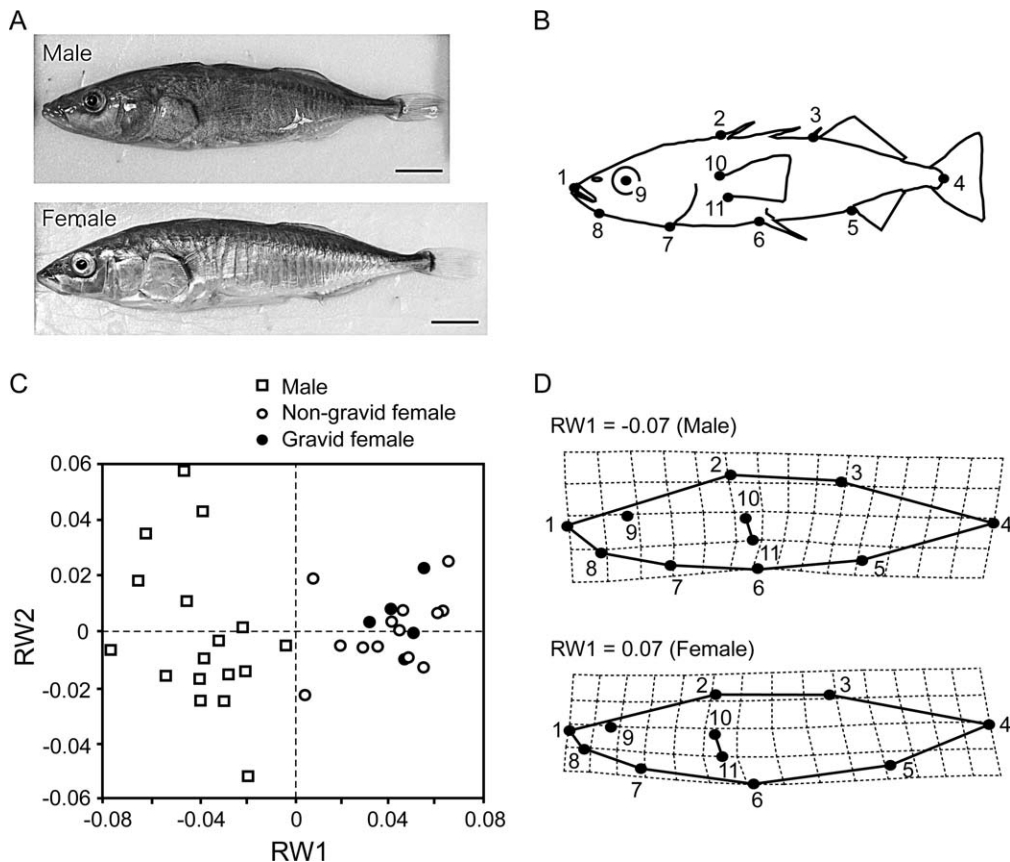


Fig. 3. (A) Representative images of male and female anadromous forms of the Akkeshi Pacific Ocean population (APA). Scale bar = 1 cm. (B) Location of landmarks used for geometric morphometrics. Numbers in the figure indicate the eleven landmarks: (1) anterior tip of upper lip, (2) anterior base of the first dorsal spine, (3) anterior base of the third dorsal spine, (4) caudal end of caudal keel, (5) base of anal spine, (6) anterior base of pelvic spine, (7) ventral border of operculum, (8) posterior edge of angular, (9) center of eye, (10) upper end of pectoral-fin base, and (11) lower end of pectoral-fin base. (C) Scatter plot of first and second relative warp scores (RW1 and RW2, respectively) for APA males ( $n = 17$ ; open square), APA non-gravid females ( $n = 12$ ; open circle), and APA gravid females ( $n = 5$ ; black circles). (D) Thin-plate spline deformation grids visualizing the body shape with RW1 of  $-0.07$  and RW1 of  $0.07$ , which reflect the male- and female-specific body shapes, respectively.

general, males had larger heads and mouths than females. Males had larger values of body size-adjusted JL in eight populations and body size-adjusted SnL in nine populations (Table 1; Fig. 4). Slope heterogeneity between the sexes for  $\log JL$  and  $\log SnL$  in the remaining populations precluded use of ANCOVA to test for sexual dimorphism. However, males in these populations had larger absolute values in these traits, although the females had longer SL, suggesting that there is sexual dimorphism in JL and SnL. Therefore, a larger mouth in males is a shared feature among the ten populations. Sexual dimorphism in HL, GW, and ED was not universal, but when sexual dimorphism was present, males had larger values after body size

correction (Table 1; Fig. 4), suggesting that males usually have larger head structures than females. In three populations, males had deeper bodies (BD) than females after body size correction, while in no population did females have larger values in body size-adjusted BD than males (Table 1; Fig. 4).

We then conducted geometric morphometrics to analyze the variation of body shape in the APA population (Fig. 3). The first and second relative warps (RW1 and RW2, respectively) explain 58.3% and 13.7% of the variance in partial warp scores. RW1 differs significantly between males and females (Mann-Whitney U-test;  $Z = -5.0$ ;  $P < 0.001$ ), while RW2 did not differ between the sexes (Mann-Whitney U-test;  $Z = -1.1$ ;  $P = 0.274$ ). A

TABLE 1. MORPHOLOGY AND SEXUAL DIMORPHISM IN WILD-CAUGHT THREESPINE STICKLEBACKS. Means  $\pm$  SD are shown. The statistical significance of  $\alpha = 0.05$  was adjusted by sequential Bonferroni correction to avoid a Type I error, because we conducted 90 comparisons in this table. Although absolute values of morphological traits are shown in the table, bold letters indicate the sex that has a significantly larger body-size corrected value for the trait even after sequential Bonferroni correction. The superscript a indicates that there is a difference between the sexes in the trait at  $P < 0.05$ , but this significance is not significant after sequential Bonferroni correction. When slope heterogeneity (indicated by superscript b) was detected for a trait, the presence or absence of the sexual dimorphism was not determined.

Population	SL (mm)	HL (mm)	BD (mm)	JL (mm)	SnL (mm)	GW (mm)	ED (mm)	IDS (mm)	2DS (mm)	PS (mm)	PG (mm)	n
LCA male	57.06 $\pm$ 1.81	<b>18.39</b> $\pm$ 0.51	<b>13.41</b> $\pm$ 0.43	<b>4.41</b> $\pm$ 0.30	<b>5.82</b> $\pm$ 0.23	<b>3.86</b> $\pm$ 0.36	4.81 $\pm$ 0.24 <sup>a</sup>	5.57 $\pm$ 0.48	6.19 $\pm$ 0.51	9.20 $\pm$ 0.56	12.99 $\pm$ 2.22	23–31
LCA female	<b>60.95</b> $\pm$ 3.31	17.19 $\pm$ 0.93	13.56 $\pm$ 0.83	3.84 $\pm$ 0.27	5.05 $\pm$ 0.37	3.59 $\pm$ 0.29	4.71 $\pm$ 0.36	5.80 $\pm$ 0.56	6.83 $\pm$ 0.62	10.04 $\pm$ 0.96	15.15 $\pm$ 1.50 <sup>a</sup>	24
LCS male	50.41 $\pm$ 3.53	<b>16.61</b> $\pm$ 1.13	13.04 $\pm$ 0.97	<b>4.24</b> $\pm$ 0.46	<b>4.73</b> $\pm$ 0.43	<b>4.14</b> $\pm$ 0.47	4.71 $\pm$ 0.32 <sup>a</sup>	3.59 $\pm$ 0.45	4.26 $\pm$ 0.44	5.54 $\pm$ 0.51	9.24 $\pm$ 0.72	25–28
LCS female	<b>58.70</b> $\pm$ 2.76	17.82 $\pm$ 0.79	15.37 $\pm$ 0.98	4.08 $\pm$ 0.25	4.61 $\pm$ 0.32	4.19 $\pm$ 0.31	4.97 $\pm$ 0.26	3.97 $\pm$ 0.36	4.94 $\pm$ 0.41	6.15 $\pm$ 0.66	11.12 $\pm$ 1.04	22
LCH male	49.41 $\pm$ 3.79	16.27 $\pm$ 1.23 <sup>b</sup>	12.00 $\pm$ 1.11 <sup>b</sup>	<b>3.90</b> $\pm$ 0.50	<b>4.68</b> $\pm$ 0.45	3.78 $\pm$ 0.44	<b>4.57</b> $\pm$ 0.33	4.33 $\pm$ 0.51	4.78 $\pm$ 0.44	6.57 $\pm$ 0.70	10.30 $\pm$ 0.87	13
LCH female	49.10 $\pm$ 3.44	15.03 $\pm$ 1.58 <sup>b</sup>	11.98 $\pm$ 1.45 <sup>b</sup>	3.32 $\pm$ 0.40	4.02 $\pm$ 0.42	3.43 $\pm$ 0.33	4.20 $\pm$ 0.35	4.37 $\pm$ 0.56	4.85 $\pm$ 0.60	6.94 $\pm$ 0.97	11.45 $\pm$ 1.35 <sup>a</sup>	12
CL male	50.27 $\pm$ 3.12	16.85 $\pm$ 0.96 <sup>b</sup>	10.51 $\pm$ 0.68	<b>4.19</b> $\pm$ 0.37	<b>4.68</b> $\pm$ 0.37	<b>3.65</b> $\pm$ 0.38	<b>5.14</b> $\pm$ 0.32	2.74 $\pm$ 0.29	3.38 $\pm$ 0.35	3.96 $\pm$ 0.34	8.22 $\pm$ 0.72	34
CL female	51.73 $\pm$ 4.91	16.22 $\pm$ 1.57 <sup>b</sup>	10.91 $\pm$ 1.06	3.66 $\pm$ 0.41	4.17 $\pm$ 0.48	3.36 $\pm$ 0.39	4.89 $\pm$ 0.52	3.00 $\pm$ 0.25 <sup>a</sup>	<b>3.75</b> $\pm$ 0.28	4.06 $\pm$ 0.38	<b>8.96</b> $\pm$ 0.80	<b>29</b>
APA male	75.88 $\pm$ 2.64	<b>24.55</b> $\pm$ 0.73	18.05 $\pm$ 0.73	6.37 $\pm$ 0.39 <sup>b</sup>	<b>8.45</b> $\pm$ 0.42	5.25 $\pm$ 0.37 <sup>a</sup>	5.64 $\pm$ 0.23	8.05 $\pm$ 0.61 <sup>b</sup>	8.54 $\pm$ 0.62	11.51 $\pm$ 0.72	17.27 $\pm$ 0.72	12–14
APA female	<b>82.90</b> $\pm$ 2.71	23.80 $\pm$ 0.81	18.86 $\pm$ 0.73	5.50 $\pm$ 0.37 <sup>b</sup>	7.95 $\pm$ 0.40	4.72 $\pm$ 0.39	5.68 $\pm$ 0.23	8.26 $\pm$ 0.70 <sup>b</sup>	8.97 $\pm$ 0.86	12.42 $\pm$ 0.69	19.67 $\pm$ 0.93 <sup>a</sup>	29–31
HPA male	74.46 $\pm$ 2.35	23.35 $\pm$ 0.84 <sup>a</sup>	17.35 $\pm$ 0.63	<b>6.16</b> $\pm$ 0.37	<b>7.95</b> $\pm$ 0.27	4.86 $\pm$ 0.42	5.62 $\pm$ 0.24 <sup>a</sup>	7.24 $\pm$ 0.55	8.20 $\pm$ 0.64	11.27 $\pm$ 0.50	16.66 $\pm$ 0.51	5
HPA female	<b>80.68</b> $\pm$ 2.96	23.48 $\pm$ 0.75	18.08 $\pm$ 0.67	5.52 $\pm$ 0.27	7.57 $\pm$ 0.39	4.86 $\pm$ 0.49	5.54 $\pm$ 0.25	7.96 $\pm$ 0.60	8.85 $\pm$ 0.74	11.89 $\pm$ 0.69	<b>18.63</b> $\pm$ 0.58	20–23
HP male	57.06 $\pm$ 3.72	<b>19.28</b> $\pm$ 1.24	<b>13.63</b> $\pm$ 0.94	<b>4.75</b> $\pm$ 0.39	<b>6.24</b> $\pm$ 0.59	<b>3.88</b> $\pm$ 0.36	<b>5.09</b> $\pm$ 0.29	<b>5.48</b> $\pm$ 0.58	6.14 $\pm$ 0.56 <sup>a</sup>	8.60 $\pm$ 0.75 <sup>a</sup>	13.04 $\pm$ 0.82	24–26
HP female	<b>62.45</b> $\pm$ 5.59	18.47 $\pm$ 1.54	14.03 $\pm$ 1.21	4.24 $\pm$ 0.37	5.52 $\pm$ 0.56	3.37 $\pm$ 0.23	5.07 $\pm$ 0.39	5.39 $\pm$ 0.66	6.35 $\pm$ 0.69	8.73 $\pm$ 0.89	14.95 $\pm$ 1.50 <sup>a</sup>	23–25
NS male	57.37 $\pm$ 3.73	<b>18.29</b> $\pm$ 2.04	13.04 $\pm$ 1.15 <sup>b</sup>	<b>4.86</b> $\pm$ 0.44	5.73 $\pm$ 0.59 <sup>b</sup>	3.54 $\pm$ 0.43 <sup>b</sup>	<b>5.37</b> $\pm$ 0.45	4.84 $\pm$ 0.65 <sup>a</sup>	5.56 $\pm$ 0.67	8.28 $\pm$ 0.60	12.40 $\pm$ 0.90	20
NS female	<b>63.16</b> $\pm$ 4.14	17.97 $\pm$ 1.05	13.66 $\pm$ 0.86 <sup>b</sup>	4.36 $\pm$ 0.52	5.14 $\pm$ 0.39 <sup>b</sup>	3.43 $\pm$ 0.31 <sup>b</sup>	5.29 $\pm$ 0.28	4.83 $\pm$ 0.62	5.80 $\pm$ 0.60	8.80 $\pm$ 0.85	<b>14.63</b> $\pm$ 1.28	19–20
AJS male	61.60 $\pm$ 2.17	<b>19.51</b> $\pm$ 0.75	<b>14.11</b> $\pm$ 0.67	<b>5.03</b> $\pm$ 0.28	<b>6.60</b> $\pm$ 0.33	4.00 $\pm$ 0.37 <sup>b</sup>	5.18 $\pm$ 0.24	7.24 $\pm$ 0.65 <sup>b</sup>	<b>7.67</b> $\pm$ 0.69	9.45 $\pm$ 0.77 <sup>b</sup>	13.78 $\pm$ 0.76	35–39
AJS female	<b>67.14</b> $\pm$ 2.57	19.24 $\pm$ 0.86	14.42 $\pm$ 0.76	4.65 $\pm$ 0.27	6.07 $\pm$ 0.34	3.74 $\pm$ 0.39 <sup>b</sup>	5.28 $\pm$ 0.24	6.40 $\pm$ 0.73 <sup>b</sup>	7.20 $\pm$ 0.93	9.71 $\pm$ 0.82 <sup>b</sup>	15.23 $\pm$ 1.17	32–36
SJS male	59.24 $\pm$ 2.59	18.30 $\pm$ 0.75 <sup>a</sup>	12.92 $\pm$ 0.60	4.76 $\pm$ 0.27 <sup>b</sup>	5.97 $\pm$ 0.28	3.73 $\pm$ 0.23	4.98 $\pm$ 0.29	6.69 $\pm$ 0.46 <sup>a</sup>	7.54 $\pm$ 0.56 <sup>a</sup>	9.26 $\pm$ 0.78	12.44 $\pm$ 0.64	17–18
SJS female	<b>65.86</b> $\pm$ 3.69	18.91 $\pm$ 0.79	14.19 $\pm$ 0.84	4.68 $\pm$ 0.26	5.94 $\pm$ 0.27	4.04 $\pm$ 0.42	5.09 $\pm$ 0.21	6.74 $\pm$ 0.61	7.70 $\pm$ 0.67	10.19 $\pm$ 0.85	14.65 $\pm$ 1.25 <sup>a</sup>	26

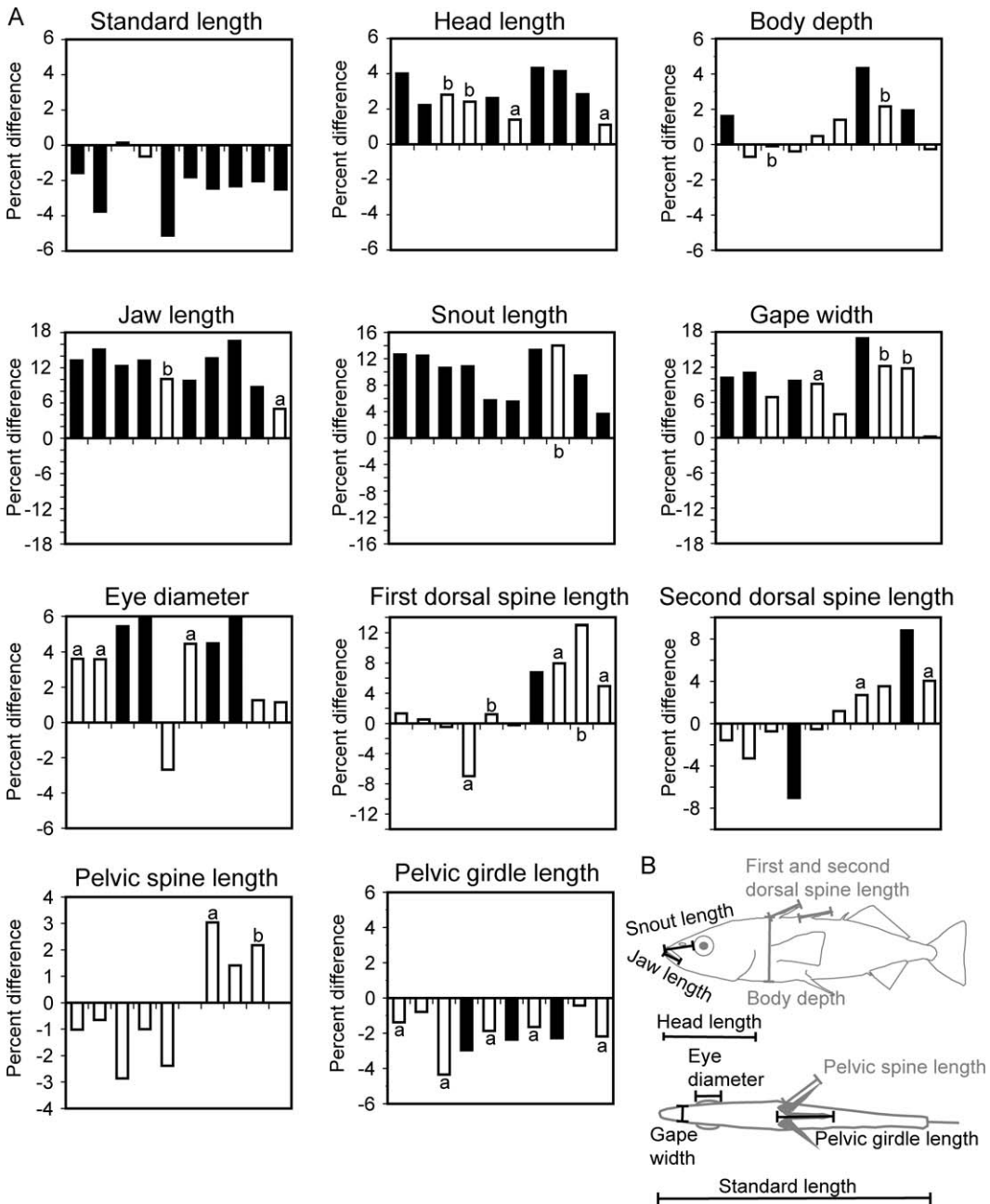


Fig. 4. (A) Summary of sexual dimorphism in ten populations of wild-caught threespine sticklebacks. The differences between male and female means are expressed as a percentage of the female mean for each population, where a negative value indicates that females are larger and a positive value indicates that males are larger. All traits except standard length are corrected for body size. In each panel, bars are arranged from left to right in the order of LCA, LCS, LCH, CL, APA, HPA, HP, NS, AJS, and SJS populations. Black bars indicate that the sexual difference is significant, even after sequential Bonferroni correction. Sexual dimorphism that is significant at  $P < 0.05$ , but not significant after sequential Bonferroni correction is indicated by a above or below each bar. The traits for which sexual dimorphism was not tested due to slope heterogeneity are indicated by b. (B) Morphological traits that show sexual dimorphism in most populations are depicted in gray, while other traits are depicted in black. The upper panel is a schematic figure of a left side of a threespine stickleback, while the lower panel is a schematic figure of a ventral side of a threespine stickleback.

scatter plot of RW1 and RW2 shows that the males and females have distinct clusters along the axis of RW1. Figure 3D shows deformation grids that visualize the fish that have RW1 of  $-0.07$  and RW2 of  $0.07$ , which reflect the male- and female-specific body shape, respectively. This analysis shows that in the males, the head is larger and the base of pectoral fin is located in a more caudal position than in the females. This sexual dimorphism of body shape is not due to the gravidity of females, because the males and females make distinct clusters along the RW1, regardless of whether the females were gravid or not (Fig. 3C).

Females had consistently larger values of body size-adjusted PG length, although sequential Bonferroni correction left only three populations as significantly dimorphic (Table 1; Fig. 4). Sexual dimorphism in dorsal-spine length was variable among populations (Table 1; Fig. 4): the males had longer dorsal spines in the HP and AJS populations, while females had longer dorsal spines in the CL population. No significant sexual dimorphism was seen in pelvic spine length (PS). GRN, left LPN, and right LPN were not sexually dimorphic in any population (Mann–Whitney U-test,  $P > 0.05$ ; Table 2). Although slight left–right asymmetry of LPN was observed in CL females (one sample  $t$ -test,  $P = 0.036$ ) and SJS males (one sample  $t$ -test;  $P = 0.007$ ), with left LPN greater than right LPN, asymmetry (R–L) did not significantly differ between the sexes in any population (Student  $t$ -test,  $P > 0.05$ ). Serration on the first dorsal spine was greater in males in all of the six populations we analyzed (Fig. 2B).

*Sexual dimorphism in laboratory-reared fish.*—None of the morphological traits we analyzed were significantly sexually dimorphic in the pre-breeding fish of both the AxF and AxA crosses (Table 3). However, all body shape traits except PG became larger in males after they started breeding in both the AxF and AxA crosses, regardless of whether they were cultured at high density or low density (Table 3). Sexual dimorphism in SL was not observed in either cross, regardless of whether the fish were in reproductive condition or not.

#### DISCUSSION

Different populations of threespine sticklebacks have shared features of sexual dimorphism: males have larger heads and mouths, while females have larger body sizes and pelvic girdles. Sexual dimorphism in the head and mouth seems to be a general feature in threespine sticklebacks, because adult males have larger

heads than adult females not only in the populations we analyzed, but also in other populations of threespine sticklebacks from Japan (Mori, 1984, 1987a; Mori and Takamura, 2004), Canada (McPhail, 1992; Reimchen and Nosil, 2006), Iceland (Kristjánsson et al., 2002a), and Europe (Kristjánsson et al., 2002b). Similarly, males have larger heads than females in Asian populations of the related species *Pungitius pungitius*, *P. tymensis*, and *P. sinensis* (Kobayashi, 1959; Chae and Yang, 1990). Female-biased sexual dimorphism in body size may be another general feature in sticklebacks. Adult females are larger than adult males not only in the populations we analyzed, but also in other populations of threespine sticklebacks from Japan (Ikeda, 1933; Mori, 1984, 1990; Mori and Takamura, 2004) and Canada (Moodie and Reimchen, 1976; Blouw and Hagen, 1990; Lavin and McPhail, 1993; Reimchen and Nosil, 2006) as well as other species of stickleback such as *Gasterosteus wheatlandi* (Sargent et al., 1984), *Culaea inconstans* (Moodie, 1986), *Apeltes quadracus* (Blouw and Hagen, 1984), *P. pungitius*, *P. tymensis*, and *P. sinensis* (Ikeda, 1933; McKenzie and Keenleyside, 1970). However, exceptional cases of male-biased size dimorphism are reported in lacustrine populations of Canadian threespine sticklebacks (Bentzen and McPhail, 1984; Schluter and McPhail, 1992; Boughman et al., 2005). Adult females also have larger pelvic girdles than adult males in Canadian lacustrine populations (Reimchen and Nosil, 2006).

Larger heads may be adaptive for male threespine sticklebacks. During the breeding season, males use their mouths to collect fibers and sand to build their nests (Wootton, 1984). In addition, biting behavior frequently occurs in male–male antagonistic interactions and during male courtship behavior (Wootton, 1984). In contrast, nest care and biting behavior is rarely observed in female threespine sticklebacks (Wootton, 1984). In addition to divergence in reproductive roles, niche divergence between the sexes may be a factor contributing to sexual dimorphism (Slatkin, 1984; Shine, 1989). During the breeding season, breeding males inhabit benthic zones, while breeding females are prevalent in open water (Reimchen, 1980; Reimchen and Nosil, 2004). Longer jaws and wider gapes are characteristic of sticklebacks in benthic zones and adaptive for feeding on benthic invertebrates (Bentzen and McPhail, 1984; Lavin and McPhail, 1986; Schluter and McPhail, 1992), although snout length is usually shorter in benthic sticklebacks.

Body size does play a significant role in reproduction of threespine sticklebacks. Larger

TABLE 2. GILL RAKER NUMBER (GRN), LEFT AND RIGHT LATERAL PLATE NUMBER (LPN), AND LPN ASYMMETRY (R-L) IN WILD-CAUGHT THREESPINE STICKLEBACKS. MEANS  $\pm$  SD ARE SHOWN.

Population	Male				Female				n	
	GRN	Left LPN	Right LPN	R-L	GRN	Left LPN	Right LPN	R-L		
LCA	22.09 $\pm$ 1.41	33.59 $\pm$ 0.80	33.73 $\pm$ 0.94	0.136 $\pm$ 0.889	22	22.13 $\pm$ 1.33	33.29 $\pm$ 0.75	33.25 $\pm$ 0.68	-0.042 $\pm$ 0.55	24
LCS	16.76 $\pm$ 0.97	5.56 $\pm$ 0.65	5.40 $\pm$ 0.71	-0.160 $\pm$ 0.688	25	16.46 $\pm$ 1.18	5.27 $\pm$ 0.99	5.27 $\pm$ 0.99	0.000 $\pm$ 0.756	22
LCH	18.77 $\pm$ 1.17	15.82 $\pm$ 1.17	15.29 $\pm$ 1.07	-0.529 $\pm$ 1.505	17	18.25 $\pm$ 0.97	16.33 $\pm$ 10.65	16.08 $\pm$ 10.66	-0.250 $\pm$ 2.137	12
CL	21.47 $\pm$ 1.38	6.44 $\pm$ 0.81	6.19 $\pm$ 0.91	-0.25 $\pm$ 0.683	16	21.24 $\pm$ 1.15	6.66 $\pm$ 0.72	6.35 $\pm$ 0.72	-0.310 $\pm$ 0.76	29
APA	21.36 $\pm$ 1.22	34.14 $\pm$ 0.77	33.91 $\pm$ 0.83	-0.273 $\pm$ 1.104	11	21.50 $\pm$ 1.37	33.57 $\pm$ 0.77	33.39 $\pm$ 0.50	-0.214 $\pm$ 0.63	28
HPA	21.80 $\pm$ 1.79	33.40 $\pm$ 0.55	33.80 $\pm$ 0.450	0.400 $\pm$ 0.548	5	21.96 $\pm$ 1.30	32.82 $\pm$ 2.97	33.36 $\pm$ 0.73	0.536 $\pm$ 2.76	28
HP	22.16 $\pm$ 1.28	33.92 $\pm$ 0.70	33.85 $\pm$ 0.67	-0.200 $\pm$ 0.616	20	21.52 $\pm$ 1.04	33.81 $\pm$ 0.81	34.00 $\pm$ 0.84	-0.059 $\pm$ 0.748	17
NS	21.55 $\pm$ 0.94	34.45 $\pm$ 0.76	34.55 $\pm$ 0.76	0.100 $\pm$ 0.64	20	21.00 $\pm$ 1.37	34.05 $\pm$ 0.61	34.10 $\pm$ 0.72	0.050 $\pm$ 0.686	20
AJS	25.11 $\pm$ 1.43	33.73 $\pm$ 0.67	33.50 $\pm$ 0.93	-0.333 $\pm$ 0.856	21	24.86 $\pm$ 1.14	33.76 $\pm$ 0.77	33.73 $\pm$ 0.53	0.000 $\pm$ 0.798	23
SJS	25.17 $\pm$ 1.89	33.67 $\pm$ 0.91	33.22 $\pm$ 0.81	-0.444 $\pm$ 0.616	18	25.58 $\pm$ 1.63	33.35 $\pm$ 0.63	33.16 $\pm$ 0.80	-0.200 $\pm$ 0.645	25

TABLE 3. MORPHOLOGY AND SEXUAL DIMORPHISM IN LABORATORY-REARED THREESPINE STICKLEBACKS. MEANS  $\pm$  SD ARE SHOWN. THE STATISTICAL SIGNIFICANCE OF  $\alpha = 0.05$  WAS ADJUSTED BY SEQUENTIAL BONFERRONI CORRECTION TO AVOID A TYPE I ERROR, BECAUSE WE CONDUCTED 55 COMPARISONS IN THIS TABLE. ALTHOUGH ABSOLUTE VALUES OF MORPHOLOGICAL TRAITS ARE SHOWN IN THE TABLE, BOLD LETTERS INDICATE THE SEX THAT HAS A SIGNIFICANTLY LARGER BODY-SIZE CORRECTED VALUE FOR THE TRAIT EVEN AFTER SEQUENTIAL BONFERRONI CORRECTION. THE SUPERScript A INDICATES THAT THERE IS A DIFFERENCE BETWEEN THE SEXES IN THE TRAIT AT  $P < 0.05$ , BUT THIS SIGNIFICANCE IS NOT SIGNIFICANT AFTER SEQUENTIAL BONFERRONI CORRECTION. WHEN SLOPE HETEROGENEITY (INDICATED BY SUPERScript b) WAS DETECTED FOR A TRAIT, THE PRESENCE OR ABSENCE OF THE SEXUAL DIMORPHISM WAS NOT DETERMINED.

	SL				HL				BD				JL				SnL				CW				ED				IDS				2DS				PS				PG				n
	SL	HL	BD	JL	SnL	CW	ED	IDS	2DS	PS	PG	SL	HL	BD	JL	SnL	CW	ED	IDS	2DS	PS	PG	SL	HL	BD	JL	SnL	CW	ED	IDS	2DS	PS	PG												
Pre-breeding AxF male	32.94 $\pm$ 4.27	10.94 $\pm$ 1.50	7.27 $\pm$ 0.98	2.09 $\pm$ 0.39	2.66 $\pm$ 0.47	1.85 $\pm$ 0.29 <sup>b</sup>	3.50 $\pm$ 0.47	3.42 $\pm$ 0.57	4.02 $\pm$ 0.60	5.76 $\pm$ 0.76	7.13 $\pm$ 0.11	16																																	
Pre-breeding AxF female	31.96 $\pm$ 3.94	10.58 $\pm$ 1.22	2.06 $\pm$ 0.79	1.93 $\pm$ 0.32	2.53 $\pm$ 0.44	1.86 $\pm$ 0.15 <sup>b</sup>	3.29 $\pm$ 0.30	3.13 $\pm$ 0.58	3.92 $\pm$ 0.59	5.88 $\pm$ 0.80	7.10 $\pm$ 0.95	12																																	
Breeding AxF male	50.91 $\pm$ 3.54	<b>17.58</b> $\pm$ <b>1.50</b>	<b>11.62</b> $\pm$ <b>0.95</b>	3.77 $\pm$ 0.60 <sup>b</sup>	<b>4.84</b> $\pm$ <b>0.61</b>	3.65 $\pm$ 0.45 <sup>b</sup>	<b>5.12</b> $\pm$ <b>0.40</b>	<b>4.09</b> $\pm$ <b>0.34</b>	<b>4.90</b> $\pm$ <b>0.43</b>	<b>7.12</b> $\pm$ <b>0.40</b>	11.30 $\pm$ 0.84	15																																	
Breeding AxF female	49.64 $\pm$ 3.19	15.48 $\pm$ 1.00	10.89 $\pm$ 0.66	3.04 $\pm$ 0.24 <sup>a</sup>	3.93 $\pm$ 0.36	3.31 $\pm$ 0.26 <sup>b</sup>	4.78 $\pm$ 0.29	3.30 $\pm$ 0.35	4.03 $\pm$ 0.37	5.0 $\pm$ 0.56	11.24 $\pm$ 0.92	20																																	
Pre-breeding AxA male	26.03 $\pm$ 2.19	8.32 $\pm$ 0.76	5.82 $\pm$ 0.62 <sup>b</sup>	1.98 $\pm$ 0.33	2.15 $\pm$ 0.28	1.68 $\pm$ 0.25	2.73 $\pm$ 0.22	2.39 $\pm$ 0.42	3.07 $\pm$ 0.40	4.68 $\pm$ 0.50 <sup>b</sup>	5.68 $\pm$ 0.59 <sup>a</sup>	23																																	
Pre-breeding AxA female	25.59 $\pm$ 1.73	8.33 $\pm$ 0.53	5.66 $\pm$ 0.38 <sup>b</sup>	1.87 $\pm$ 0.27	2.22 $\pm$ 0.19 <sup>a</sup>	1.61 $\pm$ 0.16	2.78 $\pm$ 0.22	2.48 $\pm$ 0.42	2.93 $\pm$ 0.30	4.62 $\pm$ 0.30 <sup>b</sup>	5.33 $\pm$ 0.37	18																																	
High-density AxA male	48.18 $\pm$ 4.57	<b>16.64</b> $\pm$ <b>1.84</b>	<b>11.09</b> $\pm$ <b>1.16</b>	3.86 $\pm$ 0.63 <sup>b</sup>	<b>5.17</b> $\pm$ <b>0.64</b>	<b>3.43</b> $\pm$ <b>0.37</b>	<b>4.88</b> $\pm$ <b>0.43</b>	<b>4.07</b> $\pm$ <b>0.47</b>	<b>5.00</b> $\pm$ <b>0.54</b>	<b>6.02</b> $\pm$ <b>0.71</b>	7.78 $\pm$ 1.05	16																																	
High-density AxA female	46.97 $\pm$ 2.56	15.06 $\pm$ 0.95	8.59 $\pm$ 0.59	3.43 $\pm$ 0.28 <sup>b</sup>	4.35 $\pm$ 0.39	3.00 $\pm$ 0.28	4.63 $\pm$ 0.23	3.46 $\pm$ 0.38	4.32 $\pm$ 0.40	2.30 $\pm$ 0.33	9.85 $\pm$ 0.69 <sup>a</sup>	22-24																																	
Low-density AxA male	58.84 $\pm$ 2.95	<b>21.27</b> $\pm$ <b>1.30</b>	<b>14.24</b> $\pm$ <b>0.69</b>	<b>5.18</b> $\pm$ <b>0.43</b>	<b>6.64</b> $\pm$ <b>0.46</b>	<b>4.01</b> $\pm$ <b>0.31</b>	<b>5.95</b> $\pm$ <b>0.20</b>	<b>5.19</b> $\pm$ <b>0.58</b>	5.95 $\pm$ 0.53 <sup>a</sup>	3.25 $\pm$ 0.65 <sup>a</sup>	10.99 $\pm$ 0.80	8																																	
Low-density AxA female	56.97 $\pm$ 8.56	18.47 $\pm$ 1.24	11.67 $\pm$ 0.57	4.02 $\pm$ 0.28	5.56 $\pm$ 0.42	3.41 $\pm$ 0.19	5.40 $\pm$ 0.23	4.43 $\pm$ 0.52	5.38 $\pm$ 0.49	3.73 $\pm$ 0.53	12.56 $\pm$ 0.69 <sup>a</sup>	41																																	

females are suggested to have higher reproductive success both because they produce more eggs and are preferred by courting males. For example, there exists a positive correlation between female body length and fecundity in many wild populations (Wootton, 1973; Mori, 1987b; Baker et al., 1998; for a review, see Baker, 1994). However, it should also be noted that a negative association has been suggested between egg number and egg size in studies of wild-caught sticklebacks from Alaska (Baker et al., 1998, 2005), suggesting a potential trade-off between offspring number and the quality of zygotes (Smith and Fretwell, 1974; Wootton, 1979; Parker and Begon, 1986; Stearns, 1992). Male preference for larger females is prevalent in stickleback populations and can be a selective force (Rowland, 1989a, 1994; Kraak and Bakker, 1998). However, in one of the Canadian lacustrine stickleback species pairs, limnetic males prefer smaller females (Albert and Schluter, 2004), and in this lake the limnetic females are smaller than the limnetic males (Bentzen and McPhail, 1984; Schluter and McPhail, 1992; Boughman et al., 2005).

Female threespine sticklebacks also prefer larger males in several populations (Moodie, 1982; Rowland, 1989b). Larger males are likely to succeed in male–male contests for nesting territories (Rowland, 1989c; Dufresne et al., 1990; Candolin, 1998) and less likely to be victims of sneaking than smaller males (Parker, 1992; Lurgiader et al., 2001; Zbinden et al., 2001). In addition, the presence of paternal care may increase male body size, because size has high advantage in guarding nests (Parker, 1992). However, there are also advantages for smaller males because they can mature and start breeding earlier than larger males (Andersson, 1994). In field studies of threespine sticklebacks, it was found that smaller males start breeding earlier, occupy better breeding territories, and eventually achieve higher reproductive success than larger males, which breed later (Mori, 1993; Candolin and Voigt, 2003). Males may be able to attain higher reproductive success by investing more energy into territoriality, nesting, and parental care rather than allocating energy to their own growth. Further research on the balance between different components of male and female reproductive success will lead to a better understanding of the evolution of sexual dimorphism in body size in threespine sticklebacks.

Larger pelvic girdles in females may be adaptive. Because the pelvic girdle is a bony structure covering a part of the ventral surface of the fish (Bell and Foster, 1994), it may be a primary defensive armor to protect the eggs

in the abdominal cavity. Other armor structures such as dorsal and pelvic spines show various patterns of sexual dimorphism. Females in Boulton Lake have a greater mean spine number than the males (Reimchen, 1980), and this is attributed to divergent predation regimes between the sexes: breeding males usually inhabit the benthic zones and are likely to be preyed upon by benthic invertebrates, while breeding females are prevalent in open water and subject to avian predation (Reimchen and Nosil, 2004). In the present investigation, we did not see any great variation in spine number or a consistent pattern in sexual differences of dorsal-spine length. However, dorsal spines are more serrated in males than in females across multiple populations (Ikeda, 1937). It is suggested that the serration makes penetration of the dorsal spine into the soft tissues of the mouth of a predator more difficult when a stickleback is trapped in its mouth (Gross, 1978). Besides protection against predators, male dorsal spines have another function; the males raise their dorsal spines and prick the females during courtship (ter Pelkwijk and Tinbergen, 1937; Wilz, 1970). Despite the consistent pattern that males have more serrated spines than females, little is known about the role of serration in stickleback reproductive behavior. Although lateral plate number is sexually dimorphic in a related species, *Gasterosteus wheatlandi* (Sargent et al., 1984), and left–right asymmetry of lateral plate number is more frequent in females than in males in a Canadian population of threespine sticklebacks (Reimchen and Nosil, 2001), in the present investigation we did not observe sexual dimorphism in lateral plate number or lateral plate asymmetry. Further research is required to understand the relationship between sexual dimorphism and variation in fitness and reproductive success as well as the ecological factors that contribute to variation in sexual dimorphism in skeletal armor.

Laboratory-reared threespine sticklebacks had patterns of sexual dimorphism similar to those found in wild-caught fish. Since the males and the females were grown under similar environmental conditions, these data strongly suggest that sexual dimorphism in body shape has a genetic basis. In contrast to body shape, sexual dimorphism of body size was not observed in our laboratory-reared fish. This suggests that environmental factors are more important for the expression of sexual dimorphism in body size than of body shape in sticklebacks. This is consistent with previous studies, which suggest that body size is greatly influenced by rearing environment (Mori and Nagoshi, 1987; Wootton, 1994; McKinnon et al., 2004), although it is

genetically controlled to some extent (McPhail, 1977; Snyder and Dingle, 1989, 1990; Snyder, 1991; Colosimo et al., 2004).

Sexual dimorphism in body shape only became significant after the fish started breeding. This suggests that sexual dimorphism in body shape is a secondary sexual character that may be regulated by reproductive hormones. Although sex in threespine sticklebacks is genetically determined (Peichel et al., 2004), it is currently unknown what genes or hormones might regulate sexual dimorphism of body shape in these fish. Further analysis of the genetic and developmental mechanisms that underlie sexual dimorphism in threespine sticklebacks will be possible by using the recently established genomic tools (Peichel et al., 2001; Peichel, 2005) and will provide a complement to ecological studies to discern the functional significance of sexual dimorphism in threespine sticklebacks.

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#### LITERATURE CITED

- ALBERT, A. Y. K., AND D. SCHLUTER. 2004. Reproductive character displacement of male stickleback mate preference: reinforcement or direct selection? *Evolution* 58:1099–1107.
- ANDERSSON, M. 1994. *Sexual Selection*. Princeton University Press, Princeton, New Jersey.
- ARAI, T., A. GOTO, AND N. MIYAZAKI. 2003. Migratory history of the threespine stickleback *Gasterosteus aculeatus*. *Ichthyological Research* 50:9–14.
- BAKER, J. A. 1994. Life history variation in female threespine stickleback, p. 144–187. *In: The Evolutionary Biology of the Threespine Stickleback*. M. A. Bell and S. A. Foster (eds.). Oxford University Press, New York.
- BAKER, J. A., W. A. CRESKO, S. A. FOSTER, AND D. C. HEINS. 2005. Life-history differentiation of benthic and limnetic ecotypes in a polytypic population of threespine stickleback (*Gasterosteus aculeatus*). *Evolutionary Ecology Research* 7:121–131.
- BAKER, J. A., S. A. FOSTER, D. C. HEINS, M. A. BELL, AND R. W. KING. 1998. Variation in female life-history traits among Alaskan populations of the threespine stickleback, *Gasterosteus aculeatus* L. (Pisces: Gasterosteidae). *Biological Journal of the Linnean Society* 63:141–159.
- BAKKER, T. C. M., AND B. MUNDWILDER. 1999. Pectoral fin size in a fish species with parental care: a condition-dependent sexual trait revealing infection status. *Freshwater Biology* 41:543–551.
- BELL, M. A., AND S. A. FOSTER. 1994. *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press, New York.
- BENTZEN, P., AND J. D. MCPHAIL. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): specialization for alternative trophic niches in the Enos Lake species pair. *Canadian Journal of Zoology* 62:2280–2286.
- BLAIS, J., C. RICO, AND L. BERNATCHEZ. 2004. Non-linear effects of female mate choice in wild threespine sticklebacks. *Evolution* 58:2498–2510.
- BLOUW, D. M., AND D. W. HAGEN. 1984. The adaptive significance of dorsal spine variation in the four-spine stickleback, *Apeltes quadracus*. III. Correlated traits and experimental evidence on predation. *Heredity* 53:371–382.
- BLOUW, D. M., AND D. W. HAGEN. 1990. Breeding ecology and evidence of reproductive isolation of a widespread stickleback fish (*Gasterosteidae*) in Nova Scotia, Canada. *Biological Journal of the Linnean Society* 39:195–217.
- BOUGHMAN, J. W., H. D. RUNDLE, AND D. SCHLUTER. 2005. Parallel evolution of sexual isolation in sticklebacks. *Evolution* 59:361–373.
- CALDECUTT, W. J., AND D. C. ADAMS. 1998. Morphometrics of trophic osteology in the threespine stickleback, *Gasterosteus aculeatus*. *Copeia* 1998:827–838.
- CALDECUTT, W. J., M. A. BELL, AND J. A. BUCKLAND-NICKS. 2001. Sexual dimorphism and geographic variation in dentition of threespine stickleback, *Gasterosteus aculeatus*. *Copeia* 2001:936–944.
- CANDOLIN, U. 1998. Reproduction under predation risk and the trade-off between current and future reproduction in the threespine stickleback. *Proceedings of the Royal Society of London. Series B* 265:1171–1175.
- CANDOLIN, U., AND H.-R. VOIGT. 2003. Size-dependent selection on arrival times in sticklebacks: why small males arrive first. *Evolution* 57:862–871.

- CHAE, B. S., AND H. J. YANG. 1990. Sexual dimorphism in eightspine stickleback, *Pungitius sinensis*: Gasterosteidae. Korean Journal of Zoology 33:260–265.
- COLOSIMO, P. F., C. L. PEICHEL, K. NERENG, B. K. BLACKMAN, M. D. SHAPIRO, D. SCHLUTER, AND D. M. KINGSLEY. 2004. The genetic architecture of parallel armor plate reduction in threespine sticklebacks. PLoS Biology 2:635–641.
- DARWIN, C. 1874. The Descent of Man; and Selection in Relation to Sex. Humbolt, New York.
- DUFRESNE, F., G. J. FITZGERALD, AND S. LACHANCE. 1990. Age and size-related differences in reproductive success and reproductive costs in threespine sticklebacks (*Gasterosteus aculeatus*). Behavioral Ecology 1:140–147.
- FOSTER, S. A. 1988. Diversionary displays of paternal stickleback: defenses against cannibalistic groups. Behavioral Ecology and Sociobiology 22:335–340.
- GROSS, H. P. 1978. Natural selection by predators on the defensive apparatus of the three-spined stickleback, *Gasterosteus aculeatus* L. Canadian Journal of Zoology 56:398–413.
- HAGEN, D. W. 1967. Isolating mechanisms in three-spine sticklebacks (*Gasterosteus*). Journal of the Fisheries Research Board of Canada 24:1637–1692.
- HAGLUND, T. R., D. G. BUTH, AND R. LAWSON. 1992. Allozyme variation and phylogenetic relationships of Asian, North American, and European populations of the threespine stickleback, *Gasterosteus aculeatus*. Copeia 1992:432–443.
- HIGUCHI, M., AND A. GOTO. 1996. Genetic evidence supporting the existence of two distinct species in the genus *Gasterosteus* around Japan. Environmental Biology of Fishes 47:1–16.
- IKEDA, K. 1933. The distribution and morphological variations of the stickleback in Japan. Zoological Magazine (Tokyo) 46:553–572.
- IKEDA, K. 1937. Effect of castration on the secondary sexual characters of anadromous three-spined stickleback, *Gasterosteus aculeatus* (L.). Japanese Journal of Zoology 5:135–157.
- ISHIKAWA, M., AND S. MORI. 2000. Mating success and male courtship behaviours in three populations of the threespine stickleback. Behaviour 137:1065–1680.
- KOBAYASHI, H. 1959. Cross-experiments with three species of stickleback, *Pungitius pungitius* (L.), *Pungitius tymensis* (Nikolsky), and *Pungitius sinensis* (Guichenot), with special reference to their systematic relationship. Journal of Hokkaido Gakugei University, Section B 10:363–384.
- KRAAK, S. B. M., AND T. C. M. BAKKER. 1998. Mutual mate choice in sticklebacks: attractive males choose big females, which lay big eggs. Animal Behaviour 56:859–866.
- KRISTJANSSON, B. K., S. SKULASON, AND D. L. G. NOAKES. 2002a. Morphological segregation of Icelandic threespine stickleback (*Gasterosteus aculeatus* L.). Biological Journal of the Linnean Society 76:247–257.
- KRISTJANSSON, B. K., S. SKULASON, AND D. L. G. NOAKES. 2002b. Rapid divergence in a recently isolated population of threespine stickleback (*Gasterosteus aculeatus* L.). Evolutionary Ecology Research 4:659–672.
- KUME, M., T. KITAMURA, H. TAKAHASHI, AND A. GOTO. 2005. Distinct spawning migration patterns in sympatric Japan Sea and Pacific Ocean forms of threespine stickleback *Gasterosteus aculeatus*. Ichthyological Research 52:189–193.
- LANDE, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:1210–1226.
- LARGIADER, C. R., V. FRIES, AND T. C. M. BAKKER. 2001. Genetic analysis of sneaking and egg-thievery in a natural population of the three-spined stickleback (*Gasterosteus aculeatus* L.). Heredity 86:459–468.
- LAVIN, P. A., AND J. D. MCPHAIL. 1986. Adaptive divergence of trophic phenotype among freshwater populations of the threespine stickleback (*Gasterosteus aculeatus*). Canadian Journal of Fisheries and Aquatic Sciences 43:2455–2463.
- LAVIN, P. A., AND J. D. MCPHAIL. 1993. Parapatric lake and stream sticklebacks on northern Vancouver Island: disjunct distribution or parallel evolution? Canadian Journal of Zoology 71:11–17.
- MCKENZIE, J. A., AND M. H. A. KEENLEYSIDE. 1970. Reproductive behavior of ninespine stickleback (*Pungitius pungitius* (L.)) in South Bay, Manitoulin Island, Ontario. Canadian Journal of Zoology 48:55–61.
- MCKINNON, J. S., AND H. D. RUNDLE. 2002. Speciation in nature: the threespine stickleback model systems. Trends in Ecology and Evolution 17:480–488.
- MCKINNON, J. S., S. MORI, B. K. BLACKMAN, L. DAVID, D. M. KINGSLEY, L. JAMIESON, J. CHOU, AND D. SCHLUTER. 2004. Evidence for ecology's role in speciation. Nature 429:294–298.
- MCPHAIL, J. D. 1977. Inherited interpopulation differences in size at first reproduction in threespine stickleback, *Gasterosteus aculeatus*. Heredity 38:53–60.
- MCPHAIL, J. D. 1992. Ecology and evolution of sympatric stickleback (*Gasterosteus*): evidence for a species-pair in Paxton Lake, Texada Island, British Columbia. Canadian Journal of Zoology 70:361–369.
- MOODIE, G. E. E. 1982. Why asymmetric mating preferences may not show the direction of evolution. Evolution 36:1096–1097.
- MOODIE, G. E. E. 1986. The population biology of *Culaea inconstans*, the brook stickleback, in a small prairie lake. Canadian Journal of Zoology 64:1709–1717.
- MOODIE, G. E. E., AND T. E. REIMCHEN. 1976. Phenetic variation and habitat differences in *Gasterosteus* populations of the Queen Charlotte Islands. Systematic Zoology 25:49–61.
- MORI, S. 1984. Sexual dimorphism of the landlocked three-spined stickleback *Gasterosteus aculeatus microcephalus* from Japan. Japanese Journal of Ichthyology 30:419–425.
- MORI, S. 1987a. Geographical variations in freshwater populations of the three-spined stickleback, *Gaster-*

- osteus aculeatus*, in Japan. Japanese Journal of Ichthyology 34:33–46.
- MORI, S. 1987b. Divergence in reproductive ecology of the three-spined stickleback, *Gasterosteus aculeatus*. Japanese Journal of Ichthyology 34:165–175.
- MORI, S. 1990. Two morphological types in the reproductive stock of the three-spined stickleback, *Gasterosteus aculeatus*, in Lake Harutori, Hokkaido Island. Environmental Biology of Fishes 27:21–31.
- MORI, S. 1993. The breeding system of the three-spined stickleback, *Gasterosteus aculeatus* (forma leiura) with reference to spatial and temporal patterns of nesting activity. Behaviour 126:97–124.
- MORI, S., AND M. NAGOSHI. 1987. Growth and maturity size of the three-spined stickleback in a rearing pool. Bulletin of the Faculty of Fisheries, Mie University 14:1–10.
- MORI, S., AND N. TAKAMURA. 2004. Changes in morphological characteristics of an introduced population of the threespine stickleback *Gasterosteus aculeatus* in Lake Towada, northern Japan. Ichthyological Research 51:295–300.
- ORTÍ, G., M. A. BELL, T. E. REIMCHEN, AND A. MEYER. 1994. Global survey of mitochondrial DNA sequences in the threespine stickleback: evidence for recent migrations. Evolution 48:608–622.
- PARKER, G. A. 1992. The evolution of sexual size dimorphism in fish. Journal of Fish Biology 41, (Supplement B):1–20.
- PARKER, G. A., AND M. BEGON. 1986. Optimal egg size and clutch size: effects of environment and maternal phenotype. American Naturalist 128:573–591.
- PEICHEL, C. L. 2005. Fishing for the secrets of vertebrate evolution in threespine sticklebacks. Developmental Dynamics 234:739–752.
- PEICHEL, C. L., K. S. NERENG, K. A. OHGI, B. L. E. COLE, P. F. COLOSIMO, C. A. BUERKLE, D. SCHLUTER, AND D. M. KINGSLEY. 2001. The genetic architecture of divergence between threespine stickleback species. Nature 414:901–905.
- PEICHEL, C. L., J. A. ROSS, C. K. MATSON, M. DICKSON, J. GRIMWOOD, J. SCHMUTZ, R. M. MYERS, S. MORI, D. SCHLUTER, AND D. M. KINGSLEY. 2004. The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. Current Biology 14:1416–1424.
- POTAPOVA, T. L. 1972. Intraspecific variability of the three-spined stickleback (*Gasterosteus aculeatus* L.). Journal of Ichthyology 12:20–33.
- REIMCHEN, T. E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators? Canadian Journal of Zoology 68:1232–1244.
- REIMCHEN, T. E., AND P. NOSIL. 2001. Lateral plate asymmetry, diet and parasitism in threespine stickleback. Journal of Evolutionary Biology 14:632–645.
- REIMCHEN, T. E., AND P. NOSIL. 2004. Variable predation regimes predict the evolution of sexual dimorphism in a population of threespine stickleback. Evolution 58:1274–1281.
- REIMCHEN, T. E., AND P. NOSIL. 2006. Replicated ecological landscapes and the evolution of morphological diversity among *Gasterosteus* populations from an archipelago on the west coast of Canada. Canadian Journal of Zoology 84:643–654.
- RICE, W. R. 1984. Sex chromosomes and the evolution of sexual dimorphism. Evolution 38:735–742.
- RICE, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.
- ROHLF, J., AND L. F. MARCUS. 1993. A revolution in morphometrics. Trends in Ecology and Evolution 8:129–132.
- ROWLAND, W. J. 1989a. The ethological basis of mate choice in male threespine sticklebacks, *Gasterosteus aculeatus*. Animal Behaviour 38:112–120.
- ROWLAND, W. J. 1989b. Mate choice and the supernormality effect in female sticklebacks (*Gasterosteus aculeatus*). Behavioral Ecology and Sociobiology 24:433–438.
- ROWLAND, W. J. 1989c. The effects of body size, aggression and nuptial coloration on competition in male threespine sticklebacks, *Gasterosteus aculeatus*. Animal Behaviour 37:282–289.
- ROWLAND, W. J. 1994. Proximate determinants of stickleback behaviour: an evolutionary perspective, p. 297–344. In: The Evolutionary Biology of the Threespine Stickleback. M. A. Bell and S. A. Foster (eds.). Oxford University Press, New York.
- SARGENT, R. C., M. A. BELL, W. H. KRUEGER, AND J. V. BAUMGARTNER. 1984. A lateral plate cline, sexual dimorphism, and phenotypic variation in the black-spotted stickleback, *Gasterosteus wheatlandi*. Canadian Journal of Zoology 62:368–376.
- SCHLUTER, D. 2001. Ecology and the origin of species. Trends in Ecology and Evolution 16:372–380.
- SCHLUTER, D., AND J. D. MCPHAIL. 1992. Ecological character displacement and speciation in sticklebacks. American Naturalist 140:85–108.
- SHINE, R. 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. The Quarterly Review of Biology 64:419–461.
- SLATKIN, M. 1984. Ecological causes of sexual dimorphism. Evolution 38:622–630.
- SMITH, C. C., AND S. D. FRETWELL. 1974. The optimal balance between size and number of offspring. American Naturalist 108:499–506.
- SNYDER, R. J. 1991. Migration and life histories of the threespine stickleback: evidence for adaptive variation in growth rate between populations. Environmental Biology of Fishes 31:381–388.
- SNYDER, R. J., AND H. DINGLE. 1989. Adaptive, genetically based differences in life history between estuary and freshwater threespine sticklebacks (*Gasterosteus aculeatus* L.). Canadian Journal of Zoology 67:2448–2454.
- SNYDER, R. J., AND H. DINGLE. 1990. Effects of freshwater and marine overwintering environments on life histories of threespine sticklebacks: evidence for adaptive variation between anadromous and resident freshwater populations. Oecologia 84:386–390.
- STEARNS, S. C. 1992. The Evolution of Life Histories. Oxford University Press, Oxford.
- TER PELKWIJK, J. J., AND N. TINBERGEN. 1937. Eine reizbiologische analyse einiger verhaltensweisen

- von *Gasterosteus aculeatus* L. Zeitschrift für Tierpsychologie 1:193–200.
- TINBERGEN, N. 1951. The Study of Instinct. Oxford University Press, Oxford.
- WALKER, J. A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. Biological Journal of the Linnean Society 61:3–50.
- WILZ, K. J. 1970. Causal and functional analysis of dorsal pricking and nest activity in the courtship of the three-spined stickleback *Gasterosteus aculeatus*. Animal Behaviour 18:115–124.
- WOOTTON, R. J. 1973. Fecundity of the three-spined stickleback, *Gasterosteus aculeatus* (L.). Journal of Fish Biology 5:683–688.
- WOOTTON, R. J. 1976. The Biology of the Stickleback. Academic Press, London.
- WOOTTON, R. J. 1979. Energy costs of egg production and environmental determinants of fecundity in teleost fishes. Symposium of the Zoological Society of London 44:133–159.
- WOOTTON, R. J. 1984. A Functional Biology of Sticklebacks. Croom Helm, London.
- WOOTTON, R. J. 1994. Energy allocation in the threespine stickleback, p. 144–143. *In*: The Evolutionary Biology of the Threespine Stickleback. M. A. Bell and S. A. Foster (eds.). Oxford University Press, Oxford.
- ZBINDEN, M., C. R. LARGIADER, AND T. C. M. BAKKER. 2001. Sperm allocation in the three-spined stickleback. Journal of Fish Biology 59:1287–1297.
- ZELDITCH, M. L., D. L. SWIDERSKI, H. D. SHEETS, AND W. L. FINK. 2004. Geometric Morphometrics for Biologists. Elsevier, California.
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