

10 000 years later: evolution of body shape in Haida Gwaii three-spined stickleback

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The body shape of 1303 adult male three-spined stickleback *Gasterosteus aculeatus* from 118 populations on Haida Gwaii archipelago off the mid-coast of British Columbia was investigated using discriminant function analysis on partial warp scores generated from 12 homologous landmarks on a digital image of each fish. Results demonstrated geographical differences in adult body shape that could be predicted by both abiotic and biotic factors of the habitat. Populations with derived shape (CV1–), including thick peduncles, posterior and closely spaced dorsal spines, anterior pelvis, small dorsal and anal fins, were found in small, shallow, stained ponds, and populations with less derived shape (CV1+), with small narrow peduncles, anterior and widely spaced dorsal spines, posterior pelvis, large dorsal and anal fins were found in large, deep, clear lakes. This relationship was replicated between geographic regions; divergent mtDNA haplotypes in lowland populations; between predation regimes throughout the archipelago, and in each geographical region and between predation regimes in lowland populations monomorphic for the Euro and North American mtDNA haplotype. There were large-bodied populations with derived shape (CV2–), including small heads and shallow elongate bodies in open water habitats of low productivity, and populations with smaller size and less derived shape (CV2+), with large heads and deeper bodies in higher productivity, structurally complex habitats. This relationship was replicated between geographic regions, and partially between divergent mtDNA haplotypes in lowland populations. Field tests for phenotypic plasticity of body shape suggest that <10% of the total variation in body shape among populations throughout the archipelago can be attributed to plasticity. © 2007 The Authors

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Key words: adaptation; *Gasterosteus*; geometric morphometrics; hydrodynamic landscape; phenotypic plasticity; Queen Charlotte Islands.

INTRODUCTION

Environmental heterogeneity in the selective landscape can lead to adaptive radiations with predictable evolutionary outcomes (Lack, 1947; Grant *et al.*, 1976; Boag & Grant, 1981; Grant & Grant, 1995). Typically, adaptive radiations have been quantified through measurements of a few discrete trait characteristics (Lack, 1947; Williams, 1969, 1972; Grant *et al.*, 1976; Losos, 1990). All traits, however, are related to the fitness of an organism and many may be

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integrated (interactive response of multiple characters to specific selection regimes) (Schlichting, 1989; Bell & Foster, 1994). Body shape reflects phenotypic integration as shape is often quantified by numerous correlated characters (Strauss & Bookstein, 1982; Baumgartner *et al.*, 1988; Rohlf, 1990; Bookstein, 1991; Baumgartner, 1992; Walker, 1997; Walker & Bell, 2000; Klingenberg *et al.*, 2003), which conserves shape during evolutionary diversification.

In aquatic organisms, drag reduction has been an important process in body shape evolution evident through the convergence of form in many diverse aquatic chordate groups (Aleev, 1977). Organisms differ in the niche space they occupy, and in aquatic species, the optimal body shape for ensuring survival is often correlated to physical and biological characteristics of the habitat (Walker, 1997; Guill *et al.*, 2003a; Klingenberg *et al.*, 2003; Langerhans *et al.*, 2003; McGuigan *et al.*, 2003). Variation in shape often reflects ecological and behavioural differences, which bear directly on traits important for fitness such as feeding efficiency, vulnerability to predators and reproductive success (Guill *et al.*, 2003b; Klingenberg *et al.*, 2003). In fishes, these traits have been linked directly to locomotor performance and efficiency, allowing organisms to occupy various habitats successfully (Swain, 1992; Guill *et al.*, 2003a; McGuigan *et al.*, 2003). Therefore, the body shape of fishes can be expected to be of particular ecological and evolutionary relevance (Klingenberg *et al.*, 2003).

Locomotion and body shape are intrinsically linked, and shape can often be used to predict how a particular type of fish moves through the water (Webb, 1982; Sfakoitakis *et al.*, 1999). Most often, fishes swim by lateral undulation of the body and tail fin, which can be used for both sustained and burst swimming (Breder, 1926; McGuigan *et al.*, 2003). A streamlined body and a narrow caudal peduncle are characteristics of shape that confer maximum performance in sustained swimming, while a deep flexible body and deep caudal peduncle characterize shape adapted to maximize burst performance (Webb, 1982). Obviously, fishes with a body shape characteristic of sustained swimming can also use burst acceleration and *vice versa*. Shape adapted for maximum performance in one predominant mode of locomotion, however, can preclude maximum performance at the other (Reidy *et al.*, 2000). Because of this trade-off, selection to increase sustained performance, for example, could result in streamlining of body shape which can lead to a proportionate decrease in burst performance (McGuigan *et al.*, 2003).

Ecological interactions in different environments can have substantive effects on the evolution of body shape. Local environments provide the template on which evolution forges characteristic life-history strategies (Southwood, 1977; Townsend & Hildrew, 1994). Following this premise, individuals from a single species in adjacent habitats can develop different morphologies in response to environmental cues, and these differences can often be attributed to phenotypic plasticity (Andersson, 2003). A genetic component to body shape, however, has been suggested (Walker, 1997; Guill *et al.*, 2003a; Langerhans & DeWitt, 2004) through the diversification of body shape in a few closely related fish species. The results from these investigations are consistent with principles of functional morphology, linking body shape to fitness through correlations with ecological factors of the habitat.

One of the more striking examples of evolutionary radiation of vertebrates in the temperate latitudes are the isolated populations of three-spined stickleback *Gasterosteus aculeatus* L. from the Haida Gwaii archipelago (formerly known as the Queen Charlotte Islands), 100 km off the west coast of Canada. These populations of three-spined stickleback are ideal to study the evolution of body shape as many of the lakes, which are *c.* 10 000 years old, were each colonized by marine three-spined stickleback (trachurus) (Moodie & Reimchen, 1976a). There is recent mitochondrial evidence that some of the lakes had two colonization events (O'Reilly *et al.*, 1993; Orti *et al.*, 1994; Deagle *et al.*, 1996). The Haida Gwaii three-spined stickleback populations inhabit geographically isolated, relatively pristine lakes and streams, which encompass a wide range of abiotic and biotic variation (Moodie & Reimchen, 1976a; Reimchen, 1994; Bergstrom & Reimchen, 2002). This broad selective landscape has led to substantial diversification in three-spined stickleback among lakes including differences in maximum body size (30–115 mm standard length, L_s) (Moodie, 1972a, b; Reimchen, 1991), nuptial colouration (Reimchen, 1989) and defensive morphology (Moodie & Reimchen, 1976a; Reimchen *et al.*, 1985). The differences among lake populations within this small area exceed that found throughout the rest of the circumboreal distribution including Alaska, eastern North America as well as northern Europe. The present study used geometric morphometrics to examine body shape variation among Haida Gwaii three-spined stickleback populations and assesses potential associations with biophysical variables of each habitat.

MATERIALS AND METHODS

SAMPLING AND STUDY AREA

Samples of three-spined stickleback from 99 lake, 19 stream and six marine localities on Haida Gwaii were collected from 1969 to 2003. These sites (Figs 1 and 2) encompass three major geographical zones (lowland, plateau and mountain). Three-spined stickleback were collected in the spring and summer using standard-mesh minnow traps baited with cheddar cheese and placed in the littoral zones (Reimchen *et al.*, 1985; Reimchen, 1989, 1992a, 1994). Fish were fixed in 10% formalin and stored in 95% ethanol. Six populations (two from streams and four from lakes) were excluded from the analysis of shape because there were no suitable male three-spined sticklebacks in the samples (*i.e.* shape compromised from previous investigations). Habitat biophysical data were also collected from a sub-set of localities during the fish collections [pH, specific conductance, calcium concentration (mmol), T400 (per cent 400 nm light transmission), lake area (ha), maximum depth (m), volume (m³), elevation (m), distance to the ocean (m) and predation regime (salmonid, bird and macroinvertebrate)].

QUANTIFICATION OF BODY SHAPE

Adult male three-spined stickleback ($n = 1303$) from 118 different populations on Haida Gwaii were photographed, and the digital images were used in the programme TPSDig (Rohlf, 2004) to produce data from 12 homologous landmarks (Fig. 3) (Bookstein, 1991) to quantify body shape. The programme TPSRegr (Rohlf, 2000) was used to align and scale the data in concordance with the generalized-least squares orthogonal Procrustes method, to generate partial warp scores (corresponding to variation in body shape at smaller scales), and the uniform component of shape change

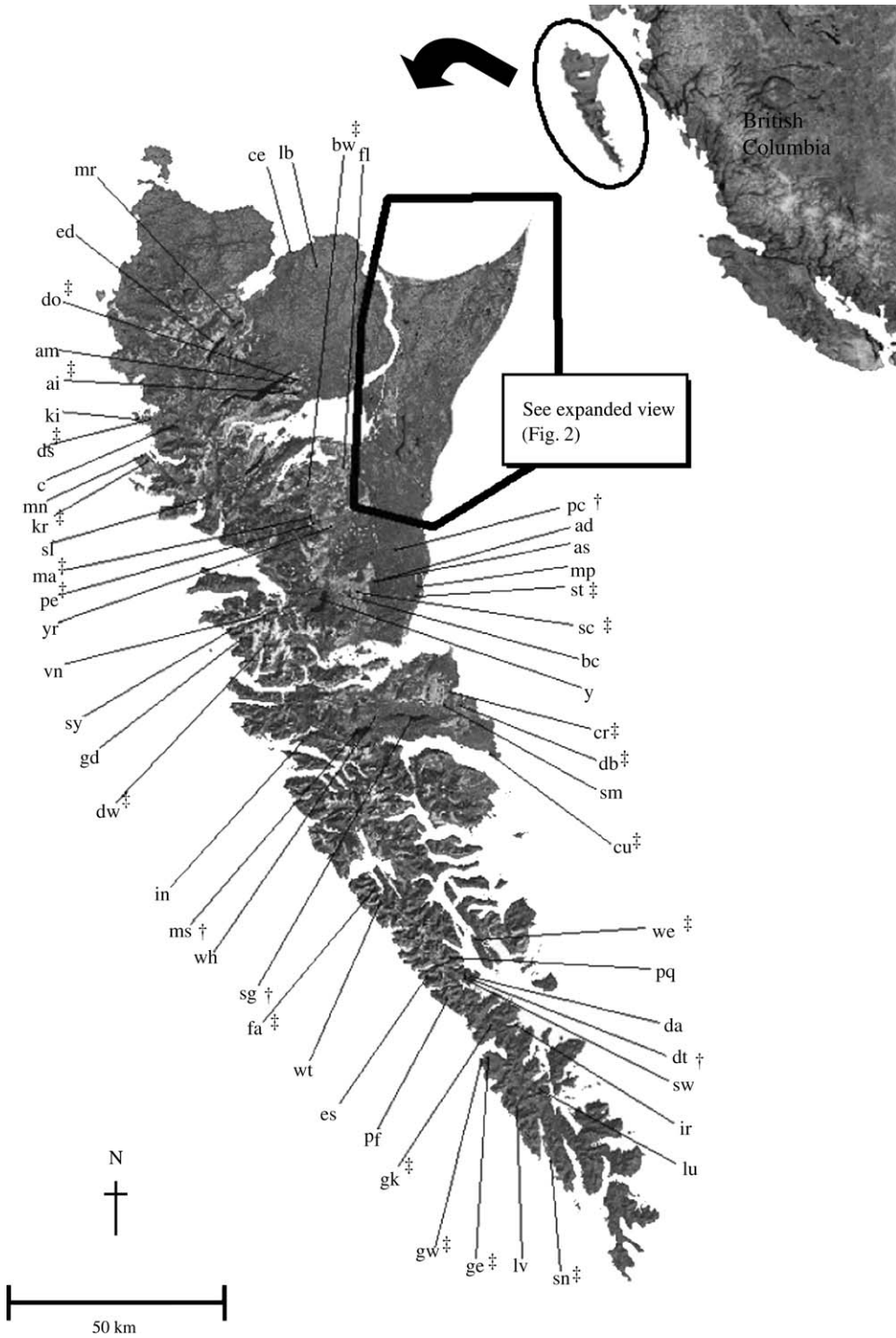


FIG. 1. Localities (see appendix) where three-spined stickleback were collected on Haida Gwaii. †, Populations not used in the quantification of shape; ‡, populations with $n < 10$.

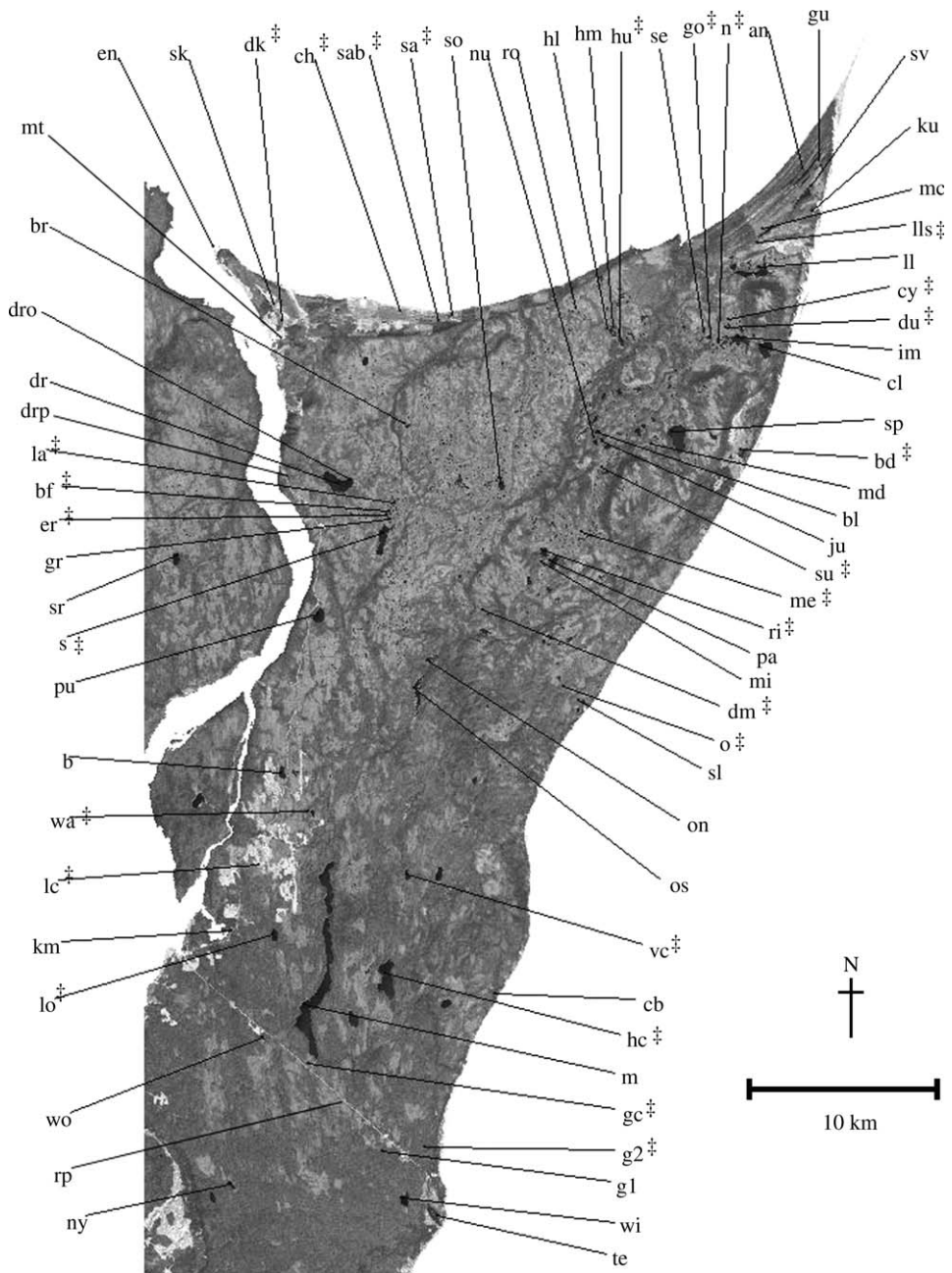


FIG. 2. Localities (see appendix) where three-spined stickleback were collected from the north-east corner of Haida Gwaii. †, Populations not used in the quantification of shape; ‡, populations with $n < 10$.

(large scale variation in shape from the alignment procedure; Rohlf, 1990, 1996, 1999). Discriminant function analysis was used on the partial warp and uniform component scores to predict group membership and quantify multivariate measures of body shape along canonical vectors (CVs), among specimens throughout the archipelago. The

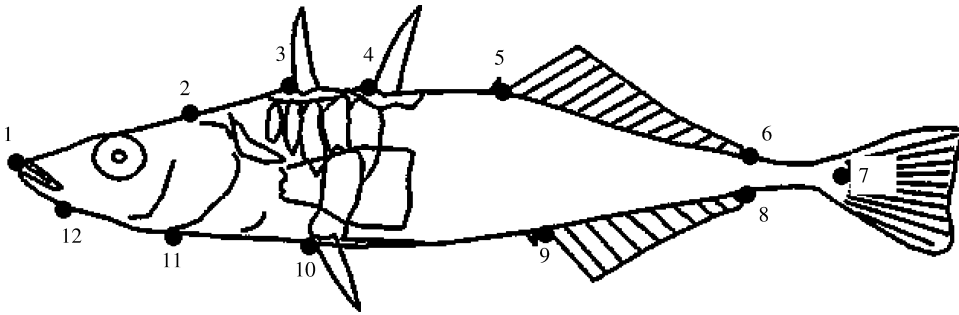


FIG. 3. Lateral view of three-spined stickleback showing the placement of landmarks used in the body shape analysis: 1, anterior tip of premaxilla; 2, supraoccipital notch immediately left of the dorsal midline (DML); 3, anterior junction of first dorsal spine and basal plate along the DML; 4, anterior junction of the second dorsal spine and basal plate along the DML; 5, base of the first dorsal fin ray at the DML; 6, insertion of the dorsal fin membrane on the DML; 7, caudal border of hypural plate at lateral midline; 8, insertion of anal fin membrane on ventral midline (VML); 9, base of first anal fin ray on VML; 10, posterior tip of ectocoracoid; 11, anterior border of ectocoracoid on VML; 12, point of articulation between angular and quadrate.

uniform component was included to attain the total within population variation of body shape differences (Rohlf, 1993; Bookstein, 1996). Canonical vectors without the uniform component were tightly correlated to the CV scores used in the subsequent analysis (Pearson's correlation: CV1: $n = 118$, $r = 0.98$, $P < 0.001$; CV2: $n = 118$, $r = 0.89$, $P < 0.001$). Pearson correlation was used to describe body shape along each shape vector from log-linear regression transformed measurement (Reimchen *et al.*, 1985) of standard morphometric variables.

To minimize ontogenetic effects and variation due to possible sexual shape dimorphism, the largest male three-spined stickleback from the samples were used for the shape analysis. Two analyses were performed: the first on all 118 populations regardless of male sample size (n ranges from 1 to 81), and the second on 72 samples which had $n \geq 10$ male three-spined stickleback. Although each of the test statistics differed between the two datasets, the statistically significant results were the same and therefore only the results for all 118 samples are reported. Univariate ANOVA was used to determine if there was any difference in body shape among populations. Population means for CV1 and CV2 were plotted against biophysical data to ascertain any ecological associations between body shape and habitat.

The configuration of landmarks for an individual specimen (*i.e.* the figure) has a mathematical centre (centroid). The size of a figure (centroid size) is the square root of the sums of squared distances of a set of landmarks from their centroid (Bookstein, 1991). Pearson's correlations between populations mean \ln of centroid size and mean shape scores were used to determine if body shape quantified by canonical vectors was related to size.

PHENOTYPIC PLASTICITY OF BODY SHAPE

In 1992, a common garden experiment was started as part of the long-term investigation of three-spined stickleback from Haida Gwaii to investigate evolutionary responses to novel predation regimes and potential phenotypic plasticity. Giant melanistic three-spined stickleback from Mayer Lake, which is large (400 ha, maximum depth 10 m) and dystrophic with high numbers of predatory fishes (Moodie, 1972*b*), were transplanted into a nearby small eutrophic pond (0.3 ha and shallow maximum depth 1 m), which lacked fishes. The biological and physical differences between Mayer Lake and the experimental pond are ecological opposites and span the range of habitat

types on Haida Gwaii. A few years later, the experiment was replicated and giant melanistic three-spined stickleback from Drizzle Lake (97 ha with a maximum depth of 20 m) were transplanted into another experimental pond (0.5 ha with a maximum depth of 1 m). Again the source lake and recipient pond are ecological opposites (extreme limnetic niche *v.* extreme benthic niche) and provided an opportunity to assess the extent of phenotypic plasticity in body shape.

Tests for plasticity were conducted with several methods. Mean adult body shape of the source populations were compared to those of the first generation that had developed from eggs to adult in the experimental ponds. Shape comparisons of the experimental pond three-spined stickleback collected after 10 generations were compared with those of the source populations. In both instances, the same range of L_S of fish between the source and the experimental ponds were used. The per cent differences in population mean CV scores between the experimental ponds and source populations were calculated and compared against the range of body shapes found on the archipelago.

ECOLOGICAL ASSOCIATIONS AND BODY SHAPE

A principal component analysis of ln transformed lake biophysical data (Table I) was performed. The first component explained 38.9% of the total variation and represented mainly lake morphometry, while the second component explained 25.2% of the total variation and represented lake chemistry. Previous work has shown lake types within each geographical region are more similar than lake types between regions (Reimchen, 1989, 1992b).

Body shape variables (CV1 and CV2) were regressed against the principal components to look for potential ecological relationships with body shape. The data were also partitioned to determine whether there was concordance among geographical regions.

The Japanese (trans-North Pacific) three-spined stickleback mitochondrial haplotype occurs in some of the Haida Gwaii populations primarily from the lowlands (O'Reilly *et al.*, 1993; Deagle *et al.*, 1996). Tests were performed (ANCOVA) to determine whether there were any body shape and habitat (PC1 and PC2) associations between populations with and without the Japanese haplotype.

Tests were performed to determine whether body shape could be predicted by the predation regime of the habitat. Predator state is defined as either salmonid or bird and invertebrate (Reimchen, 1994). Body shape and predation were examined for the whole archipelago, for each geographical region and for presence of European and

TABLE I. Principal component matrix of loading scores for physical characteristics of lake habitat ($n = 70$) on Haida Gwaii, British Columbia. PC1 explains 38.9% of the variation among habitats and represents lake morphometry and PC2 explains 25.2% of the variation and represents lake chemistry or conductivity. All of the variables except pH and T400 were ln transformed prior to the principal component analysis

Variable	Lake morphometry (PC1)	Lake chemistry (PC2)
pH	0.66	0.57
Conductivity	-0.20	0.75
Calcium	0.10	0.86
T400	-0.70	-0.44
Depth	0.93	-0.18
Area	0.86	-0.29
Volume	0.92	-0.26
Elevation	0.18	-0.32
Distance from the ocean	-0.23	-0.39

North American mtDNA haplotype in lowland lakes. The five lowland populations with the Japanese mtDNA haplotype present do not have salmonid predation; therefore, any ecological relationship detected in the presence of the Japanese haplotype includes effects of bird and invertebrate predation.

RESULTS

QUANTIFICATION OF BODY SHAPE

Discriminant function analysis on partial warp and uniform component scores predicted group membership (*i.e.* lake sample) 83.7% of the time. The first CV accounted for 34.7% of the total variation and contrasted more derived fish on the negative extent of the vector, characterized by thick peduncles, posterior and closely spaced dorsal spines, anterior pelvis and small dorsal and anal fins (Table II), represented by Rouge Lake [Fig. 4(a)] with ancestral marine three-spined stickleback [Fig. 4(b)], on the positive end of the vector with a small narrow peduncle, anterior and widely spaced dorsal spines, posterior pelvis and large dorsal and anal fins. The second vector accounted for 20.3% of the total variation and contrasted derived three-spined stickleback with negative loadings characterized by small heads and shallow bodies, represented by Mayer Lake [Fig. 4(c)] with less derived fish with large heads and deeper bodies, represented by fish from Fairfax Lake [Fig. 4(d)].

TABLE II. Pearson correlations for CV1 and CV2 between ln linear regression standardized morphometric measurement calculated from inter-landmark distances for 118 populations of adult three-spined stickleback from Haida Gwaii, British Columbia. CV1 explained 33.4% of the variation among populations of adult stickleback, and CV2 explained 21.7% of the variation

Character	CV1		CV2	
	Pearson correlation (<i>r</i>)	<i>P</i> value	Pearson correlation (<i>r</i>)	<i>P</i> value
Pelvis	0.69	<0.001	0.42	<0.001
Pelvis position	0.62	<0.001	0.63	<0.001
Head area	0.16	0.09	0.49	<0.001
Head length	0.42	<0.001	0.48	<0.001
Head depth	0.04	0.68	0.58	<0.001
Gape	-0.10	0.29	0.15	0.11
Dorsal spine 1 (D1) position	-0.41	<0.001	0.20	0.03
Distance between D1 and D2	0.92	<0.001	0.44	<0.001
Body depth (anterior)	0.26	0.005	0.38	<0.001
Body depth (posterior)	0.08	0.37	0.25	0.007
Dorsal fin length	0.35	<0.001	-0.04	0.64
Anal fin length	0.47	<0.001	-0.29	0.002
Caudal depth (anterior)	-0.08	0.37	0.04	0.7
Caudal depth (posterior)	-0.64	<0.001	-0.25	0.005
Caudal peduncle size	-0.72	<0.001	-0.41	<0.001

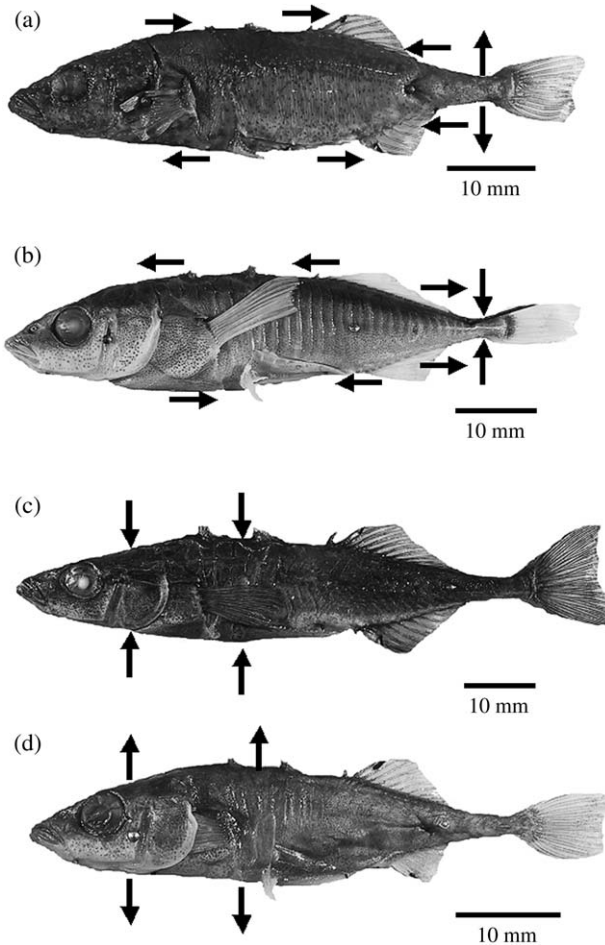


FIG. 4. Photographs of representative adult male three-spined stickleback from: (a) Rouge Lake (CV1-), (b) Entry Point (CV1+), (c) Mayer Lake (CV2-) and (d) Fairfax Lake (CV2+). Arrows indicate differences in shape as quantified by canonical vectors.

There were significant differences among population in the mean body shapes (ANOVA, CV1: $F_{118,1302}$, $P < 0.001$; CV2: $F_{118,1302}$, $P < 0.001$; Fig. 5).

Adult body size differed among the populations (30–115 mm L_S), so tests were conducted to determine whether there were any corresponding differences in body shape. The first vector was not significantly correlated with centroid size ($n = 118$, $r = 0.13$, $P > 0.05$). Body shape means (CV2) for each population, however, were negatively correlated with adult body size ($n = 118$, $r = -0.48$, $P < 0.001$), showing that larger fish at the negative extreme of CV2 had a more derived shape than the marine ancestral populations.

ESTIMATES OF PHENOTYPIC PLASTICITY IN BODY SHAPE

Differences in body shape between source and transplanted populations of three-spined stickleback were detected. The first generation of three-spined

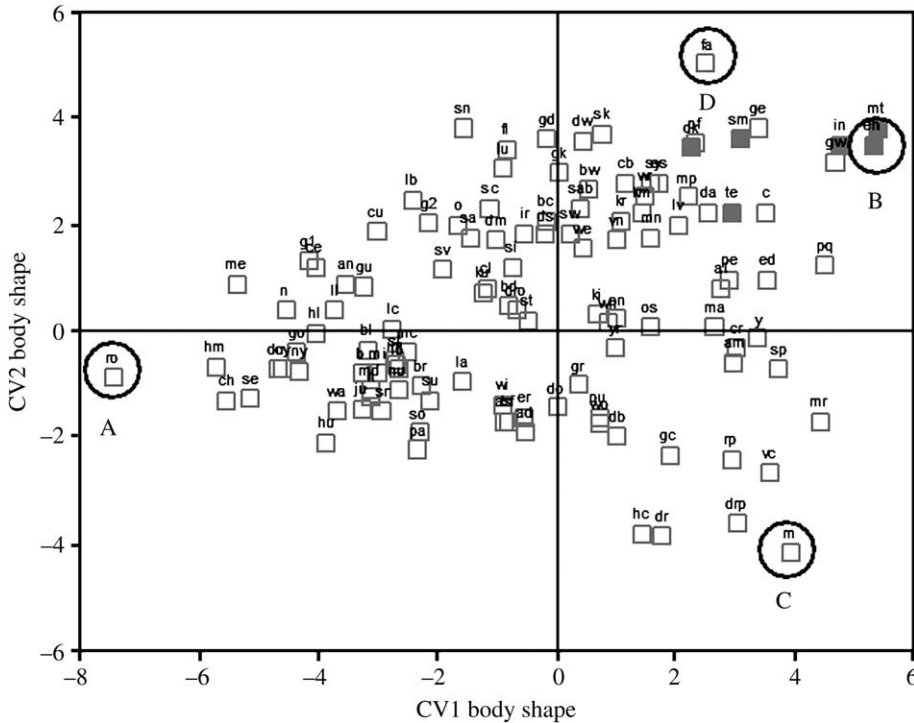


Fig. 5. Body shape quantified by canonical vector 1 (33.4% of total variation) and canonical vector 2 (21.7% of total variation) of adult male three-spined stickleback from Haida Gwaii. ■, Anomomous populations. Populations with extreme shape are A, Rouge Lake; B, Entry Point; C, Mayer Lake; D, Fairfax Lake.

stickleback from Drizzle experimental pond ($n = 22$) had marginally higher positive loadings for CV1 and CV2 than did the source population ($n = 25$) and differed by 11.3% of the total CV1 variation and 4.6% of the CV2 variation among Haida Gwaii populations. Similar results occurred for the transplanted population from Mayer Lake. Although visually indistinguishable, the first generation from the experimental pond ($n = 8$) had slightly higher CV1 and CV2 values relative to the source population ($n = 38$) and differed by 3.9% of the total CV1 variation and 9.4% of the CV2 variation among Haida Gwaii populations. Body shape was examined following 10 years of habitation and yearly reproduction in the experimental ponds. There were no further shifts in CV1 or CV2 for the Drizzle experimental pond. At the Mayer experimental pond ($n = 11$), however, there was a slight increase in both CV1 and CV2 relative to the source population, which accounted for 8.9% of total CV1 variation and 21.2% of total CV2 variation among all Haida Gwaii populations.

BODY SHAPE AND ECOLOGICAL ASSOCIATIONS

Derived fish with high negative loadings of CV1 (thick peduncles, posterior positioned and closely spaced dorsal spines, anterior pelvis and small dorsal

and anal fins) were found in small, shallow, stained ponds (negative PC1 loadings), and less derived three-spined stickleback with high positive loadings (opposite characteristics) were found in large, deep, clear lakes (positive PC1 loadings; linear regression, d.f. = 65, $r^2 = 0.45$, $P < 0.001$). The slopes did not differ between geographical region (ANCOVA, $F_{1,64}$, $P > 0.05$; Fig. 6) and between predator grouping within each geographical region (plateau and mountain region: ANCOVA, $F_{1,20}$, $P > 0.05$; lowlands: ANCOVA, $F_{1,39}$, $P > 0.05$). Furthermore, the slopes did not differ between mitochondrial haplotypes in lowland populations (ANCOVA, $F_{1,31}$, $P > 0.05$).

Body shape (CVI) was also related to the predation regime of a given habitat. Populations of three-spined stickleback from predatory fish lakes had higher CV values across all lake conditions relative to bird and invertebrate lakes ($F_{1,41}$, $P < 0.001$). Most of the lakes with bird and invertebrate predators are in the lowlands and when body shape was examined against lake morphometry within this region, the same associations were observed in that three-spined stickleback from predatory fish lakes had higher CV scores relative to bird and invertebrate predation for all lake sizes ($F_{1,23}$, $P < 0.001$).

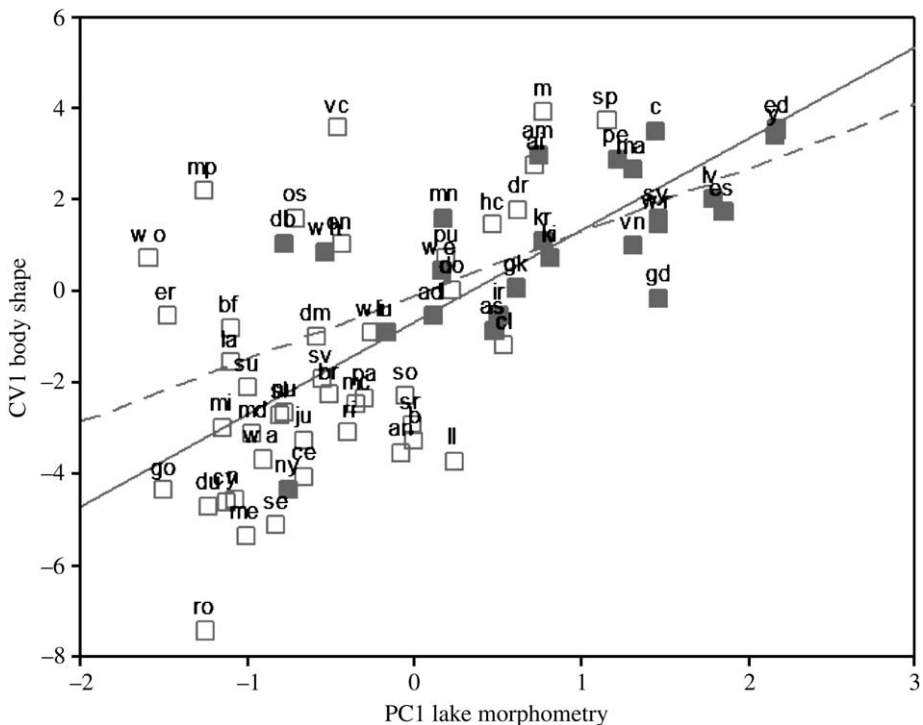


FIG. 6. Relationship between body shape of adult male three-spined stickleback (CVI: 34.7% of total variation), lake morphometry (PC1: 38.9% of total variation) and geographic region for populations of fish from the lowlands (—□—), and from the plateau and mountain region (---■---). Lakes that are small, shallow and stained contained three-spined stickleback with derived body shape, compared to large, deep and clear lakes which had less derived fish (overall slope $r^2 = 0.45$, d.f. = 65, $P < 0.001$; slope comparisons for geographic region, ANCOVA $F_{1,64}$, $P > 0.5$).

Body shape quantified by CV2 could also be predicted by lake chemistry. Three-spined stickleback with derived shape (high negative CV2 loadings) characterized by small heads and shallow bodies were found in habitats with low conductivity (negative PC2 loadings), and fish with less derived shape (high positive CV2 loadings) distinguished by large heads and deeper bodies were often found in lakes of higher conductivity (positive PC2 loadings; linear regression, d.f. = 65, $r^2 = 0.22$, $P < 0.001$). This relationship was observed when the data were partitioned by geographical area, but a significant interaction effect between lake physical characteristics and geographical region suggests that the relationship is strongest in the lowlands (ANCOVA, $F_{1,63}$, $P < 0.001$; Fig. 7). The relationship between shape (CV2) and lake chemistry (PC2) was consistent within lowland populations between mitochondrial haplotypes (ANCOVA, $F_{1,31}$, $P > 0.05$). Three-spined stickleback with derived shape (high negative CV2 loadings) were found in habitats of low conductivity (negative PC2 loadings) and less derived shape (high positive CV2 loadings) in lakes of higher conductivity (positive PC2 loadings) (Fig. 8).

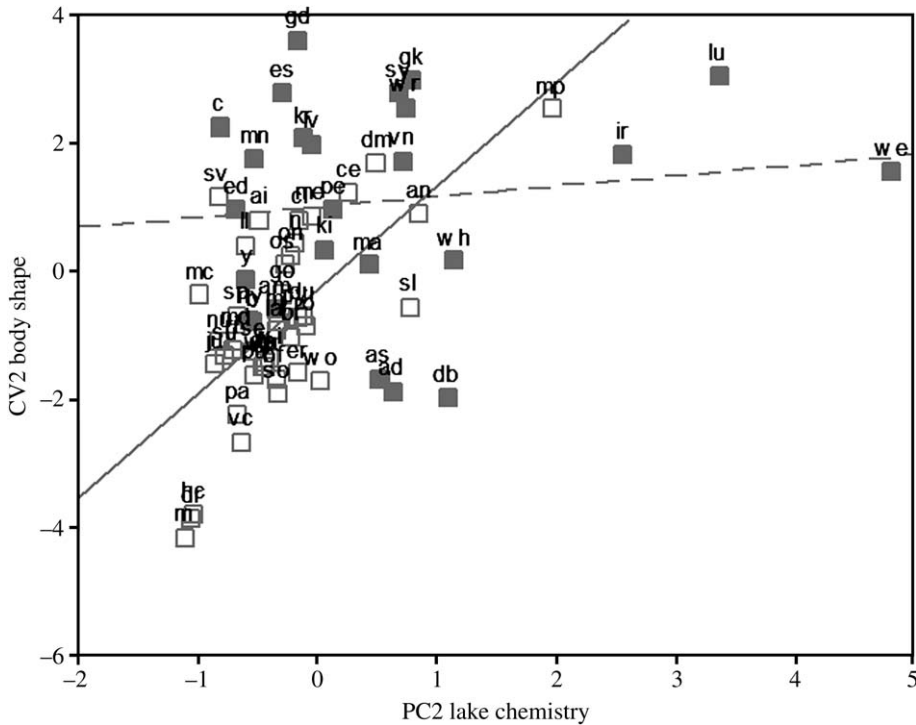


FIG. 7. Relationship between body shape of adult male three-spined stickleback (CV2: 20.3% of total variation), lake chemistry (PC2: 25.2% of total variation) and geographic region for populations of fish from the lowlands (—□—), from the plateau and mountain region (---■---). In each geographic region lakes of low conductivity had derived three-spined stickleback with small heads and shallow bodies compared to lakes of higher conductivity that supported less derived fish with large heads and deeper bodies. The relationship is strongest in the lowlands (lowlands $r^2 = 0.45$, d.f. = 42, plateau and mountain $r^2 = 0.02$, d.f. = 23; slope comparisons for geographical region, ANCOVA $F_{1,63}$, $P < 0.001$).

DISCUSSION

The modern marine three-spined stickleback has remained morphologically conserved since the Miocene (Bell, 1977; Bell & Foster, 1994; Walker & Bell, 2000) and therefore represents the ancestral or less derived condition. The marine populations have streamlined bodies and with extreme positive loadings for CV1 and high positive loadings for CV2, which can be characterized as having large heads, deeper bodies, narrow peduncles, anterior dorsal spines, widely spaced dorsal spines, posterior pelvis and long dorsal and anal fins. Combined, these attributes typify streamlined three-spined stickleback which have evolved in association with the pelagic habitat and migratory lifestyle of these anadromous fish (Wootton, 1976; Taylor & McPhail, 1986; Walker, 1997).

Coastal freshwater habitats in the circumboreal zone were colonized by marine three-spined stickleback and have diversified since the retreat of the Pleistocene glaciers 10 000 years ago (Bell & Foster, 1994). Morphological diversity in freshwater three-spined stickleback populations has been widely discussed and includes gigantism (Moodie, 1972*b*; Bell, 1984; Reimchen

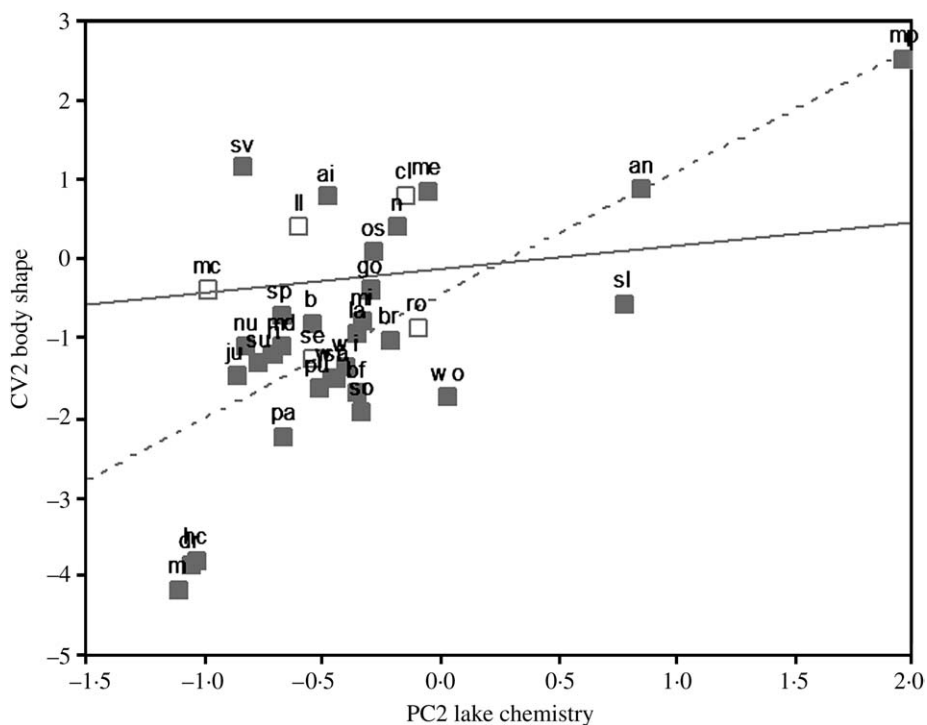


FIG. 8. Relationship between body shape of adult male three-spined stickleback (CV2: 20.3% of total variation), lake chemistry (PC2: 25.2% of total variation) and mtDNA haplotype for populations of fish with the Japanese mtDNA haplotype present (—□—) and populations that are monomorphic for the Euro and North American mtDNA haplotype (—■—). Populations within each lineage were less derived in lakes of higher conductivity (European and North American haplotype $r^2 = 0.44$, d.f. = 29, Japanese haplotype $r^2 = 0.02$, d.f. = 4; slope comparisons for mitochondrial lineage, ANCOVA $F_{1,31}$, $P > 0.5$).

et al., 1985), and armour reduction (Reimchen, 1994). Differentiation in body shape of freshwater populations was initially described within lakes for benthic and limnetic ecotypes from south-western British Columbia (McPhail, 1984) and among lakes in Alaskan populations (Walker, 1997; Walker & Bell, 2000). Although body shape is highly conserved, principal component analysis of 40 Alaskan freshwater populations showed derived three-spined stickleback possessing posterior and closely spaced dorsal spines, short median fins, thick peduncles and anterior positioned pelvic girdle (Walker & Bell, 2000). The present canonical vector data from 118 Haida Gwaii freshwater populations show concordant trends to those observed by Walker & Bell (2000) with respect to each of these morphological characters and each principal component factor but encompass a broader range of habitat.

Intraspecific differences in shape observed among freshwater fish populations may reflect heritable or phenotypically plastic traits or some combination of the two. Within cichlids, trophic morphological characters can be highly variable and can be partially explained by differences in diet and phenotypic plasticity (Meyer, 1987; Klingenberg *et al.*, 2003). A slight alteration of growth rates for different bones due to availability and abundance of prey types during development can have substantive effects on adult phenotype (Liem, 1973; Alberch *et al.*, 1979; Meyer, 1987). In the present study, large bodied, highly streamlined three-spined stickleback from a large dystrophic lake with zooplankton and a full suite of vertebrate predators were transplanted to a small and shallow clear-water eutrophic pond with benthic prey and macroinvertebrate predators as a field assessment of environment-dependent phenotypic expression. The subsequent generations of three-spined stickleback in these experimental ponds were very similar in appearance and shape to their parental populations. With detailed shape analysis, however, it was evident that *c.* 10% of the variation in body shape among all of the Haida Gwaii three-spined stickleback populations can be attributed to phenotypic plasticity. The possibility exists that there were gene frequency changes within the first generation that resulted in the new phenotype. This seems unlikely, however, given the similarity in body shape within each experimental population after 10 years monitoring.

The body shape of fishes can be expected to be associated with hydrodynamic factors for both foraging and predator evasion. In the analysis of shape in Alaskan populations, Walker (1997) found that three-spined stickleback from deep lakes were streamlined, those from shallow lakes had deeper body shape, but these effects were accentuated in populations with the presence of native predatory fishes. On Haida Gwaii, there was a similar association, but the relationship between shape and lake depth was also found in lakes without native predatory fishes. Specifically, the body shapes of three-spined stickleback found in large, deep lakes were similar to that of marine three-spined stickleback in adjacent waters. As such, where the freshwater habitat was similar to that of the marine three-spined stickleback (large and deep with zooplankton as the primary food source), the body shape was conserved. Populations of three-spined stickleback in habitats least like the marine environment (small, shallow and stained ponds with benthic prey) had the most divergent body shapes. Throughout Haida Gwaii, populations of three-spined stickleback subject to predation regimes most similar to the ancestral marine (toothed predators such

as salmonids) had less derived attributes of shape, while populations subject to predation from primarily birds and macroinvertebrates had derived body shape. What is most important is that populations from either predation regime (primarily salmonids or bird and invertebrate) had characteristics of the less derived streamlined-shape in larger, deeper, clearer lakes and more derived attributes of shape (towards CV1–) when found in smaller, shallower, stained ponds, highlighting the efficacy of hydrodynamic processes on the evolution of shape.

The streamlined shape of three-spined stickleback inhabiting large lakes is conducive to lower pressure drag during steady swimming and should therefore increase open water foraging performance (Aleev, 1977; Vogel, 1981; Webb, 1984; Walker, 1997). Patchily distributed zooplankton (George, 1981; Pinel-Alloul *et al.*, 1988) are the primary food source in large deep lakes (Hart & Gill, 1994) and in large lakes of low conductivity (Moodie, 1972a; Moodie & Reimchen, 1976b). Therefore, three-spined stickleback in such habitats would have to constantly swim to find food, which increases the risk of predation by limiting the amount and type of cover. The large median fins of populations with less derived body shape effectively increase caudal depth, without the additive affects of drag associated with a thick caudal peduncle (Walker, 1997), thus facilitating prey capture and increasing the probability of escape from predators (salmonids and birds and invertebrates) with improved fast start performance (Reimchen, 1992a; Bergstrom, 2002). Long median fins of the streamlined three-spined stickleback in the marine and open water habitats suggest stabilizing selection has maintained this less derived condition in freshwater three-spined stickleback that forage in open water (Walker, 1997).

In contrast, freshwater three-spined stickleback found in small and shallow ponds, which are most different from marine habitat, were derived and distinctive from the marine form. Small shallow ponds offer habitats that are very heterogeneous compared to the uniformity of the open water environment (Hart & Gill, 1994). The larvae and pupae of the Chironomidae are the primary food source in littoral habitats (Wootton, 1994), and the rate at which three-spined stickleback encounter prey is strongly influenced by the density and structural complexity of woody debris through which they must search (Hart & Gill, 1994). Predation experiments indicate that structural complexity in littoral areas can provide a refuge from predators, and predation risk can affect both choice of feeding site and the amount and type of prey eaten (Werner *et al.*, 1983a, b; Ibrahim & Huntingford, 1989). The shorter median fins of populations found in small shallow ponds potentially compromise acceleration (Aleev, 1977). Benthic prey, however, are relatively sedentary and do not require the same fast acceleration that three-spined stickleback exhibit in attack in pelagic habitats (Walker, 1997). Possibly, the fin reduction is associated with calcium limitations and bone reduction (Giles, 1983; Bourgeois *et al.*, 1994) or to reduced surface area as a result of selection from grappling macroinvertebrates (Reimchen, 1980).

The ecomorphological relationships were similar in both the lowland and plateau and mountain regions, and between the two mitochondrial haplotypes in the lowland region, suggesting the parallel evolution of body shape through adaptations to similar hydrodynamic landscapes and selection regimes imposed by habitat. The associations exhibited by the Haida Gwaii three-spined stickleback

are consistent with results from previous studies, which link body shape adaptations to multiple aspects of ecology in Alaskan three-spined stickleback populations (Walker, 1997; Walker & Bell, 2000) and to other three-spined stickleback populations of the world (Hermida *et al.*, 2005). The body shape associations detected in this study are also consistent with those reported for feeding habits in serranids (Cavalcanti *et al.*, 1999) and populations of cichlids (Klingenberg *et al.*, 2003). Population body shape differentiation has been shown to occur as a response to predation in poecilids (Langerhans *et al.*, 2004; Langerhans & DeWitt, 2004), as well as cyprinids (Brönmark & Miner, 1992).

What has happened after 10 000 years of evolution? On Haida Gwaii, the three-spined stickleback has colonized 118 lakes comprising a wide variety of isolated habitats and has diversified in maximum body size (Reimchen, 1991), nuptial colouration (Reimchen, 1989) and defensive morphology (Moodie & Reimchen, 1976a; Reimchen *et al.*, 1985), yet has maintained a relatively conserved body shape. Haplochrome cichlids from Lake Victoria in East Africa have diversified within the last 15 000 years into >500 endemic species (Seehausen, 2002; Verheyen *et al.*, 2003), which inhabit adaptive zones throughout the lake (Liem, 1973) and exhibit marked differences in body shape (Stiassny & Meyer, 1999; Joyce *et al.*, 2005). The results of the present study on three-spined stickleback from Haida Gwaii suggest that a phenotypic integration conserves body shape, but responds to biophysical processes, indicating local adaptation to the foraging, predator and hydrodynamic landscape in the adaptive radiation of fishes.

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APPENDIX. DEFINITION OF SITE ABBREVIATIONS

ai ‡, Ain; am, Amber; ad, Anderson North; as, Anderson South; an, Anser; bf ‡, Bigfish; bw ‡, Blackwater Creek; bl, Blowdown; bd ‡, Blue Danub; b, Boulton; br, Branta; bc, Brent Creek; cb, Cape Ball; ce, Cedar; ch ‡, Chown Creek; cl, Clearwater; c, Coates; cr ‡, Copper River; cu ‡, Cumshewa; cy ‡, Cygnet; dm ‡, Dam; da, Darwin; dw ‡, Dawson Pond; dt †, Dead Toad Creek; db ‡, Debris; dk ‡, Delkatla; ds ‡, Desolate; do ‡, Down tree; dr, Drizzle; dro, Drizzle Outlet Creek; drp, Drizzle Pond; du ‡, Duck; ed, Eden; en, Entry Point; er ‡, Eriophore; es, Escarpment; fa ‡, Fairfax; fl, Florence Creek; g1, Geike Creek; g2 ‡, Geike #2 Creek; gc ‡, Gold Creek; gk ‡, Goski; go ‡, Gosling; ge ‡, Gowgaia; gw ‡, Gowgaia West; gr, Gross; gu, Grus; gd, Gudal; hu ‡, Harelda Upper; hl, Harelda Lower; hm, Harelda Middle; hc ‡, Hickey; im, Imber; in, Inskip Lagoon; ir, Irridens; ju, Juno; ki, Kiokathli; kr ‡, Krajina; ku, Kumara; km, Kumdis Creek; lb, Labrador Creek; la ‡, Laurel Pond; lo ‡, Loon; lc ‡, Loon Creek; lv, Lower Victoria; ll, Lumme; lls †, Lumme Swamp Creek; lu, Lutea; ma ‡, Marie; mr, Marion; mt, Masset Inlet; m, Mayer; mn, Menyanthes; me ‡, Mesa; mc, Mica; mi ‡, Middle; md, Midge; mp, Molitors Pond; ms †, Mosquito; n ‡, Naked; ny, New Years; nu, Nuphar; o ‡, Oeanda River; on, Otter North; os, Otter South; pa, Parkes; pe ‡, Peter; pc †, Pontoon Center; pq, Poque; pf, Puffin; pu, Pure; ri ‡, Richter; rp, Mayer Pond; ro, Rouge; sa ‡, Sangan River; sab ‡, Sangan Backwater River; si, Seal Inlet; se, Serendipity; sm, Sheldon Meadows; sv, Silver; sk ‡, Skaters; sg †, Skidgate; s †, Skonun; sl, Slim; sn ‡, Snub; so, Solstice; sp, Spence; sr, Spraint; st ‡, Stellata; sy, Stiu; su ‡, Stump; sw, Sundew; sc ‡, Survey Creek; te, Tlell Estuary; vc ‡, Vaccinium; vn, Van Inlet; wa ‡, Watt; we ‡, Wegner; wh, White Swan; wi, Wiggins; wo, Woodpile; wr, Wright; y, Yakoun; yr, Yakoun River.