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Reduction of sexual dimorphism in stream-resident forms of three-spined stickleback *Gasterosteus aculeatus*

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Abstract

Sexual dimorphism in geometric body shape and external morphology was compared between marine and stream-resident forms of three-spined stickleback *Gasterosteus aculeatus* collected from North America and Japan. Some aspects of sexual dimorphism were shared between ecotypes: males had larger heads than females with no significant effect of ecotype on the magnitude of sexual dimorphism. By contrast, a significant sex-by-ecotype interaction was found for body depth. Males tended to have deeper bodies than females in both forms, but the magnitude of sexual dimorphism was reduced in stream-resident forms. Although females were generally larger in standard length and had larger pelvic girdles, significant sexual dimorphism in these traits was not consistently found across populations or ecotypes. These results suggest that some aspects of sexual dimorphism were shared between ecotypes, while others were unique to each population. The results further suggest that ecology may influence the evolution of sexual dimorphism in some external morphological traits, such as body depth.

Keywords

ecology; evolution; geometric morphometrics; sex chromosome; sexual selection

INTRODUCTION

Phenotypic traits that enhance fitness in one sex do not necessarily increase fitness in the other sex. When selection on males and females differs, sexual dimorphism can evolve. Because different trait values can differentially affect mating success in the two sexes, sexual selection is one of the most important drivers of sexual dimorphism (Darwin, 1871). Ecology can also influence the evolution of sexual dimorphism. For example, when the sexes exploit divergent ecological resources, divergent adaptation can also result in sexual dimorphism (Selander, 1966; Slatkin, 1984; Shine, 1989; Dayan *et al.*, 1990; Temeles *et al.*,

2000; Bolnick & Doebeli, 2003; Butler *et al.*, 2007). Even in cases where sexual selection is the primary cause of sexual dimorphism, selective pressures or constraints derived from predation, locomotion, nutrition and sensory perception can influence the evolutionary trajectory and magnitude of sexual dimorphism (Endler, 1980, 1992; Quinn *et al.*, 2001; Boughman, 2002, 2007; Reimchen & Nosil, 2004; Langerhans *et al.*, 2005; Hendry *et al.*, 2006). Comparing patterns of sexual dimorphism between different ecotypes is a first step towards a better understanding of the ecological factors that contribute to the evolution of sexual dimorphism.

The three-spined stickleback *Gasterosteus aculeatus* L. 1758 species complex contains a variety of ecotypes that have adapted to diverse environments (Bell & Foster, 1994), providing a model for investigating the ecological factors that facilitate or constrain the evolution of sexual dimorphism in fishes. Previous studies have revealed that male and female *G. aculeatus* differ in several morphological traits (Jones *et al.*, 2006; Reimchen & Nosil, 2006; Kitano *et al.*, 2007; Aguirre *et al.*, 2008; Albert *et al.*, 2008; Spoljaric & Reimchen, 2008; Aguirre & Akinpelu, 2010). Some traits, such as head length and jaw length, are sexually dimorphic in virtually all populations of *G. aculeatus* analysed thus far. In all cases, males have larger heads and jaws. This may be due to divergent reproductive roles because males construct nests with their jaws, and to ecological niche divergence, because males exploit benthic habitats during the breeding season (Kitano *et al.*, 2007; Spoljaric & Reimchen, 2008). By contrast, sexual dimorphism in dorsal and pelvic spine length is quite variable among populations and may result from a combination of sexual selection and niche divergence between the sexes (Reimchen & Nosil, 2004; Kitano *et al.*, 2007, 2009).

Spoljaric & Reimchen (2008) compared sexual dimorphism in body shape among a variety of ecotypes of Canadian *G. aculeatus* and found that *G. aculeatus* in large lakes as well as in marine habitats tend to exhibit a greater magnitude of sexual dimorphism than *G. aculeatus* inhabiting shallow ponds. Spoljaric & Reimchen (2008) suggested that a reduction of sexual dimorphism in populations of *G. aculeatus* inhabiting shallow ponds may result from a lack of ecological opportunity. Similarly, Albert *et al.* (2008) reported a lower magnitude of sexual dimorphism in a benthic (bottom-dwelling lacustrine) population of *G. aculeatus* than in an ancestral marine population. No previous studies, however, have comprehensively compared sexual dimorphism between multiple marine and stream-resident populations of *G. aculeatus*. Because freshwater colonization by marine *G. aculeatus* has occurred independently in different geographical regions (Haglund *et al.*, 1992; McKinnon *et al.*, 2004; Colosimo *et al.*, 2005), marine and stream-resident *G. aculeatus* represent multiple replicates of two contrasting ecotypes. In the present investigation, geometric body shape and external morphological traits were compared between multiple marine and stream-resident populations collected from North America and Japan to further investigate the role of ecology in the evolution of sexual dimorphism in *G. aculeatus*. Most of the marine and stream-resident populations analysed here were previously investigated to characterize parallel evolution of body size (McKinnon *et al.*, 2004), armour plates (Schluter *et al.*, 2004; Colosimo *et al.*, 2005; Kitano *et al.*, 2008) and thyroid hormone levels (Kitano *et al.*, 2010). Previous phylogenetic studies demonstrated that Japanese and North American stream-resident populations are independently derived from Japanese and North American marine ancestors, respectively (McKinnon *et al.*, 2004; Colosimo *et al.*, 2005).

MATERIALS AND METHODS

All wild-caught *G. aculeatus* were sampled during the breeding season with minnow traps and seines, as described previously (Fig. 1 and Table I) (Mori, 1987; Kitano *et al.*, 2007, 2008, 2009, 2010). For body shape analysis, digital pictures were taken of the left side of

anaesthetized and straightened fish. A tripod was used to take each picture at a fixed 90° angle. Fourteen landmarks were chosen to capture the overall body shape (Kume *et al.*, 2010) and digitized with tpsDig2 (Rohlf, 2006a) [Fig. 2(a)]: (1) anterior tip of upper lip, (2) anterior base of the first dorsal spine, (3) anterior base of the second dorsal spine, (4) anterior base of the third dorsal spine, (5) dorsal base of caudal fin, (6) caudal end of caudal keel, (7) ventral base of caudal fin, (8) anterior base of anal spine, (9) anterior base of pelvic spine, (10) ventral border of operculum, (11) posterior edge of angular, (12) centre of eye, (13) dorsal base of pectoral fin and (14) ventral base of pectoral fin. All images were aligned, rotated and scaled by the orthogonal least squares superimposition with tpsRelw (Rohlf, 2006b). Relative warp scores (RW), which are principal components of partial warp scores (Zelditch *et al.*, 2004), were calculated with tpsRelw. For relative warp analysis, all fish images ($n = 354$) were pooled. Centroid size was also calculated with tpsRelw software and normalized with $\log_{10}$ -transformation before statistical analysis. Next, for each sex of each population, the mean relative warp scores and centroid size were calculated and used for further analysis. Using ANCOVA, mean relative warp scores were tested for the effects of sex, ecotype, centroid size and their interactions. Because there was no significant interaction between centroid size and any other factors for RW scores (except RW3; interaction between centroid size and ecotype; $F_{1,22} = 7.5$, $P < 0.05$), interactions with centroid size were excluded from the final model for the analysis of RW1, 2 and 4 (Table II).

Standard length (L_S), head length, body depth, upper jaw length, snout length, snout width, eye diameter, first dorsal spine length, second dorsal spine length, pelvic spine length and pelvic girdle length were measured from the left side of formalin-fixed samples with vernier callipers (Fig. 3), as described previously (Kitano *et al.*, 2007). The mean values of each trait were calculated for each sex of each population (Table III). To test for sexual dimorphism, L_S was $\log_{10}$ -transformed ($\log_{10} L_S$) and analysed with ANOVA; all other traits were $\log_{10}$ -transformed, then ANCOVA was conducted with $\log_{10} L_S$ as a covariate. When the interaction between sex and $\log_{10} L_S$ was not significant ($P > 0.05$), ANCOVA was conducted without the interaction term to examine sexual dimorphism. When the sex $\times$ $\log_{10} L_S$ interaction was significant (*i.e.* the slope was heterogeneous between sexes), no test was made for sexual dimorphism (Table IV).

To further examine the patterns of sexual dimorphism among ecotypes, the mean trait values for each sex and each population were used to test the effects of sex, ecotype, $\log_{10} L_S$ and their interactions by ANCOVA. Because interactions between $\log_{10} L_S$ and sex or interactions between $\log_{10} L_S$ and ecotype were significant for eye diameter, first dorsal spine length, second dorsal spine length and pelvic spine length, these traits were not tested for sexual dimorphism. For other traits, ANCOVA was conducted without the $\log_{10} L_S$ interaction term to examine the patterns of sexual dimorphism (Table V). To visualize these patterns, body length-adjusted trait values were calculated using a common regression slope across both sexes and both ecotypes; all mean trait values were adjusted to a grand mean of L_S (50.9 mm) by regression (Spoljaric & Reimchen, 2008).

RESULTS

GEOMETRIC MORPHOMETRICS

Marine forms had significantly larger centroid sizes than stream-resident forms: means $\pm$ s.d. of average centroid sizes were 77.2 ± 16.9 for marine females, 71.8 ± 14.0 for marine males, 61.4 ± 7.5 for stream-resident females and 56.9 ± 7.0 for stream-resident males (ANOVA; $F_{1,26} = 12.67$, $P < 0.01$). Although females tended to have larger centroid sizes, the sexual difference was not significant (ANOVA; $F_{1,26} = 1.28$, $P > 0.05$). The interaction

between sex and ecotype was not significant for centroid size (ANOVA; $F_{1,26} = 0.001$, $P > 0.05$).

A scatter plot of RW1 and RW2 revealed that marine males, marine females, stream-resident males and stream-resident females diverged in body shape [Fig. 2(b)]. The first relative warp score (RW1) explained the largest variation in shape (27.08%). RW1 significantly differed between sexes, but not between ecotypes (Table II). In fish with smaller RW1 scores, landmarks 9, 13 and 14 were located at more posterior positions than in fish with larger RW1 scores [see the deformation grids in Fig. 2(b)], suggesting that fish with smaller RW1 scores had larger heads; these fish were males [Fig. 2(b)]. No significant interaction between sex and ecotype was found (Table II), suggesting that the magnitude of sexual dimorphism along RW1 did not differ between ecotypes.

The RW2 explained 16.96% of shape variation. The RW2 differed not only between sexes, but also between ecotypes (Table II). Fish with larger RW2 scores had deeper bodies [see the deformation grids in Fig. 2(b)], suggesting that stream fish had deeper body shapes than marine fish. Therefore, stream-resident forms had deeper bodies than marine forms for each sex, while males had deeper bodies than females for each ecotype. Importantly, the interaction between sex and ecotype was also significant for RW2 (Table II). This indicates that the magnitude of sexual dimorphism along RW2 was reduced in stream-resident forms [Fig. 2(b)].

The RW3 and RW4 explain 13.43 and 10.13% of the variation in shape. The variation along RW3 seemed to reflect artificial body bending, because both the snout tip (landmark 1) and the caudal peduncle end (landmark 6) were dislocated in the same direction [see the deformation grids in Fig. 2(c)]. Furthermore, there was a significant interaction between centroid size and ecotype for RW3 (see Materials and Methods), so sexual dimorphism in RW3 was not analysed further. The RW4 reflected the caudal part, including the shifts in position of the anal spine (landmark 8) and the base of the caudal fin (landmarks 5, 6 and 7) [Fig. 2(a)]. Although there was a significant difference in RW4 between ecotypes, no significant sexual dimorphism or sex-by-ecotype interaction was found (Table II).

MORPHOLOGICAL MEASUREMENTS

Eleven morphological traits were measured from formalin-fixed specimens (Fig. 3 and Table III). First, each trait in each population was tested for the presence of sexual dimorphism (Table IV). The most consistent sexual dimorphism was found for head length: in all populations, including both marine and stream forms, males had larger heads than females. In most populations, males also had larger values in other traits related to head morphology, such as jaw length, snout length, snout width and eye diameter. The L_s and pelvic girdle length were sexually dimorphic in several populations, where females had larger values. Dorsal spine length, pelvic spine length and body depth did not exhibit consistent patterns of sexual dimorphism.

To further investigate these patterns of sexual dimorphism, the effects of size, ecotype, sex and interactions between ecotype and sex were analysed for each trait. Most of the traits examined were different between ecotypes and between sexes (Table V). Importantly, a significant interaction between sex and ecotype was found for body depth. This indicates that sexual dimorphism in body depth was reduced in stream-resident fish, which is consistent with the results of the geometric morphometric analysis. Plots of body length-adjusted trait values visualized the substantial reduction of sexual dimorphism in relative body depth, but not in other traits in stream-resident forms [Fig. 3(b)].

DISCUSSION

These results are mostly consistent with previous studies conducted on sexual dimorphism in a variety of ecotypes of *G. aculeatus* (Jones *et al.*, 2006; Reimchen & Nosil, 2006; Kitano *et al.*, 2007; Aguirre *et al.*, 2008; Albert *et al.*, 2008; Spoljaric & Reimchen, 2008; Aguirre & Akinpelu, 2010). Males had larger heads than females in almost all populations examined thus far. Females were often larger in L_s and had larger pelvic girdles than males, although this pattern of sexual dimorphism was not as consistent as that for dimorphism in head size. Sexual dimorphism in spine lengths was variable among populations. One of the most interesting findings in the present study was that sexual dimorphism in body depth was reduced in stream-resident *G. aculeatus*, suggesting a possible role of ecology in the evolution of sexual dimorphism in body depth.

Body depth can be a target of both natural and sexual selection. In fishes, including *G. aculeatus*, body depth is known to influence swimming performance (Webb, 1984; Taylor & McPhail, 1986; Taylor & Foote, 1991; Walker, 1997; Blake, 2004; Langerhans, 2009; Hendry *et al.*, 2011). Shallower body depth is thought to reduce drag during sustained swimming and thus be favoured in open water (*i.e.* marine) habitats. By contrast, deeper body depth is thought to increase manoeuvrability and burst swimming speed, and thus is favoured in habitats with many hiding places (*i.e.* streams). Therefore, parallel divergence in body depth between marine and stream-resident *G. aculeatus* in multiple geographical regions is probably the result of adaptation to divergent habitats (Schluter *et al.*, 2004). Although the effect of body depth on male reproductive success has not yet been investigated in *G. aculeatus*, body depth is known to be a target of sexual selection in *Oncorhynchus nerka* (Walbaum 1792). Male *O. nerka* with deeper bodies (*i.e.* a larger dorsal hump) are dominant in spawning grounds and can have higher reproductive success than males with shallower bodies (Quinn & Foote, 1994). Rapid water currents and shallow water depth can constrain the evolution of the sexually dimorphic hump (Hendry *et al.*, 2000; Quinn *et al.*, 2001). Thus, body depth in *O. nerka* is under the influence of both natural and sexual selection, highlighting the difficulty in disentangling the effects of these forces on the evolution of sexual dimorphism.

A contrasting pattern of natural selection between habitats might be one of the factors related to the reduction of sexual dimorphism in stream-resident *G. aculeatus*. In small stream habitats, deeper bodies are probably favourable for both male and female *G. aculeatus*, and the optimal trait value of body depth might not differ between sexes. By contrast, males and females in the marine habitats may have different optimal trait values of body depth. It is possible that male marine *G. aculeatus* are exposed to two contrasting selective pressures: natural selection driving the evolution of slender body shape and sexual selection driving the evolution of deeper body shape. If sexual selection does not necessarily favour the evolution of deeper bodies in females, male and female marine *G. aculeatus* are expected to have different optimal trait values. Therefore, the presence of sexual conflict in marine habitats might lead to the evolution of sexual dimorphism in body depth in the ancestral marine *G. aculeatus*, but the absence of sexual conflict in stream habitats might lead to the reduction of sexual dimorphism in derived stream-resident *G. aculeatus*.

A lack of ecological opportunity might be another factor related to the reduction of sexual dimorphism in stream-resident *G. aculeatus*. Reduction of sexual dimorphism has been also found for North American freshwater populations living in shallow lakes or benthic habitats (Albert *et al.*, 2008; Spoljaric & Reimchen, 2008). For example, Spoljaric & Reimchen (2008) reported that ecology influences the magnitude of sexual dimorphism in dorsal fin length and posterior caudal depth. They suggested that the reduction of sexual dimorphism might result from limited niche availability in shallow ponds. When a resource distribution

is unimodal, sexual dimorphism is less likely to evolve as a result of differential adaptation to divergent ecological resources by the sexes (Bolnick & Doebeli, 2003; Bolnick & Lau, 2008). Therefore, as suggested for Canadian *G. aculeatus* (Spoljaric & Reimchen, 2008), stream habitats may have less niche diversity than marine habitats, which might constrain the evolution of sexual dimorphism. Although the ecology of marine *G. aculeatus* is less well known than that of freshwater populations, *G. aculeatus* have been caught in diverse marine habitats (Jones & John, 1978; Quinn & Light, 1989; Williams & Delbeek, 1989; Blouw & Hagen, 1990; Cowen *et al.*, 1991; Kume *et al.*, 2006), suggesting that *G. aculeatus* may exploit diverse ecological habitats in marine environments. One of the best ways to test this hypothesis is to investigate divergence in habitat choice and trophic ecology between the sexes (Reimchen, 1980, 2001; Reimchen *et al.*, 2008). Although ecological differences between the sexes have been demonstrated in lacustrine *G. aculeatus* populations (Reimchen, 1980, 2001; Reimchen *et al.*, 2008), it is not known whether there are ecological differences between the sexes in either stream-resident or marine *G. aculeatus*.

A previous morphological study on a Canadian lacustrine population of *G. aculeatus* suggested that sexual dimorphism could be a source of phenotypic variation for natural selection to act upon during colonization of a novel environment (Albert *et al.*, 2008). This would result in a reduction of sexual dimorphism in the novel environment. Consistent with this prediction, the results presented here suggest that sexual dimorphism along RW2, which reflects body depth, could be a source for adaptive evolution in stream habitats. By contrast, sexual dimorphism along RW1, which reflects head size, is not reduced in the stream-resident ecotype, and thus might not contribute to ecological adaptation during colonization of stream habitats.

Sexual dimorphism in *G. aculeatus* has a genetic basis (Kitano *et al.*, 2007; Albert *et al.*, 2008; Spoljaric & Reimchen, 2008; Leinonen *et al.*, 2011). Because males and females share most of their genes, genetic correlations between the sexes may constrain the evolution of sexual dimorphism (Fisher, 1930; Lande, 1980). Sex-linkage can resolve this genetic constraint, and sex-linkage is predicted to facilitate the evolution of sexual dimorphism (Rice, 1984). A recent linkage mapping study demonstrated that variation in sexually dimorphic traits in *G. aculeatus* maps to the sex chromosomes (Kitano *et al.*, 2009). In addition, mutations in regulatory sequences of genes regulated by sex steroids or sex-determination genes may also contribute to the evolution of sexual dimorphism (Williams & Carroll, 2009). These possibilities should be investigated further in *G. aculeatus*. Additional genetic and ecological research on the evolution of sexual dimorphism is possible in this model organism and will contribute to a better understanding of the evolutionary mechanisms of sexual dimorphism in fishes.

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Fig. 1. Collection sites of marine *Gasterosteus aculeatus* (○) and stream-resident *G. aculeatus* (●). More details of collection sites and sample sizes are given in Table I.

Fig. 2.

Sexual dimorphism in body shape: (a) 14 landmarks (1, anterior tip of upper lip; 2, anterior base of the first dorsal spine; 3, anterior base of the second dorsal spine; 4, anterior base of the third dorsal spine; 5, dorsal base of caudal fin; 6, caudal end of caudal keel; 7, ventral base of caudal fin; 8, anterior base of anal spine; 9, anterior base of pelvic spine; 10, ventral border of operculum; 11, posterior edge of angular; 12, centre of eye; 13, dorsal base of pectoral fin; 14, ventral base of pectoral fin) used for geometric morphometrics, (b) scatter plot of relative warp scores (RW) 1 and RW2 and deformation grids indicating the body shape change along RW1 and RW2; to increase visibility, extreme values of RW1 and RW2 were used and (c) scatter plot of RW3 and RW4 and deformation grids indicating the body shape change along RW3 and RW4; to increase visibility, extreme values of RW3 and RW4

were used (Δ , marine males; $\bigcirc$, marine females; $\blacktriangle$, stream-resident males; $\bullet$, stream-resident females). The numbers next to the symbols correspond to the population number indicated in Fig. 1 and Table I.

Fig. 3.

Sexual dimorphism in morphological traits: (a) traits measured are indicated on schematic figures of the left side of a *Gasterosteus aculeatus* (left panel) and the ventral side of a *G. aculeatus* (right panel) and (b) standard length-adjusted trait values. The numbers next to the symbols correspond to the population number indicated in Fig. 1 and Table I (Δ, marine males; ○, marine females; ▲, stream-resident males; ●, stream-resident females). The horizontal bars indicate the means of each sex of each ecotype. Because interaction terms in ANCOVA were significant (*i.e.* the slope was heterogeneous between sexes or ecotypes) for eye diameter, first dorsal spine length, second dorsal spine length and pelvic spine length, these traits were not tested for sexual dimorphism and are not shown here.

Table 1

Sampling sites, year of collection and sample size (*n*)

Population	Ecotype	Population number in the figures	Location	Geometric morphometrics			Morphological measurements		
				Year	<i>n</i> (males)	<i>n</i> (females)	Year	<i>n</i> (males)	<i>n</i> (females)
Little Campbell Estuary	Marine	1	49-016° N; 122-779° W	2006	9	4	2004	31	23
Quilecene Bay	Marine	2	47-827° N; 122-858° W	2006	7	5	2006	7	5
Seabeck Bay	Marine	3	47-640° N; 122-829° W	2006	23	20	2006	23	20
Clam Bay	Marine	4	47-572° N; 122-549° W	2006	6	13	NA	NA	NA
Duwamish Estuary	Marine	5	47-531° N; 122-319° W	2006	22	2	2006	34	3
Akkeshi Pacific Ocean	Marine	6	43-051° N; 144-859° E	2007	6	7	2003	10	25
Akkeshi Japan Sea	Marine	7	43-051° N; 144-859° E	2007	21	22	2003	29	24
Little Campbell Stream	Stream	8	49-011° N; 122-625° W	2006	10	10	2004	28	22
Allen Creek	Stream	9	48-051° N; 122-164° W	2005	10	32	2005	10	32
Kelsey Creek	Stream	10	47-601° N; 122-172° W	2007	9	11	2007	8	11
May Creek	Stream	11	47-515° N; 122-143° W	2007	9	14	2007	9	14
Black River	Stream	12	47-475° N; 122-242° W	2005	7	10	2005	7	10
Big Soos Creek	Stream	13	47-374° N; 122-144° W	2005	9	22	2005	5	20
Wapato Creek	Stream	14	47-228° N; 122-343° W	2005	9	13	2005	9	14
Hiranoi Creek	Stream	15	35-394° N; 136-619° E	2007	4	8	2009	7	8

NA, formalin-fixed specimens were not available.

Table II

Effect of centroid size, sex and ecotype on body shape

Trait*	Effect											
	Centroid size				Sex		Ecotype				Sex × ecotype	
	F	P			F	P	F	P	F	P	F	P
RW1	6.0	<0.05			83.3	<0.001			0.1	>0.05	0.3	>0.05
RW2	93.2	<0.001			35.6	<0.001			203.3	<0.05	6.4	<0.05
RW4	26.5	<0.001			4.0	>0.05			8.9	<0.01	2.7	>0.05

* RW3 was not analysed due to slope heterogeneity (see Materials and Methods).

Table III

Mean $\pm$ s.d. of morphological data

Population	Ecotype	Sex	L_s	HL	BD	Jaw	Snout	SW	Eye	DS1	DS2	PS	PG
(mm)													
Little Campbell Estuary	Marine	F	60.97 (3.38)	17.20 (0.95)	13.56 (0.84)	3.82 (0.27)	5.07 (0.38)	3.57 (0.29)	4.72 (0.37)	5.81 (0.57)	6.82 (0.64)	10.04 (0.96)	15.15 (1.50)
Little Campbell Estuary	Marine	M	57.06 (1.81)	18.39 (0.51)	13.41 (0.43)	4.41 (0.30)	5.82 (0.23)	3.86 (0.36)	4.81 (0.24)	5.57 (0.48)	6.19 (0.51)	9.20 (0.56)	13.38 (0.51)
Quileene Bay	Marine	F	45.64 (4.76)	13.37 (1.07)	9.71 (0.88)	2.88 (0.17)	3.73 (0.43)	2.83 (0.26)	4.10 (0.28)	4.13 (0.37)	4.92 (0.45)	6.86 (0.54)	10.53 (2.58)
Quileene Bay	Marine	M	42.87 (3.67)	13.52 (1.05)	9.69 (0.71)	3.17 (0.32)	3.95 (0.44)	2.99 (0.23)	4.12 (0.27)	4.29 (0.50)	5.02 (0.48)	7.14 (0.73)	9.99 (0.59)
Seabeck Bay	Marine	F	45.24 (3.93)	13.2 (1.24)	9.49 (1.08)	3.03 (0.38)	3.62 (0.44)	2.92 (0.35)	4.19 (0.26)	3.98 (0.68)	4.53 (0.81)	6.50 (0.92)	10.71 (1.19)
Seabeck Bay	Marine	M	45.00 (4.62)	14.83 (1.68)	10.21 (1.10)	3.60 (0.57)	4.43 (0.59)	3.27 (0.44)	4.45 (0.37)	4.48 (0.58)	4.93 (0.57)	6.86 (0.83)	10.38 (0.93)
Duwamish Estuary	Marine	F	56.92 (5.13)	17.41 (1.85)	12.65 (1.62)	4.26 (0.85)	5.09 (0.90)	3.69 (0.63)	4.57 (0.60)	5.49 (0.68)	5.99 (0.50)	8.71 (1.00)	13.51 (1.52)
Duwamish Estuary	Marine	M	53.67 (3.91)	17.44 (1.46)	12.79 (1.35)	4.57 (0.48)	5.43 (0.62)	4.03 (0.53)	4.75 (0.35)	5.19 (0.61)	5.55 (0.49)	8.43 (0.84)	12.35 (1.06)
Akkeshi Pacific Ocean	Marine	F	82.71 (2.42)	23.77 (0.83)	18.83 (0.60)	5.5 (0.38)	7.92 (0.33)	4.76 (0.37)	5.66 (0.23)	8.36 (0.47)	9.08 (0.70)	12.41 (0.70)	19.63 (0.86)
Akkeshi Pacific Ocean	Marine	M	76.52 (2.62)	24.61 (0.7)	18.10 (0.76)	6.29 (0.40)	8.58 (0.34)	5.18 (0.39)	5.64 (0.21)	8.14 (0.71)	8.77 (0.64)	11.68 (0.73)	17.35 (0.81)
Akkeshi Japan Sea	Marine	F	66.64 (2.45)	19.01 (0.73)	14.40 (0.70)	4.61 (0.24)	5.92 (0.22)	3.68 (0.34)	5.28 (0.21)	6.22 (0.78)	7.10 (1.03)	9.69 (0.83)	15.17 (1.10)
Akkeshi Japan Sea	Marine	M	61.48 (1.95)	19.55 (0.69)	14.15 (0.61)	5.04 (0.29)	6.61 (0.34)	4.00 (0.37)	5.18 (0.24)	7.22 (0.67)	7.59 (0.67)	9.44 (0.75)	13.83 (0.85)
Little Campbell Stream	Stream	F	58.70 (2.76)	17.82 (0.79)	15.37 (0.98)	4.08 (0.25)	4.61 (0.32)	4.19 (0.31)	4.97 (0.26)	3.97 (0.36)	4.94 (0.41)	6.15 (0.66)	11.12 (1.04)
Little Campbell Stream	Stream	M	50.41 (3.53)	16.61 (1.13)	13.04 (0.97)	4.24 (0.46)	4.73 (0.43)	4.14 (0.47)	4.71 (0.32)	3.59 (0.45)	4.26 (0.44)	5.54 (0.51)	9.24 (0.72)
Allen Creek	Stream	F	50.11 (6.75)	14.95 (2.01)	11.97 (1.72)	3.62 (0.55)	4.17 (0.68)	4.04 (0.66)	4.20 (0.48)	4.31 (0.67)	4.88 (0.76)	6.27 (0.85)	10.76 (1.54)
Allen Creek	Stream	M	44.93 (4.94)	14.79 (2.07)	11.03 (1.28)	3.81 (0.68)	4.38 (0.64)	3.87 (0.54)	4.09 (0.46)	3.89 (0.51)	4.27 (0.57)	5.84 (0.74)	9.08 (0.94)
Kelsey Creek	Stream	F	45.03 (6.56)	12.89 (1.69)	10.46 (1.49)	2.87 (0.36)	3.45 (0.46)	2.40 (0.22)	3.66 (0.33)	3.52 (0.50)	3.91 (0.54)	5.47 (0.58)	9.59 (1.46)
Kelsey Creek	Stream	M	50.64 (8.75)	16.15 (3.03)	11.21 (1.02)	3.76 (0.38)	4.48 (0.50)	3.06 (0.33)	4.15 (0.41)	4.03 (0.41)	4.19 (0.39)	6.18 (0.67)	9.70 (0.73)
May Creek	Stream	F	42.90 (4.89)	12.37 (0.87)	10.11 (1.00)	2.83 (0.32)	3.30 (0.33)	2.45 (0.25)	3.70 (0.24)	3.86 (0.42)	4.25 (0.37)	5.54 (0.53)	8.65 (0.67)
May Creek	Stream	M	41.49 (7.25)	12.7 (1.99)	9.96 (1.60)	3.19 (0.62)	3.53 (0.78)	2.54 (0.37)	3.90 (0.54)	3.97 (0.30)	4.15 (0.23)	5.50 (0.43)	8.21 (0.96)
Black River	Stream	F	47.46 (5.41)	14.35 (1.29)	11.45 (1.31)	3.51 (0.42)	3.93 (0.45)	3.33 (0.34)	4.41 (0.36)	3.96 (0.45)	4.46 (0.49)	5.85 (0.57)	9.54 (0.96)
Black River	Stream	M	45.51 (5.37)	14.57 (1.66)	11.17 (1.45)	3.74 (0.44)	4.43 (0.67)	3.53 (0.45)	4.19 (0.44)	3.31 (0.33)	3.64 (0.20)	5.15 (0.45)	8.11 (0.86)
Big Soos Creek	Stream	F	47.15 (5.09)	14.79 (1.67)	11.88 (1.31)	3.6 (0.51)	4.11 (0.56)	3.74 (0.58)	4.49 (0.44)	4.74 (0.62)	5.13 (0.61)	6.78 (0.72)	10.35 (1.08)
Big Soos Creek	Stream	M	47.16 (6.44)	15.77 (2.29)	11.56 (1.53)	3.92 (0.67)	4.64 (0.75)	3.94 (0.78)	4.73 (0.64)	4.31 (0.55)	4.37 (0.92)	6.25 (0.82)	9.57 (1.25)
Wapato Creek	Stream	F	41.53 (5.77)	12.86 (1.64)	9.82 (1.28)	3.11 (0.54)	3.46 (0.62)	3.23 (0.63)	3.70 (0.36)	3.52 (0.64)	4.00 (0.57)	5.10 (0.56)	8.63 (1.12)
Wapato Creek	Stream	M	39.31 (6.21)	12.89 (2.12)	9.35 (1.34)	3.17 (0.63)	3.72 (0.75)	3.13 (0.57)	3.75 (0.43)	3.53 (0.58)	3.85 (0.51)	5.21 (0.70)	7.78 (0.95)
Hiranoti Creek	Stream	F	52.94 (4.98)	16.92 (1.11)	12.77 (0.98)	4.38 (0.45)	5.25 (0.50)	4.18 (0.57)	4.87 (0.22)	3.13 (0.24)	3.75 (0.28)	5.39 (0.41)	10.33 (0.78)

Population	Ecotype	Sex	<i>L</i> _S	HL	BD	Jaw	Snout	SW	Eye	DS1	DS2	PS	PG
(mm)													
Hiranot Creek	Stream	M	50.45 (4.58)	16.96 (0.95)	12.89 (0.70)	4.50 (0.33)	5.28 (0.50)	4.33 (0.69)	4.76 (0.21)	3.09 (0.17)	3.53 (0.35)	5.53 (0.35)	10.11 (0.51)

F, female; M, male; *L*_S, standard length; HL, head length; BD, body depth; Jaw, jaw length; Snout, snout length; SW, snout width; Eye, eye diameter; DS1, first dorsal spine length; DS2, second dorsal spine length; PS, left pelvic spine length; PG, pelvic girdle length.

Table IV

Patterns of sexual dimorphism in each population. To test for sexual dimorphism in standard length (L_S), ANOVA was used. To test for sexual dimorphism in other traits, $\log_{10}$ -transformed L_S was used as a covariate in ANCOVA

Population	Ecotype	L_S	HL	BD	Jaw	Snout	SW	Eye	DS1	DS2	PS	PG
Little Campbell Estuary	Marine	F*	M*	M	M*	M*	M*	M	ns	ns	ns	ns
Quilcene Bay	Marine	ns	M	M	M	M	ns	M	ns	ns	M	slope
Seabeck Bay	Marine	ns	M*	M*	M*	M*	M*	M*	M	M	M	ns
Duwamish Estuary	Marine	ns	M	ns	M*	M*	M	M	ns	ns	ns	ns
Akkeshi Pacific Ocean	Marine	F*	M*	ns	M*	M*	M	ns	slope	ns	ns	F
Akkeshi Japan Sea	Marine	F*	M*	M	M*	M*	M	M	slope	M	slope	ns
Little Campbell Stream	Stream	F*	M*	ns	M*	M*	M*	M	ns	ns	ns	slope
Allen Creek	Stream	F	M*	ns	M*	M*	M	M	ns	ns	ns	F
Kelsey Creek	Stream	ns	M*	slope	M*	M*	M	M	ns	ns	ns	slope
May Creek	Stream	ns	M	ns	M*	slope	ns	slope	slope	ns	ns	ns
Black River	Stream	ns	M	ns	M	M*	M	ns	F	F	F	F
Big Soos Creek	Stream	ns	M*	F	M	M*	ns	M	ns	slope	F	F
Wapato Creek	Stream	ns	M	ns	M	M	ns	M	ns	ns	ns	F
Hiranoi Creek	Stream	F	M	ns	ns	ns	ns	ns	ns	ns	slope	ns

M, male-biased ($P < 0.05$); F, female-biased ($P < 0.05$); ns, no significant difference ($P > 0.05$); slope, slope heterogeneity ($P < 0.05$); HL, head length; BD, body depth; Jaw, jaw length; Snout, snout length; SW, snout width; Eye, eye diameter; DS1, first dorsal spine length; DS2, second dorsal spine length; PS, left pelvic spine length; PG, pelvic girdle length.

* Significant even after Bonferroni correction.

Table V
Effect of standard length (L_S) as ($\log_{10}L_S$), sex and ecotype on morphological traits

Trait	Effect											
	$\log_{10}L_S$				Sex				Ecotype			
	F	P			F	P			F	P		
Head length	818.6	<0.001	49.3	<0.001	3.4	>0.05	1.5	>0.05	1.5	>0.05	1.5	>0.05
Body depth	760	<0.001	4.8	<0.05	42.8	<0.001	5.3	<0.05	5.3	<0.05	5.3	<0.05
Jaw length	188.2	<0.001	34.3	<0.001	5.1	<0.05	1.1	>0.05	1.1	>0.05	1.1	>0.05
Snout length	385.2	<0.001	50.7	<0.001	0.01	>0.05	0.9	>0.05	0.9	>0.05	0.9	>0.05
Snout width	32.6	<0.001	4.2	<0.05	4.2	>0.05	0.4	>0.05	0.4	>0.05	0.4	>0.05
Pelvic girdle length	788.6	<0.001	4.9	<0.05	84.5	<0.001	0.8	>0.05	0.8	>0.05	0.8	>0.05