

10 000 Years later: Body shape and evolution in threespine stickleback

by

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B.Sc, University of Victoria, 2001

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Abstract

Descent with modification (Darwin, 1859) overwhelmingly occurs through the process of natural selection on genetically variable traits. Following deglaciation in the Pleistocene, morphologically conservative marine stickleback (*Gasterosteus aculeatus*) from two distinct mitochondrial DNA lineages colonized freshwater habitats on the Haida Gwaii archipelago. These freshwater populations have radiated in response to a diverse selective landscape on the archipelago and exhibit morphological diversity that equals or exceeds the known range for the species in the circumboreal distribution.

I investigated the body shape of 3808 stickleback from 125 isolated populations from Haida Gwaii using geometric morphometrics. Relative warp and discriminant function analysis were used to quantify lateral shape differences based on partial warp scores, which were generated from twelve homologous landmarks digitally placed on each specimen's image.

The possibility of ontogenetic shifts and sexual dimorphism of body shape were examined for each population on the archipelago. Stickleback body-shape changes during ontogeny were highly variable, and cannot be predicted by the volume and water clarity of the habitat. I found a slight sexual dimorphism in adult body shape, the magnitude of which could not be predicted by habitat volume and clarity.

A number of comparisons were made to elucidate the possible causes for selection for divergence of adult body shape among Haida Gwaii populations. Body shape of

parapatric lake-river stickleback populations differed significantly in concordance with hydrodynamic principles. Comparing the body shape of Haida Gwaii populations to the shape of sympatric benthic-limnetic species pairs from southwestern British Columbia, I found that benthic and limnetic body-shape ecotypes differ by up to 56% of the total variation among Haida Gwaii populations. Tests for phenotypic plasticity of body shape were conducted with two morphologically distinct stickleback populations that had been transplanted into two separate experimental ponds that were the ecological opposites of the respective source lakes. I found evidence for some phenotypic plasticity in body shape; the difference between each source and experimental population was approximately 11% of the total variation in body shape among populations throughout the archipelago.

Throughout the islands adult body shape and size can be predicted by both abiotic and biotic factors of the habitat. Populations with derived shape (CV1+), including thicker peduncles, posteriad and closely spaced dorsal spines, anterior pelvis, short dorsal and anal fins, and smaller body size occur in small, shallow, stained ponds, and populations with less derived shape (CV1-), with smaller narrow peduncles, anterior and widely spaced dorsal spines, posteriad pelvis, longer dorsal and anal fins, and larger size occur in large, deep, clear lakes. There were large-bodied populations with derived shape (CV2-), including smaller heads and shallower elongate bodies in open water habitats of low productivity, and populations with smaller size and less derived shape (CV2+), with larger heads and deeper bodies, in higher productivity, structurally complex habitats. Populations with robust defensive adaptations have less derived shape (CV1-) and larger size, in response to salmonid predation, while populations with weak defences had derived shape (CV1+), in response to bird/invertebrate predators. The ecomorphological relationships were consistent between mitochondrial lineages and replicated in each geographical region on the archipelago among geographically distant populations, suggesting the parallel evolution of body shape governed by the hydrodynamic constraints of each habitat. Although initially colonized by ancestors with conserved morphology, the stickleback populations on Haida Gwaii exhibit body shapes suited to the hydrodynamic landscape of the habitat, demonstrating the predictability of natural selection in adaptive radiations.

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Chapter 1 General introduction

Darwin's (1859) idea of descent with modification among species, is largely the result of differential selection pressures acting on genetically variable traits (Grant and Grant, 1982; Schluter and Grant, 1984). Differences in selective pressures or the selective landscape are the result of environmental heterogeneity (Lack, 1947; Grant *et al.*, 1976), and these differences can often lead to predictable evolutionary outcomes (Boag and Grant, 1981; Grant and Grant, 1995). Discovering the fundamental processes that lead to adaptation, through investigations of functional morphology, has been one of the foundations of modern biology. However, evolutionary diversification can occur through processes other than natural selection. Stochastic processes such as founder effect, population bottlenecks or genetic drift (Mayr, 1942) can lead to populations with striking phenotypic differences when compared to the ancestral population. Random processes must be addressed as a potentially confounding factor in evolutionary diversification.

One of the key components of evolutionary diversification is physical isolation. Isolation (either geographical or biological) prevents gene flow between closely related groups. Without gene flow, adaptation to novel selection pressures can lead to genetic differences and ultimately the adaptive radiation of a related populations (Mayr, 1942). Adaptive radiation is the diversification of a lineage into species that utilize a variety of different resource types, and that differ in the phenotypic traits used to exploit those resources (Huxley, 1974; Schluter, 1996). Nowhere have adaptive radiations been more apparent than on island archipelagos (Carlquist, 1974; Cox and Moore, 1993; Barrett, 1998). Colonizing organisms encounter novel habitats, open niche space, and isolation

from recurring gene flow. In other words with relaxed interspecific competition and insulation from the homogenizing effects of gene flow, colonizing organisms have the opportunity to occupy and adapt to habitats that may have been previously occupied by other groups on the mainland (Cox and Moore, 1993). Thus islands provide unique opportunities to assess the effects of stochastic processes and natural selection on adaptive radiations.

Adaptive radiation has been documented in diverse insular organisms, including numerous plant taxa, insects, and land snails (Carlquist, 1974). However, there are a few classic examples of adaptive radiations of island vertebrate fauna. The diversification of Darwin's finches of the Galápagos archipelago typifies adaptive radiation (Lack, 1947). The initial divergence of Darwin's finches began more than 2.3 million years ago, when the ancestral finches colonized the Galápagos islands from South America (Sato *et al.*, 2001; Grant and Grant, 2002). On each island, the finches have diversified into distinct segregated groups with phenotypic characters that have been correlated with environmental heterogeneity (Lack, 1947; Grant *et al.*, 1976). Specifically, the finches' food resource on the Galápagos archipelago differs on different islands concomitantly with differences in beak size, suggesting morphological differences are the result of selection pressures acting on genetically variable traits (beak size) (Grant and Grant, 1982; Schluter and Grant, 1984).

Another classic example of adaptive radiation has occurred in the West Indies. Mainland *Anolis* lizards initially colonized, and subsequently diversified on numerous Caribbean islands (Williams, 1969, 1972; Losos, 1990). Among islands, distantly related species have diverged from their mainland congeners, but have shown convergence in

ecology, morphology and behaviour (Beuttell and Losos, 1999), suggesting that the local environment, on each of these isolated islands, strongly influences the direction of adaptive radiations (Irschick *et al.*, 1997; Losos *et al.*, 1997; Beuttell and Losos, 1999). In an attempt to elucidate the mechanisms of morphological divergence Losos *et al.* (1997) introduced *Anolis sagrei* to previously uninhabited small islands from a nearby source island. Over a 10-14 year period, the introduced populations differentiated, and the magnitude of differentiation was correlated with the amount of difference between the vegetation of the source and recipient island. This example supports the argument that adaptive differentiation can be determined by the environment, and suggests divergence may occur very rapidly.

Adaptive radiation may be inevitable on islands or in an archipelago where isolation prevents gene flow and facilitates adaptation to novel selective landscapes (Mayr, 1942; Carlquist, 1974). However, adaptive radiations can occur within lakes, provided that immigrants encounter novel ecological opportunities. Cichlid fishes of the great lakes of central Africa have colonized novel habitats in each lake and morphologically diversified to an extent unmatched by any other vertebrate family (Fryer and Iles, 1972; Liem, 1973; Kornfield and Smith, 2000). For example Lake Tanganyika has more than 170 species, Lake Victoria contains over 200, and Lake Malawi has more than 500 cichlid species (Meyer *et al.*, 1990; Grant, 1998; Kornfield and Smith, 2000). With respect to the sheer number of endemic species, the radiation has been extremely rapid beginning between 12 000- 1 000 000 years before present (Johnson *et al.*, 1996; Kornfield and Smith, 2000; Sturmbauer *et al.*, 2001; Seehausen, 2002). The rapidity of the diversification may be due to an exceptional array of mechanisms (such as habitat

complexity, lake age and predation) needed to explain the extent of the radiation (see Kornfield and Smith, 2000 for review) but can mainly be attributed to the co-occurrence of a wide range of adaptive zones (ecologically distinct regions) in each lake and the presence of the extremely versatile cichlid pharyngeal jaw complex (Liem, 1973).

In the northern hemisphere a small marine teleost, threespine stickleback (*Gasterosteus aculeatus* L.), breeds along coastlines in estuaries and mouths of rivers and streams (Wootton, 1976; Francis *et al.*, 1985). The modern marine stickleback is indistinguishable from fossil stickleback from the Miocene, and suggests a static evolutionary history of the ancestral or less derived anadromous phenotype (Bell, 1977, 1994; Walker and Bell, 2000). The marine form has numerous traits for defense against predators including 30-35 lateral bony plates on each side of the body, three dorsal spines, paired pelvic spines and a small anal spine (Hagen, 1967; Bell, 1976; Reimchen, 1983). Following deglaciation in the late Pleistocene, marine stickleback have given rise to permanent freshwater populations in many low-lying lakes and streams (Münzing, 1963; Hagen, 1967; Moodie and Reimchen, 1976; Bell, 1981; Schluter and McPhail, 1992). Extensive morphological variation in freshwater populations (see Bell and Foster, 1994 for reviews) provides a unique opportunity to study the evolutionary implications of adaptive radiations.

Allopatry, or physical isolation is a consistent geographical component of the radiation of threespine stickleback (Bergstrom, 2002a), however there are 6 small lakes on islands in southern British Columbia, that contain coexisting benthic and limnetic pairs (Larson, 1976; McPhail, 1984, 1994; Lavin and McPhail, 1985; Schluter and McPhail, 1992). These pairs reside in sympatry, but the members of each pair differ

significantly in body shape and size, trophic morphology, behaviour, spatial distribution and feeding habits, suggesting these two forms have undergone extensive adaptations to very different habitats within each lake (Larson, 1976). There are several hypotheses on the origin of the species pairs (McPhail, 1994; Rundle *et al.*, 2000), but an allopatric origin through two invasions of marine stickleback is consistent with the geological history of the area, phylogeography of the populations and the local distribution (McPhail, 1993; Taylor *et al.*, 1997; Taylor and McPhail, 2000). Competition for food has played a critical role in maintaining and facilitating the divergence of the two phenotypes in each population (Schluter and McPhail, 1992), suggesting that common selective landscapes in each lake have resulted in convergent pairs of phenotypes.

On the Haida Gwaii archipelago in British Columbia (figure 1) there are numerous populations of threespine stickleback, that show a range of phenotypic variation (in size, longevity, nuptial colour display and defensive armature) that exceeds the range observed elsewhere within this widely distributed species (Moodie and Reimchen, 1976; Reimchen, 1980, 1983, 1989, 1992a; Reimchen *et al.*, 1985). Most lakes are accessible from the sea, but have been isolated from each other since the final drop in sea level over 10 000 years ago (Moodie and Reimchen, 1976). Some of the localities have high rates of predation from birds, fish and/or invertebrates, and the variability in the lakes biophysical characteristics encompass the range of biophysical variation which can support fish populations (Moodie and Reimchen, 1976; Reimchen, 1980, 1983; Bergstrom and Reimchen, 2002) in the northern hemisphere. It is the difference in the selective landscape between populations that has driven the adaptive radiation of stickleback on the Haida Gwaii archipelago.

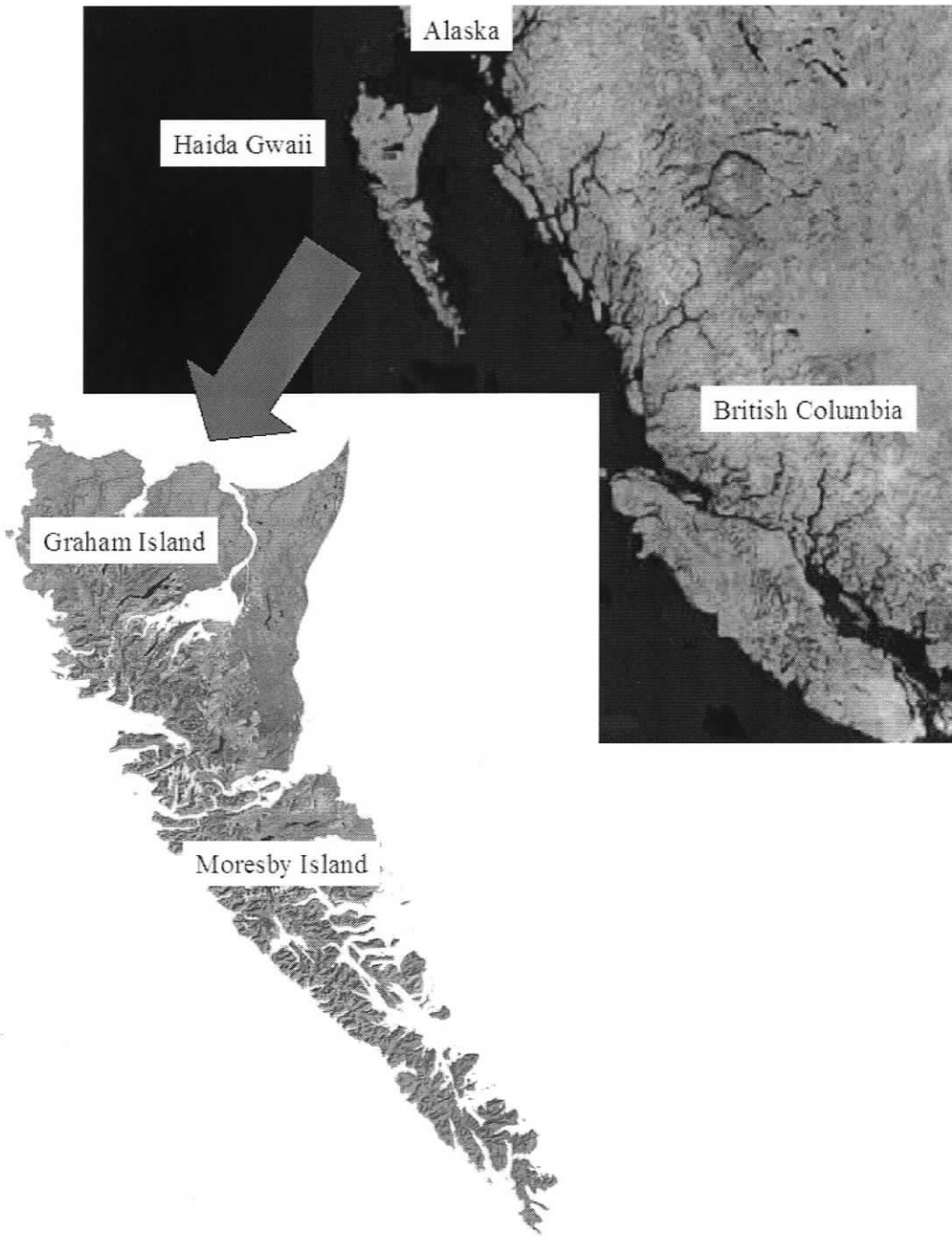


Figure 1. West coast of British Columbia and Haida Gwaii

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). However, all traits are related to the fitness of an organism, and many may be functionally integrated (interactive response of multiple characters to specific selection regimes) (Schlichting, 1989; Bell and Foster, 1994). When organisms in different habitats are exposed to different environmental conditions, the optimal response is to express the phenotype best suited to each particular set of conditions (Schlichting, 1989). An organism's body shape is an example of a trait that reflects phenotypic integration, as shape is often quantified by numerous correlated characters (Strauss and Bookstein, 1982; Baumgartner *et al.*, 1988; Rohlf, 1990; Bookstein, 1991; Baumgartner, 1992; Walker, 1997; Walker and Bell, 2000; Klingenberg *et al.*, 2003). In chapter 2 of this thesis, I briefly explain the methodology for quantifying an integrated character, such as body shape, using digital imaging and homologous landmark data.

Any organism moving in water encounters a certain degree of resistance from the environment, and the body shape in these organisms is fundamentally constrained by hydrodynamic forces associated with forward movement in a dense, viscous medium (Aleev, 1977). However, every organism inhabits slightly different niche space, and the body shape that is optimal for reducing resistance to movement changes with the functional demands of the habitat. The body-shape of fishes in particular, also show specific adaptations to specific niche space (Webb, 1982, 1984). For example, pike *Esox lucius*, and angelfish, *Pterophyllum eimekei*, both live in structurally complex habitats and show similar burst swimming performance, yet have extremely different morphologies (Domenici and Blake, 1997). Pike are elongate ambush predators, with a

narrow head and large tail, while angelfish are disk-shaped manoeuvring specialists, suggesting that the differences in integrated morphology may not be linked to burst acceleration performance, but rather to subtle differences in the ecological niche space each species inhabits.

It is not surprising that distantly related taxa have different body shapes that can be related to differences in niche space, but even within closely related species, we find this ecomorphological association. Body-shape variation among species of benthic darters (Percidae), from the southern U.S.A. is tightly coupled to variation in environmental parameters such as current speed, water depth and substrate composition (Guill *et al.*, 2003a; Guill *et al.*, 2003b). Among the *Amphilophus* (Cichlidae) species complex in Nicaragua, differences in body-shape among species is correlated with differences in feeding ecology (Klingenberg *et al.*, 2003). The association between body-shape and ecological niche space is so strong, that within species diversification can often occur. Alaskan threespine stickleback, *Gasterosteus aculeatus* (Gasterosteidae) (Walker, 1997), the mosquitofish, *Gambusia affinis* (Poeciliidae) (Langerhans *et al.*, 2004a), Coho salmon, *Oncorhynchus kisutch* (Salmonidae) (Swain and Holtby, 1989), and pupfish, *Cyprinodon tularosa* (Cyprinodontidae) (Collyer *et al.*, 2005) all exhibit predictable interspecific differentiation of body shape in relation to ecological factors. However, in order to assess the relationship between shape and ecological factors, the relative contributions of ontogeny and the possibility of sexual dimorphism have to be addressed.

As fish mature the body shape that is optimum for reducing resistance to movement through the water often changes with ontogeny (Aleev, 1977). Fish pass through distinctive size classes, which are often correlated with specific ecological

interactions (Werner and Gilliam, 1984). These interactions can be characterized as ontogenetic niche shifts, or changes in habitat or resource use during development, which provide maximum benefit for the individual (Werner *et al.*, 1983a, 1983b; Werner and Gilliam, 1984). I investigate whether there are ontogenetic shifts in body-shape in 125 stickleback populations (chapter 3), and whether any changes in shape related to ontogeny can be predicted through the volume and water clarity of the habitat.

Conspicuous sexual dimorphism is a widespread and often prominent feature of the natural world (Selander, 1966; Lande, 1980; Slatkin, 1984; Shine, 1989; Butler *et al.*, 2000; Butler and Losos, 2002). Both sexual selection (Darwin, 1874; Bonnet *et al.*, 1998; Bonnet *et al.*, 2001) and purely ecological forces can lead to, constrain or amplify sexual dimorphism and result in optimal phenotypes in each sex (Selander, 1966; Schoener, 1969; Lande, 1980; Slatkin, 1984; Shine, 1989; Reimchen and Nosil, 2001b, 2004). I examine lateral body-shape to specifically determine if there is a sexual dimorphism (chapter 4), to quantify any difference between male and female shape and to test whether any observed differences are related to the volume and water clarity of the habitat.

The relationship of body shape to ecology/habitat properties for adults in 125 stickleback populations, were investigated to elucidate mechanisms that select for body-shape differences among populations from Haida Gwaii (chapter 5). It is well known that throughout their holarctic distribution, lake stickleback populations have different morphological adaptations than do stickleback residing in streams (Hagen and Gilbertson, 1972; Gross and Anderson, 1984; Taylor and McPhail, 1986; McPhail, 1994). I compare body shape between parapatric lake-stream pairs to determine if any shape

differences are related to hydrodynamic constraints associated with habitats. To assess bi-directional versus directional selection on the evolution of form, I quantified the body-shape of stickleback from 3 populations in Southwestern British Columbia that contain coexisting benthic and limnetic pairs (Larson, 1976; McPhail, 1984; Lavin and McPhail, 1985; Schluter and McPhail, 1992), and compared the body-shape between each pair to the total variation in body-shape among Haida Gwaii populations.

There exists the possibility that body-shape differences among populations on Haida Gwaii are due to phenotypic plasticity. Simply stated, phenotypic plasticity is environment-dependent phenotypic expression that results in phenotypic changes rather than changes in allele frequency (DeWitt and Scheiner, 2004). I compared the differences in body shape between two experimental ponds and their respective source populations, to the variation in shape among Haida Gwaii populations, to estimate the magnitude of phenotypic plasticity of body shape.

There are numerous factors that have contributed to the adaptive radiation of stickleback on Haida Gwaii. Given the efficacy of hydrodynamic forces on the evolution of body-shape optima in numerous fish taxa (Webb, 1982, 1984), I explore whether the body shape of insular populations of stickleback can be predicted by biotic and abiotic characteristics of the habitat. The stickleback populations on the Haida Gwaii archipelago are ideal for elucidating the mechanisms of divergence, because of the variability in both predation regimes and biophysical characteristics among habitats (Moodie and Reimchen, 1976; Reimchen, 1980, 1983; Bergstrom and Reimchen, 2002). Additionally there are also distinct geographical regions on the archipelago where populations were initially founded by marine stickleback from two morphologically similar (Taniguchi *et al.*,

1990), yet divergent mitochondrial DNA lineages (Orti *et al.*, 1994; Deagle *et al.*, 1996). In essence each population represents one treatment in a natural evolutionary experiment (Carlquist, 1974) to study the diversification of form.

Chapter 2 Material and methods

2.1 Sampling and study area

T. E. Reimchen collected samples of stickleback from marine, lacustrine and stream localities on Haida Gwaii from 1969- 2003. Samples from 125 localities (figures 2-3) from sites in each of the three major geographical zones (lowland, plateau and mountain) on the archipelago were collected throughout the spring and summer using standard-mesh minnow traps, baited with cheddar cheese, and placed in the littoral zones (see Reimchen *et al.* 1985; Reimchen 1989, 1992c, and 1994 for detailed habitat description and collection methods). The samples included: one marine, five brackish, 19 river and 100 lake populations. Fish were fixed in 10% formalin and stored in 95% ethanol.

2.2 Lake characteristics

Physical data were also collected from a subset of localities during stickleback collection expeditions and include: pH, specific conductance, calcium concentration (mmol), T400 (percent 400nm light transmission), lake area (ha), maximum depth (m), volume (m³), elevation (m) and distance to the ocean (m). A principal component analysis was performed on the physical data to create coherent independent subsets of the data that would best describe each lake habitat type (Tabachnick and Fidell, 2001; Jones *et al.*, 2003). Variables that are correlated with one another but largely independent of other subsets of variables are combined into factors, which are thought to reflect underlying processes that have created the correlations among the variables. The first

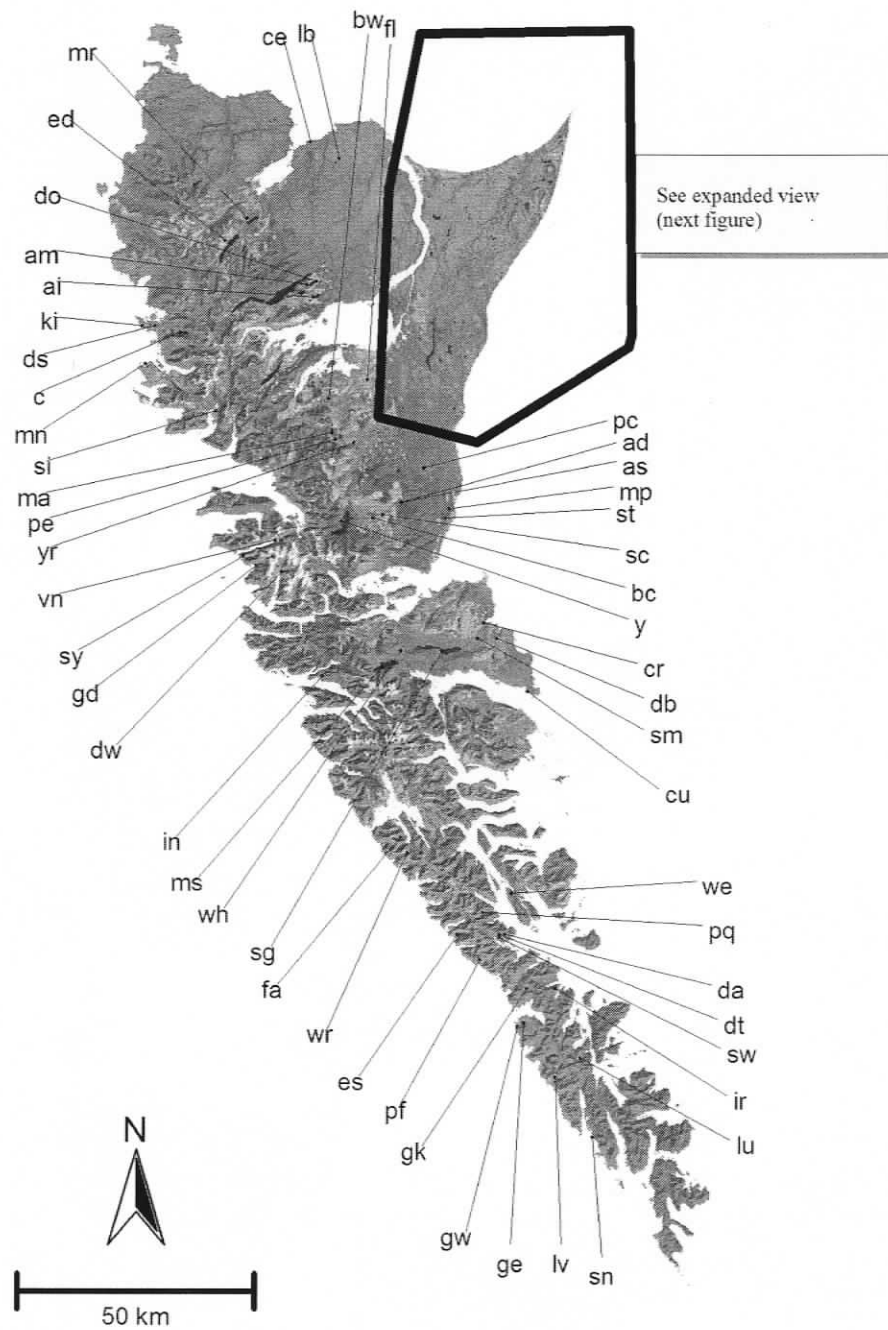


Figure 2. Localities where stickleback were collected on Haida Gwaii. Site names

listed here and in figure 3 are abbreviated as follows: 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 pd, 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= Heralda Lower, hm= Heralda 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= Ottern, 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.

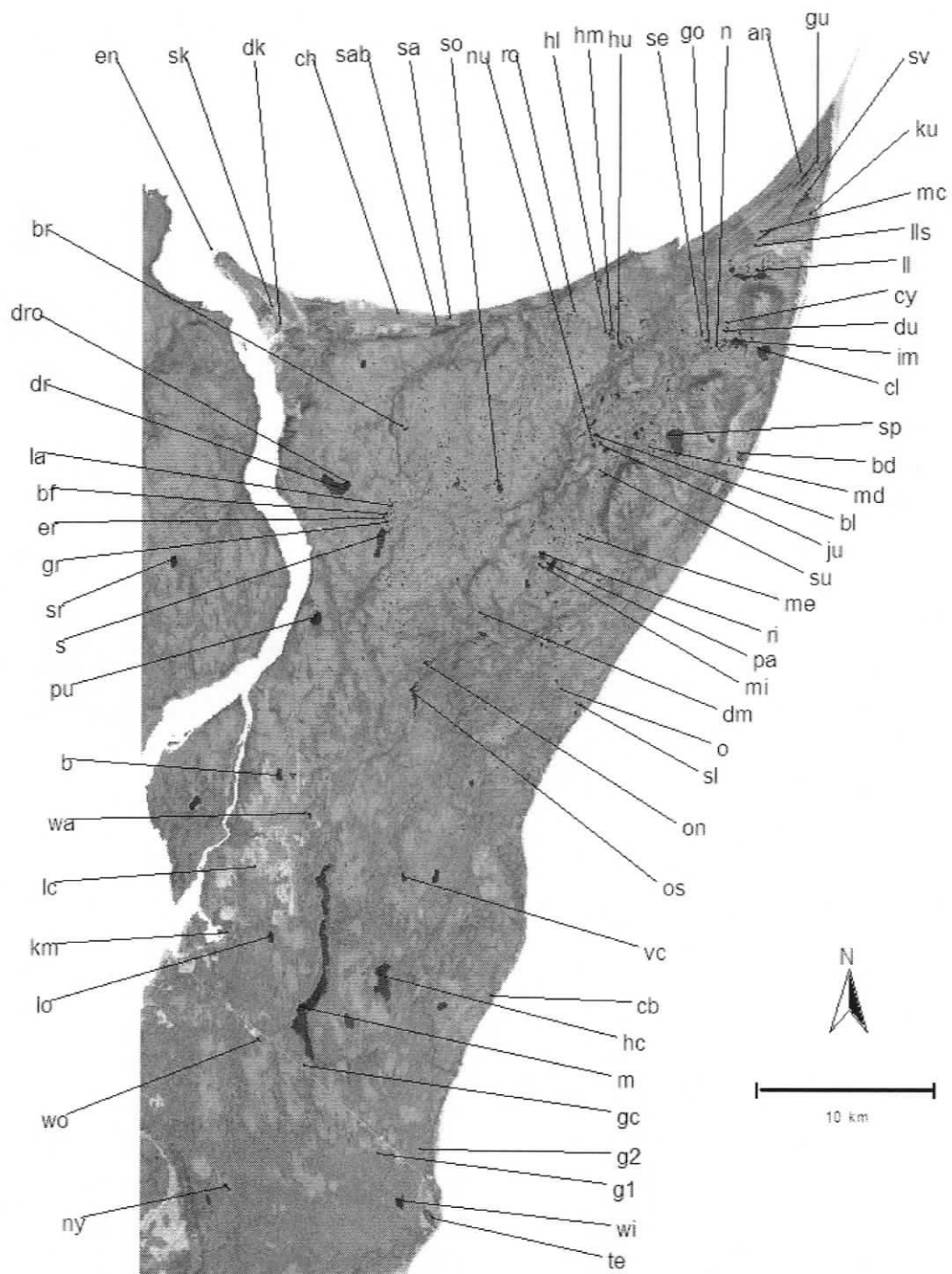


Figure 3. Localities where stickleback were collected on the North East corner of Haida Gwaii.

principal component of the physical data explains 38.9% of the variation among lake habitats and reflects lake volume/water clarity. The second principal component explains 25.2% of the variation, and captures lake conductivity (table 1). On Haida Gwaii conductivity is associated with productivity (Reimchen, 1992c), therefore I use the second PC as a proxy for productivity.

2.3 Morphometrics

2.3.1 Data collection

To determine lateral body shape, each stickleback was photographed using a Cannon Powershot G1 digital camera with 3.3 mega pixels. The camera was mounted on a Wild Heerbrugg heavy-duty stand, and I ensured the camera was level in 2 dimensions and the optical axis of the camera was perpendicular to the object using an Ace level (figure 4). Sticklebacks were pinned flat on a wax substrate with the fish's vertebral axis aligned. As a method to standardize each individual, each specimen was then oriented in the center of the camera viewfinder, with the vertebral axis parallel to the horizontal plane. A Scientific Products ruler, to quantify the scale of the image, and the specimen ID number were also included in each photo.

For the assessment of ontogenetic shifts in body-shape, I used juvenile, sub adult and adult stickleback when available. However, to get the most conservative estimate of adult stickleback body-shape, I specifically chose the largest sexually mature males and females from each sample, as allometry in stickleback body shape is small following sexual maturity (Walker, 1993).

Table 1. Principal component matrix of loading scores for physical characteristics of lake habitat, (N= 70) on Haida Gwaii. PC1_lk explains 38.9% of the variation among habitats and represents the habitat volume and clarity. PC2_lk explains 25.2% of the variation and represents the productivity of the lake. All of the variables except pH and T400, were natural log transformed prior to the principal component analysis.

	Volume/clarity (PC1)	Productivity (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 marine	-0.23	-0.39

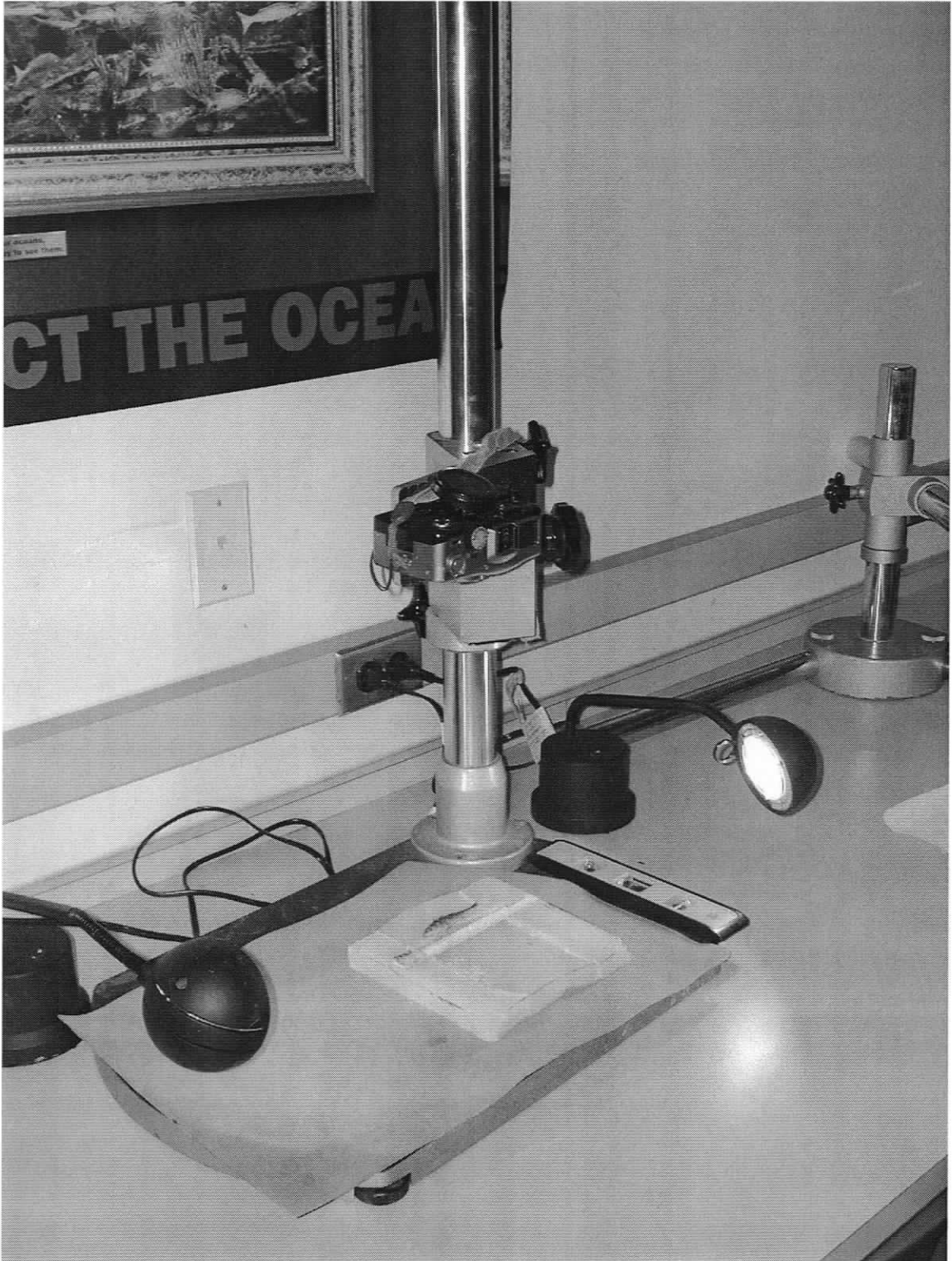
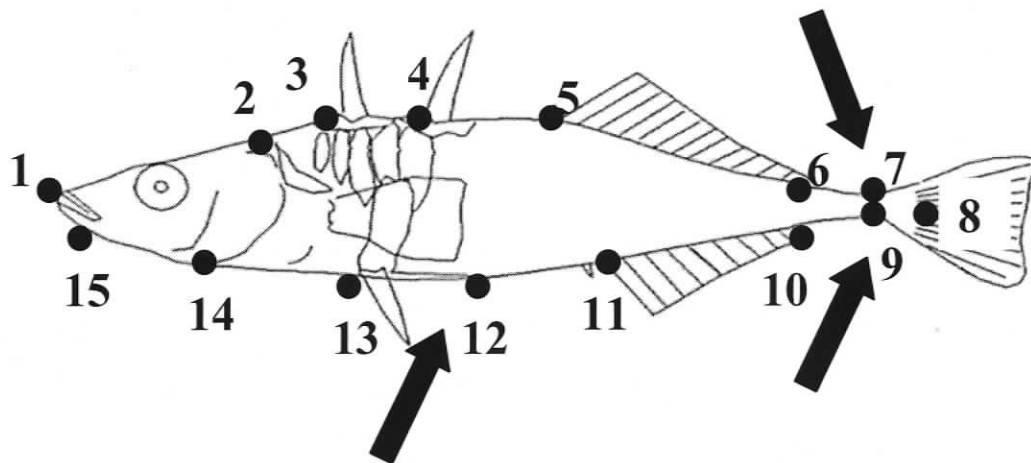


Figure 4. Photo stand and camera set up

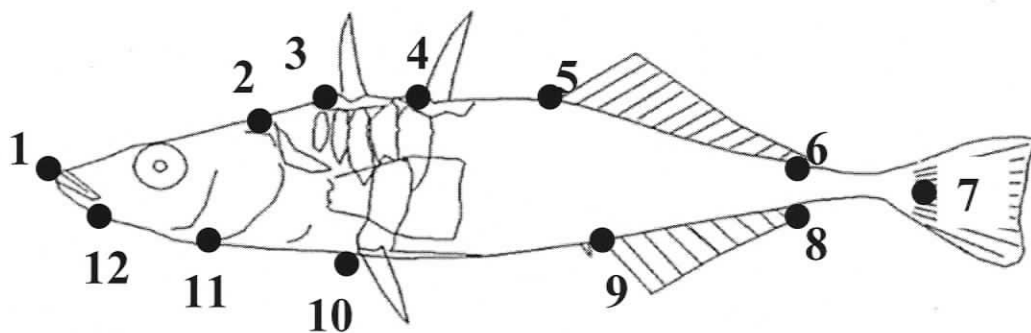
I adhered to the previous work on stickleback shape by Walker (1993, 1997) and used 15 homologous landmarks (lm) to delimit body-shape. Homologous landmarks are “juxtaposition of tissue or symphyses of bone can be found on all individuals in the study” (Bookstein, 1991). Repeatability of landmark placement was calculated for a subset of stickleback (n= 40) from both raw landmark coordinates and generalized least-squares (see section 2.3.3) aligned coordinate data (table 2). The distances from landmark 1 to each of the remaining 14 landmarks from the raw landmark data, and distances from the origin (0,0) to each of the 15 landmarks (figure 5A) from the aligned data were used in the repeatability calculations. The program TPSRelw (Rohlf, 2002) allowed me to assess the relative contribution of each landmark to the estimation of shape. I found that two landmarks, which correspond to the dorsal and ventral insertion of the caudal fin (landmarks 7 and 9; figure 5A), contributed a disproportionate amount of variation (lm7: 23%; lm9: 27%) to the shape analysis (table 3). Depending on the condition of the specimen, the neural and haemal spines that delimit the dorsal and ventral edges of the caudal fin frequently appeared in a continuous ridge extending to either the dorsal or anal fin. It was often difficult to ascertain where location of the true insertion point actually was (i.e., not a true “homologous landmark”), which would account for the unusually high variation at these positions. I decided to remove these landmarks from the assessment of body-shape, as the elevated levels of variation could affect the multivariate results and produce shapes driven disproportionately by these two landmarks. Furthermore the length of the pelvic girdle was also highly correlated with the first multivariate shape variable (Pelvis measure: $R^2 = 0.88$, $p < 0.001$; GLS transformed

Table 2 Repeatability of landmark placement. Calculated from distance measurements between landmark (1) to landmark (*i*) (for raw data) and from the origin (0, 0) to landmark (*i*) (for aligned data) as: σ^2 group/ σ^2 group+ σ^2 error.

Landmark	Raw coordinate data	Aligned coordinate data
(1) anterior tip of premaxilla	Na	93%
(2) supraoccipital notch immediately left of the dorsal midline (DML)	96%	95%
(3) anterior junction of first dorsal spine and basal plate along the DML	95%	99%
(4) anterior junction of the second dorsal spine and basal plate along the DML	95%	99%
(5) base of the first dorsal fin ray at the DML	94%	96%
(6) insertion of the dorsal fin membrane on the DML	97%	99%
(7) origin of caudal fin membrane on the DML	98%	98%
(8) caudal border of hypural plate at lateral midline	97%	99%
(9) origin of caudal fin membrane on ventral midline (VML)	99%	89%
(10) insertion of anal fin membrane on VML	74%	96%
(11) base of first anal fin ray on VML	99%	99%
(12) tip of posterior process of pelvic girdle on VML	98%	96%
(13) posterior tip of ectocoracoid	93%	67%
(14) anterior border of ectocoracoid on VML	95%	90%
(15) point of articulation between angular and quadrate	85%	92%



A: 15 landmarks



B: 12 landmarks

Figure 5. Sketch of stickleback showing landmark placement. The 15 landmarks correspond to (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) origin of caudal fin membrane on the DML (8) caudal border of hypural plate at lateral midline (9) origin of caudal fin membrane on ventral midline (VML) (10) insertion of anal fin membrane on VML (11) base of first anal fin ray on VML (12) tip of posterior process of pelvic girdle on VML (13) posterior tip of ectocoracoid (14) anterior border of ectocoracoid on VML (15) point of articulation between angular and quadrate.

Table 3. The relative contribution (proportion of total variation) of each of the 15 landmarks to the relative warp analysis.

Landmark	Relative contribution (%)
(1) anterior tip of premaxilla	0.98
(2) supraoccipital notch immediately left of the dorsal midline (DML)	0.97
(3) anterior junction of first dorsal spine and basal plate along the DML	4.0
(4) anterior junction of the second dorsal spine and basal plate along the DML	3.8
(5) base of the first dorsal fin ray at the DML	1.1
(6) insertion of the dorsal fin membrane on the DML	8.8
(7) origin of caudal fin membrane on the DML	23.4
(8) caudal border of hypural plate at lateral midline	7.5
(9) origin of caudal fin membrane on ventral midline (VML)	27.3
(10) insertion of anal fin membrane on VML	12.8
(11) base of first anal fin ray on VML	2.6
(12) tip of posterior process of pelvic girdle on VML	2.0
(13) posterior tip of ectocoracoid	0.94
(14) anterior border of ectocoracoid on VML	1.5
(15) point of articulation between angular and quadrate	2.3

pelvis measure: $R^2 = 0.87$, $p < 0.001$), indicating that the defensive condition strongly influences this measure of body-shape. Since I am interested in the stickleback body-shape independent of structural defensive adaptations, I removed the landmark that delimited the posterior maxima of the pelvic girdle (figure 5A).

I used twelve homologous landmarks to delimit body shape (figure 5B).

Landmarks were digitized on the images of stickleback-using TPSDig software (Rohlf, 2004) and were selected to represent true homologous structures and minimize the effects of the pelvic girdle.

2.3.2 Linear measurements

The original Cartesian coordinates of each specimen (figure 5A; 15 landmarks) and the scale of each fish from the digitization of the landmarks were used to calculate a number of standard morphometric measurements on each stickleback. Using the Pythagoras's formula: $\text{length} = \sqrt{((x_1 - x_2)^2 + (y_1 - y_2)^2)}$, the measurements were obtained for: standard length (lm1: lm8), gape estimate (lm1: lm15), head length (lm1: lm2), head depth (lm2:lm14), head area ((lm1: lm2) + (lm2: lm14)+ (lm14: lm15)+ (lm1: lm15)), anterior body depth (lm3: lm 13), posterior body depth (lm4: lm13), position of dorsal spine 1 (lm1: lm3), distance between dorsal spine 1 and dorsal spine 2 (lm3: lm4), pelvic position (lm1: lm13), pelvis length (lm12: lm13), dorsal fin length (lm5: lm6), anal fin length (lm10: lm11), anterior caudal depth (lm5: lm11), and posterior caudal depth (lm6:lm10), caudal area ((lm6: lm8)+ (lm8: lm10)+ (lm 6: lm10)).

Adult body-size often differs among localities, and consequently differences among populations may be inflated by allometric effects (Reimchen *et al.*, 1985). To

remove any effects of allometry, the morphometric measurements (listed above) were standardized, using the equation:

$$\log Y'_{ij} = [\log Y_{ij} - (v_j (\log x_i - \log x))]$$

Where Y'_{ij} is the adjusted value of character j for individual i , Y_{ij} is the original value, v_j is the average allometric coefficient, x_i is the standard length of individual i and x is the mean standard length averaged over all individuals in the study (Reimchen *et al.*, 1985).

2.3.3 Alignment procedure

The configuration of landmarks for an individual specimen is referred to as a figure (Goodall, 1991). The centroid is the mathematical center of a figure. The size of a figure is referred to as the centroid size, and is the square root of the sums of squared distances of a set of landmarks from their centroid. I use TPSRegr software (Rohlf, 2000) to align and scale each figure, by superimposing each figure in concordance with the generalized least-squares (GLS) orthogonal Procrustes method. Using this method each figure's centroid is aligned with the origin and scaled to a unit size of one. After alignment, a hypothetical consensus configuration is constructed, which is the average shape for a sample whose sum of squared Procrustes distances to the other figures is minimal (Rohlf, 1990, 1999). Large-scale changes in shape, due to the alignment procedure (configuration being stretched or compressed by some factor) constitute the uniform (or affine) component of shape change (Rohlf, 1996).

2.3.4 Quantification of shape variables

The TPSRegr software also creates shape variables using thin-plate spline morphometrics. This analysis involves the creation of orthogonal principal warp vectors, which correspond to differences between the consensus configuration and a given figure at different geometric scales (Bookstein, 1991). These principal warps are mathematical constructs that have no necessary biological relevance (Bookstein, 1991). Partial warps are the projections of the x and y coordinates of the deviations of a figure from the consensus onto the principal warps (Bookstein, 1991). The partial warps, or shape variables, correspond to the non-affine, small-scale changes in the consensus to those of a particular scale or principal warp. These shape variables have an advantage over conventional linear measurements used in ichthyology, because they allow one to visualize trends in shape variation as continuous non-linear deformation (Rohlf and Marcus, 1993), which can be shown in the form of a transformation grid, similar to those proposed by D'arcy Thompson (1917).

I used discriminant function and relative warp analysis to quantify lateral body-shape. I ran each analysis on the partial warp scores and uniform component to obtain an overall assessment of shape variation (Rohlf, 1993, 1996; Bookstein, 1996). The discriminant function can be used to predict group membership (i.e. stickleback sample number), based on reliable mean differences on combinations of partial warp and uniform component scores. This procedure produces linear combinations of predictors called canonical vectors, or CV's, which can be regressed, using the TPSRegr software, against the partial warp and uniform component scores to produce visualizations of trends in shape variation. The thin-plate spline function is used to construct hypothetical changes in shape (in the form of transformation grids) along each canonical variate axis that

describes the major trends in variation among samples relative to the variation found within samples (Rohlf and Marcus, 1993; Rohlf *et al.*, 1996). The relative warp analysis was performed using TPSRelw (Rohlf, 2002), to examine variation in shape among the Haida Gwaii stickleback. Relative warp analysis can be thought of as a principal component analysis of the partial warp scores and uniform component of shape change (Bookstein, 1991). The partial warps that are correlated with one another, but largely independent of other subsets, are combined into factors that summarize the patterns of correlation among the partial warp scores (Bookstein, 1991). The factors (relative warps) describe the primary axes along which shape change occurs (Bookstein, 1991).

Throughout the thesis I employed a parallel analysis of body-shape using the two multivariate methods (see above). In chapter 5, I used only the discriminant function, and associated canonical vector scores to explore the ecological relationships between body-shape and habitat. A few studies of body-shape variation among closely related species found that relative warp and discriminant function analysis produced similar results (Baumgartner *et al.*, 1988; Hood, 2000; Douglas *et al.*, 2001). When using 15 landmarks to quantify lateral body-shape, I found relative warp 1 (28.8% of total variation among Haida Gwaii samples) and canonical vector 1 (38.1% of total variation) tightly correlated ($R^2 = 0.85$, $p < 0.001$), suggesting that the two methods quantified similar aspects of body-shape. However, the length of the pelvic girdle was tightly coupled to both multivariate measures of body-shape (pg 19 of this thesis), and was subsequently removed from the shape analysis. Similarly, landmarks 7 and 9 contributed a disproportionate amount of variation and were also removed (pg 19 of this thesis). The correlation between RW1 and CV1 ($R^2 = 0.09$, $p = 0.001$) was considerably less, when using 12 landmarks to quantify

lateral body-shape. Given the disparity in these primary shape vectors, I chose to use the discriminant function analysis to explore any ecological relationships, because the multivariate result is judged by how well it predicts group membership (Tabachnick and Fidell, 2001). In principal component analysis (relative warp) there is no such criterion against which to test the solution (Tabachnick and Fidell, 2001).

2.4 Statistical considerations

The statistical tests increased probability of producing a type I error; rejecting the null hypothesis when it is inappropriate to do so (Walker, 1997; Moran, 2003). Typically the solution to this problem is the application of the sequential Bonferroni adjustment (Rice, 1989). The application of this procedure takes into account the number of statistical tests and adjusts the table-wide p-value, to keep it constant at 0.05, subsequently reducing the probability of obtaining a spurious result (Moran, 2003). However, the “irony of the sequential Bonferroni adjustment is that as one performs more detailed work (i.e. more statistical tests), the probability of finding a significant result declines dramatically” (Moran, 2003). In my study the changes in body-shape are highly correlated, and as a consequence the sequential Bonferroni adjustments are even too conservative (Walker, 1997), thus I chose not to adjust p-values for the t-tests or ANOVA’s.

Chapter 3: Ontogenetic shifts in body shape

3.1 Introduction

For fish the body-shape optimum for reducing resistance to movement through the dense aquatic medium often changes with ontogeny (Aleev, 1977). Due to their small size and low Reynolds number, many larval fish inhabit a viscous-dominated medium (Hernandez *et al.*, 2002). The Reynolds number (Re) is a “dimensionless measure of the hydrodynamic interactions on a body moving in water” (Vogel, 1981). In general terms Re is the ratio of inertia to frictional forces, where a small organism usually means slow moving, with viscous forces dominating and large means fast with inertial forces dominating (Aleev, 1977; Vogel, 1981).

For an integrated phenotypic character such as body shape, fish ontogeny can be a complex phenomenon of growth and differentiation (Koumoundouros *et al.*, 1999). A number of studies have shown that body-shape changes are not simply the result of large scale events, such as dorsoventral elongation, but rather localized small scale shape changes unique to each population (Walker, 1993; Hood and Heins, 2000; Zelditch *et al.*, 2000). Changes in size and shape often result in changes in the swimming hydrodynamics and kinematics (Wakeling *et al.*, 1999). However, once a larval fish exceeds the size at which the ratio of inertia to friction forces is large, body shape may be relatively conserved, and the organism can move efficiently through the water. Investigation of the hydrodynamic adaptations of the mullet, *Mugil saliens*, showed that the body shape did not change, over a range of standard lengths comparable to those observed in my study, even though the Reynolds number nearly doubled (Aleev, 1977). This supports the idea

that body shape may be conserved once young fish attain adult form, as there are numerous other adaptations to overcome hydrodynamic constraints.

Larvae of threespine stickleback hatch about 8 days post fertilization (Wootton, 1976). The yolk sack is still protuberant and the larval fish possess a fin fold which runs along both the dorsal and ventral mid-lines linking with the tail fin (Wootton, 1976). These low Reynolds number fish continually vibrate their pectoral fins and use their long dorsal and anal fins to move through the water with anguilliform movements (characterized as undulatory waves displacing water along the length of the body), which are adaptations to moving through the viscous medium (Wootton, 1976; Sfakoitakis *et al.*, 1999). After about 18 days of post hatching, the young stickleback loses its larval characteristics and assumes an adult-like shape (Wootton, 1976). These ontogenetic changes consist of the development of a caudal region characteristic of adult fish, and the laminarization of body shape. This can be thought of as streamlining the body with smooth flowing contours, having the maximum cross sectional area positioned one-third the total length, and tapering the posterior into an elongated cone, all of which are adaptations of increasing Re and related drag (Aleev, 1977). As stickleback grow their body-shape changes allometrically, however changes in body shape are small following sexual maturity (Walker, 1993). In laboratory conditions *G. aculeatus* can reach sexual maturity in as little as four months (Wootton, 1976). However such rapid growth is never achieved in natural populations due to the ecological interactions of a given habitat.

Ecological interactions at each stage in development, are often correlated with individual growth (specific cohorts) for a number of taxa (Werner and Gilliam, 1984). These interactions can be characterized as ontogenetic niche shifts, or changes in habitat

or resource use during development that provides the maximum benefit for the individual (Werner *et al.*, 1983a, 1983b; Werner and Gilliam, 1984). Such shifts are widespread in natural populations and introduce age or size structure in a community, with different cohorts subject to different ecological interactions, that can have very different effects on community and ecosystem level processes (Olson, 1996). In many species the timing of ontogenetic niche shifts change in response to biotic and abiotic environmental conditions (Olson, 1996), which may be adaptive responses to variable ambient conditions (Werner and Gilliam, 1984).

When the resources in one niche are scarce, growth conditions of the consumer are poor, which may lead to early ontogenetic niche shifts (Robinson *et al.*, 1993), reducing foraging by the consumer and promoting recovery of the limited resource (Takimoto, 2003). For a given species in different isolated habitats, plasticity in the ontogenetic niche shift reduces intraspecific competition for a scarce resource (Takimoto, 2003), which can lead to both variable growth rates and niche shifts in each different type of habitat.

Threespine stickleback populations from Haida Gwaii have undergone repeated, parallel evolutionary diversification in a number of isolated lakes and ponds (Moodie and Reimchen, 1976; Reimchen *et al.*, 1985; Deagle *et al.*, 1996). Numerous phenotypic characters have diversified throughout the archipelago, possibly as evolutionary responses to the selective landscape of each habitat. In this chapter I investigate the ontogenetic shifts in body shape among 125 populations of threespine stickleback from Haida Gwaii to quantify the ontogenetic changes in body shape and to determine if the populations share similar ontogenetic trajectories. Given the potential complexity of

ecological interactions during fish ontogeny, I also determine if ontogenetic shifts (isometric and allometric changes at varying degrees) in Haida Gwaii stickleback body shape are predictable in relation to the volume and clarity of habitat.

3.2 Methods

3.2.1 Quantification of body shape using discriminant function and relative warp vectors

I used digital images and landmark data (see chapter 2) of juvenile, sub-adult and adult stickleback (n= 3808) from 125 localities on Haida Gwaii. TPSRegr (Rohlf, 2000) was employed to generate partial warp scores and the uniform component of shape change, which serve as the independent variables describing morphology, in discriminant function analysis. This procedure produces canonical vectors, which quantify shape change along orthogonal axes. I regressed the canonical vector scores (CV) against the partial warp scores to generate thin plate spline plots, graphical representation, of the extreme body shapes along the first two canonical vectors. I also used relative warp (RW) analysis and the corresponding thin plate spline plots as another method to quantify body shape among populations. Pearsons correlation between vector scores (CV1 and CV2; RW1 and RW2) and log-linear regression transformed measures of standard morphometric variables (see chapter 2) was used to quantify shape differences along each of the vectors.

3.2.2 Changes in body shape during ontogeny in Mayer and Drizzle Lakes

Population CV and RW scores for stickleback from Mayer Lake (n= 185) and Drizzle Lake (n= 111) were analyzed for ontogenetic shifts in body shape. I used the

natural log of centroid size as my measure of overall body-size, as it is tightly coupled to standard length ($R^2 = 0.99$, $p < 0.001$). In each lake I used linear regression to determine if there is an association between body-shape (first two canonical and relative warp vectors), and the natural log of centroid size.

3.2.3 Comparison of ontogenetic slope among 125 stickleback populations

I performed linear regression on each populations shape variables (CV1, CV2, RW1 and RW2) against the natural log of centroid size, in all stickleback populations, and quantified the ontogenetic changes in shape through examination of the direction and significance level of each population's ontogenetic slopes. This was done for the whole archipelago, and then I partitioned that data and plotted the slopes for each geographical region.

3.2.4 Ontogenetic slope and habitat volume/clarity

To determine if there was relationship between ontogeny and habitat, I regressed each population slope against the first factor score for lake physical data (see chapter 2). The analysis was conducted for the whole archipelago, and then for each geographical region, combining the plateau/mountain areas, to determine if there were consistent associations in each region.

The sign significance of each population's slope was calculated as the slope direction (+ or -), multiplied by the significance level of each population's shape relationships with size. I also calculated the sign inverse-variance, using the inverse of the variance from each population's shape relationships with size. I regressed these

values against the lake physical factor score to determine if there was a relationship between the degree of change during ontogeny and habitat. This was done for the whole archipelago and geographical regions within the islands.

The data was partitioned into two groups corresponding to a statistically significant ($p \leq 0.05$) or highly non-significant ($p \geq 0.5$) ontogenetic slope. I then regressed population's slopes that were significant against the lake physical factor score. I also regressed population's whose slope was highly non-significant against the lakes physical factor score, to determine if there is a relationship, regardless of slope direction, between significance and habitat.

The data was then partitioned into two groups corresponding to the direction (+ or -) of the ontogenetic slope, and regressed against the lake physical factor score. This was done to determine if there was any relationship between the direction of ontogenetic shifts in body shape and habitat.

3.3 Results

3.3.1 Quantification of body shape using discriminant function and relative warp vectors

The first canonical vector (CV1) from the discriminant function analysis accounts for 33% of the total variation. The vector can be characterized by correlations with standard morphometric measures (see chapter 2) and contrasts stickleback with narrower peduncles, anterior and widely spaced dorsal spines, posterior pelvis and longer dorsal and anal fins (CV1-) versus stickleback characterized by thicker peduncles, posterior and closely spaced dorsal spines, anterior pelvis and shorter dorsal and anal fins (CV1+). The second canonical vector (CV2) accounts for 21% of the total variation and contrasts

stickleback with small heads and shallow bodies (CV2-) against stickleback with large heads deeper bodies (CV2+; figure 6; table 4). The first Relative warp (RW1) vector accounts for 24% of the total variation and contrasts stickleback with small heads, anterior dorsal spines, and shallow bodies (RW1-) against stickleback with larger heads, posterior dorsal spines, and deeper bodies (RW1+). Relative warp 2 accounts for 16% of the total variation and contrasts stickleback characterized with shorter dorsal and anal fins (RW2+) versus stickleback with longer dorsal and anal fins (RW2-; figure 7; table 5).

3.3.2 Changes in body shape during ontogeny from Mayer and Drizzle Lakes

Stickleback from Mayer Lake exhibited detectable shifts in body shape throughout ontogeny. Both the first and second canonical vectors had a significant relationship with the natural log of centroid size (body size; CV1: $R^2 = 0.1$, $p < 0.001$; CV2: $R^2 = 0.29$, $p < 0.001$). In relation to juvenile stickleback, Mayer Lake adult stickleback have relatively narrow peduncles, more anterior and widely spaced dorsal spines, posterior pelvis, larger median fins, smaller heads and shallower bodies (figure 8). I detected changes in shape during ontogeny using relative warp scores as well (RW1: $R^2 = 0.28$, $p < 0.001$; RW2: $R^2 = 0.17$, $p < 0.001$). As Mayer Lake stickleback grow they can be characterized as possessing relatively smaller heads, more anterior dorsal spines, shallower bodies and longer median fins than the juveniles (figure 9).

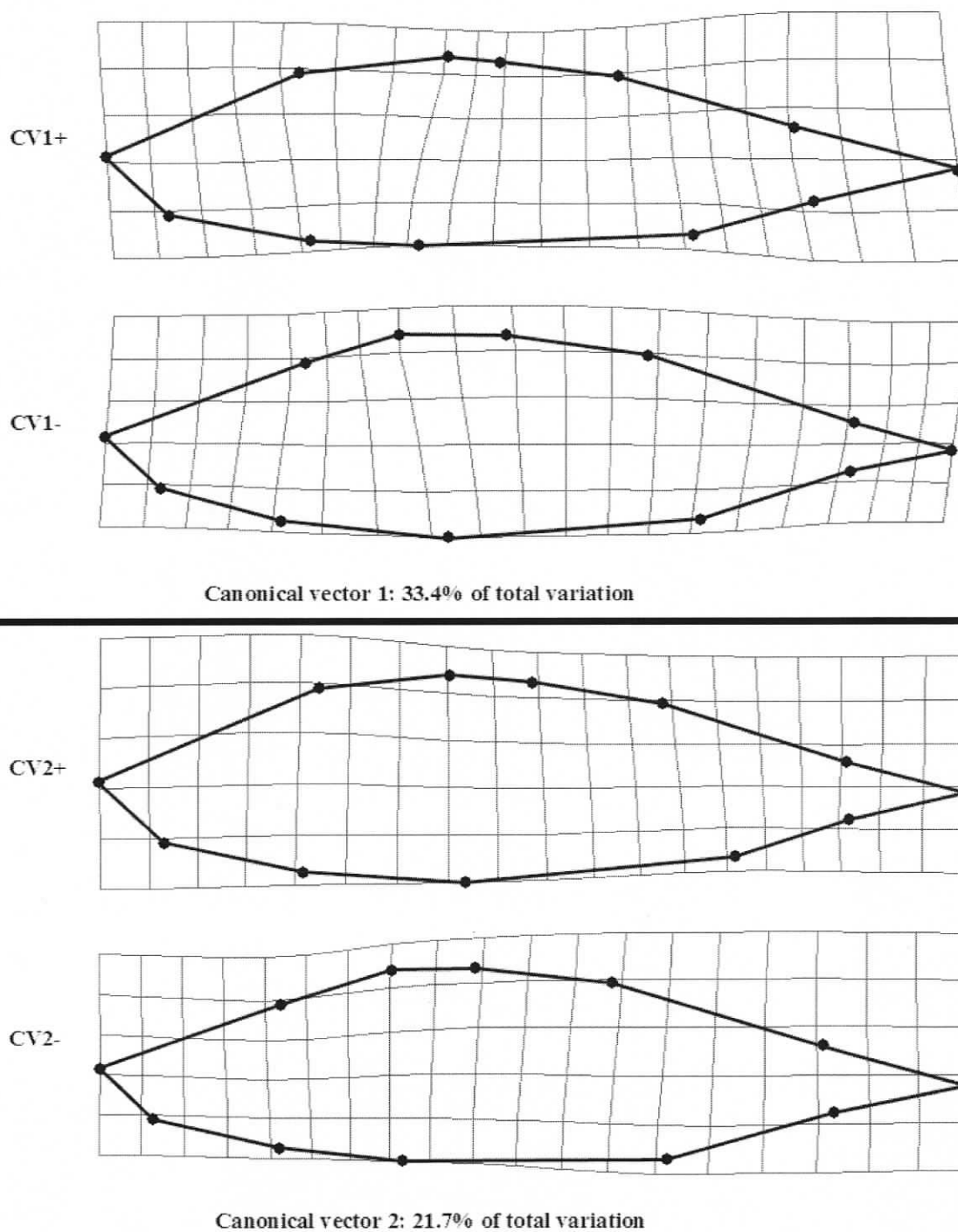


Figure 6. Thin plate spline plots of body shape of stickleback from Haida Gwaii as quantified by canonical vectors. Plots represent the actual differences between the + and - extremes of each canonical vector.

Table 4. Pearson correlations for CV1 and CV2 between log linear regression standardized morphometric measures calculated from inter-landmark distances for 125 stickleback populations. CV1 explains 33.4% of the variation among populations of adult stickleback from Haida Gwaii; CV2 explains 21.7% of the variation.

Character	CV1		CV2	
	Pearson	P value	Pearson	P value
	Correlation		Correlation	
Pelvis	-0.73	< 0.001	0.18	0.05
Pelvis position	-0.52	< 0.001	0.73	< 0.001
Head area	0.08	0.38	0.56	< 0.001
Head length	-0.23	0.01	0.55	< 0.001
Head depth	0.16	0.07	0.6	< 0.001
Gape	0.28	0.001	0.72	0.002
Dorsal spine 1 (D1) position	0.58	< 0.001	0.33	< 0.001
Distance between D1 and D2	-0.92	< 0.001	0.44	< 0.001
Body depth (anterior)	-0.15	0.10	0.24	0.006
Body depth (posterior)	-0.04	0.68	0.09	0.31
Dorsal fin length	-0.41	< 0.001	-0.08	0.36
Anal fin length	-0.45	< 0.001	-0.44	< 0.001
Caudal peduncle depth (anterior)	0.15	0.10	-0.13	0.16
Caudal peduncle depth (posterior)	0.69	< 0.001	-0.31	< 0.001
Caudal peduncle area	0.71	< 0.001	-0.43	< 0.001

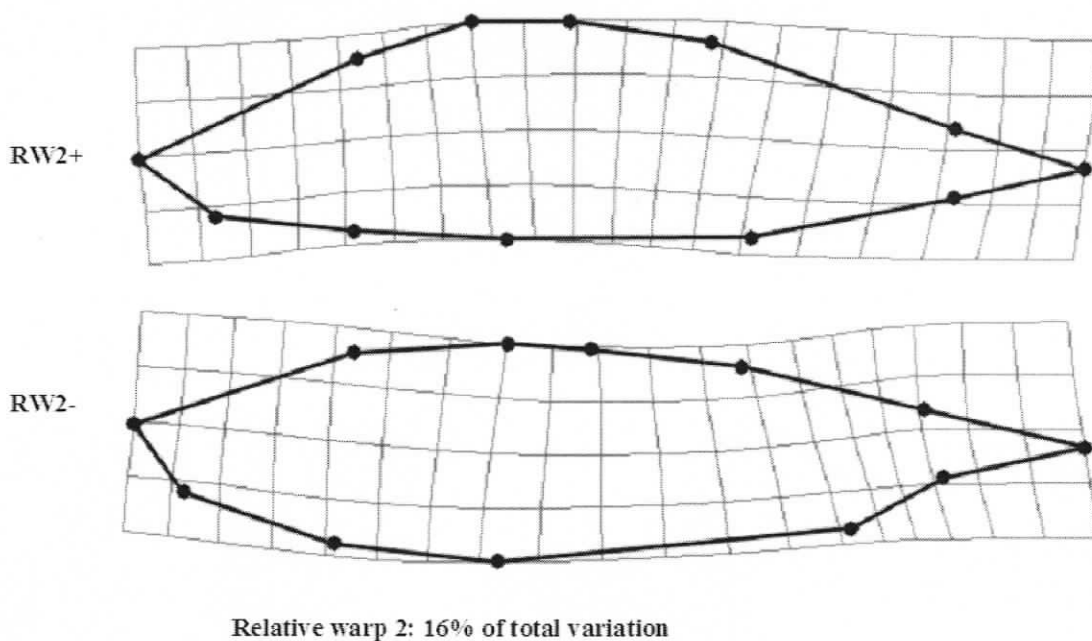
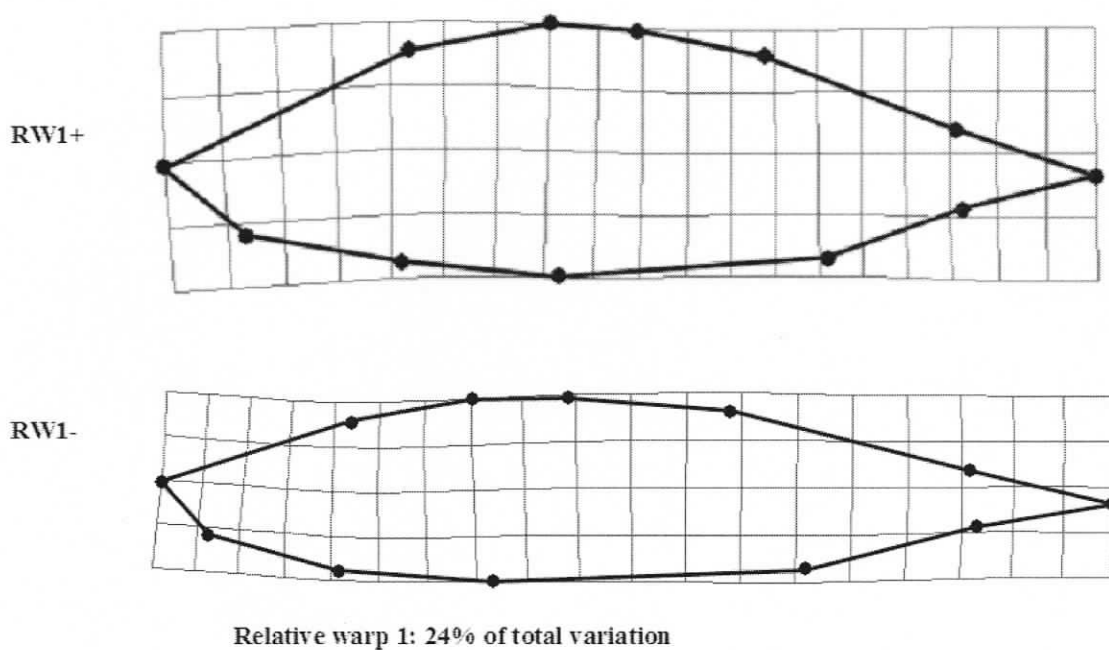


Figure 7. Thin plate spline plot of body shape of stickleback from Haida Gwaii as quantified by relative warp vectors. Plots represent the actual differences between the + and - extremes of each relative warp vector.

Table 5. Pearson correlations for RW1 and RW2 between log linear regression standardized morphometric measures calculated from inter-landmark distances for 125 stickleback populations. RW1 explains 24.8% of the variation among populations of adult stickleback from Haida Gwaii; RW2 explains 16.7% of the variation.

Character	RW1		RW2	
	Pearson	P value	Pearson	P value
	Correlation		Correlation	
Pelvis	0.09	0.30	0.30	0.001
Pelvis position	0.36	< 0.001	-0.25	0.005
Head area	0.75	< 0.001	-0.35	< 0.001
Head length	0.61	< 0.001	-0.15	0.09
Head depth	0.83	< 0.001	-0.29	0.0001
Gape	0.50	< 0.001	0.13	0.15
Dorsal spine 1 (D1) position	0.71	< 0.001	-0.39	< 0.001
Distance between D1 and D2	-0.03	0.77	0.31	0.001
Body depth (anterior)	0.69	< 0.001	0.20	0.02
Body depth (posterior)	0.64	< 0.001	0.21	0.02
Dorsal fin length	-0.38	< 0.001	0.52	< 0.001
Anal fin length	-0.49	< 0.001	0.65	< 0.001
Caudal peduncle depth (anterior)	0.49	< 0.001	0.33	0.04
Caudal peduncle depth (posterior)	0.36	< 0.001	-0.03	0.73
Caudal peduncle area	0.03	0.73	-0.19	0.04

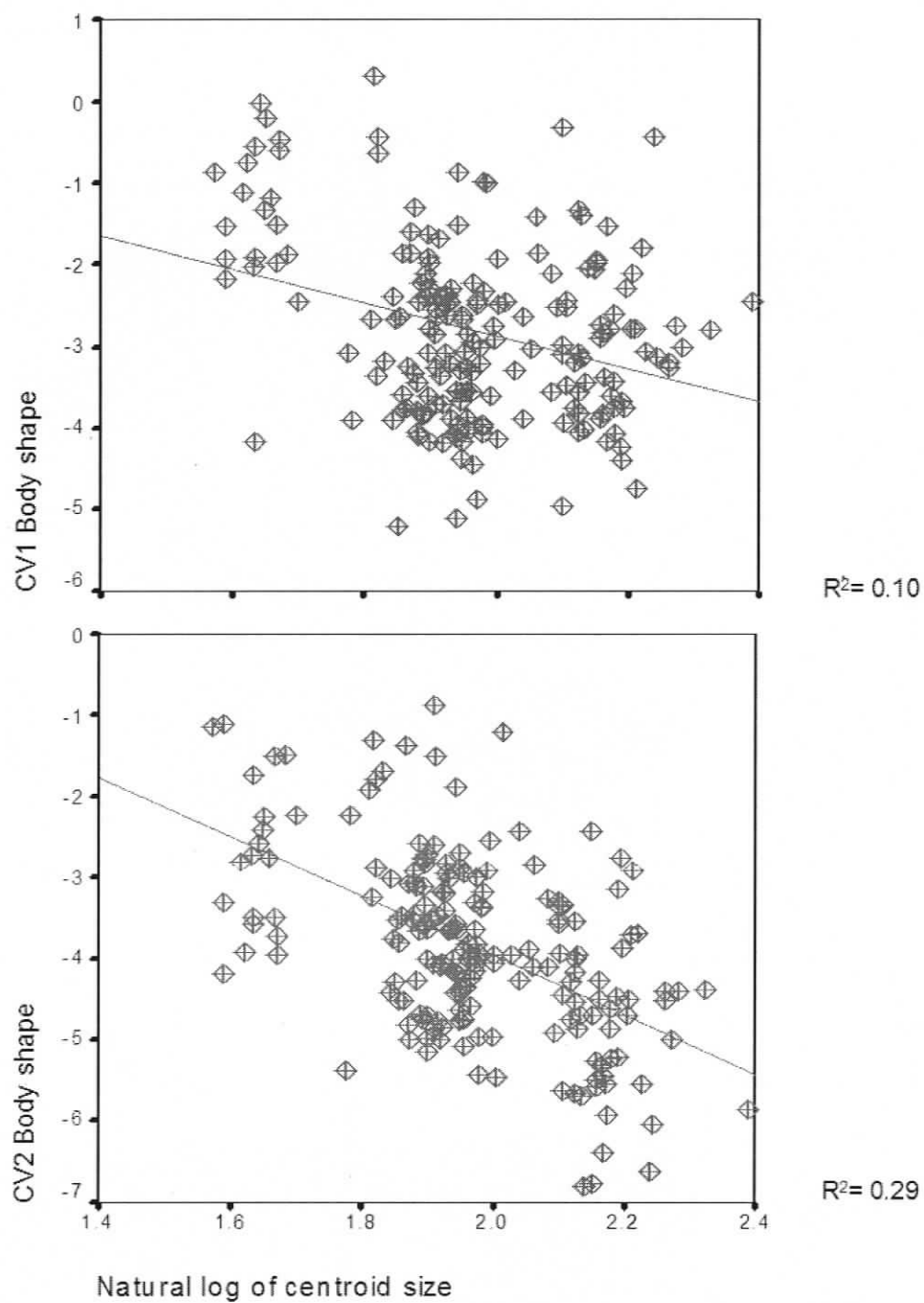


Figure 8. Relationship between CV scores and natural log of centroid size for Mayer Lake stickleback. CV1 accounts for 33.4% of total variation (top figure), and CV2 accounts for 21.7% of total variation (bottom).

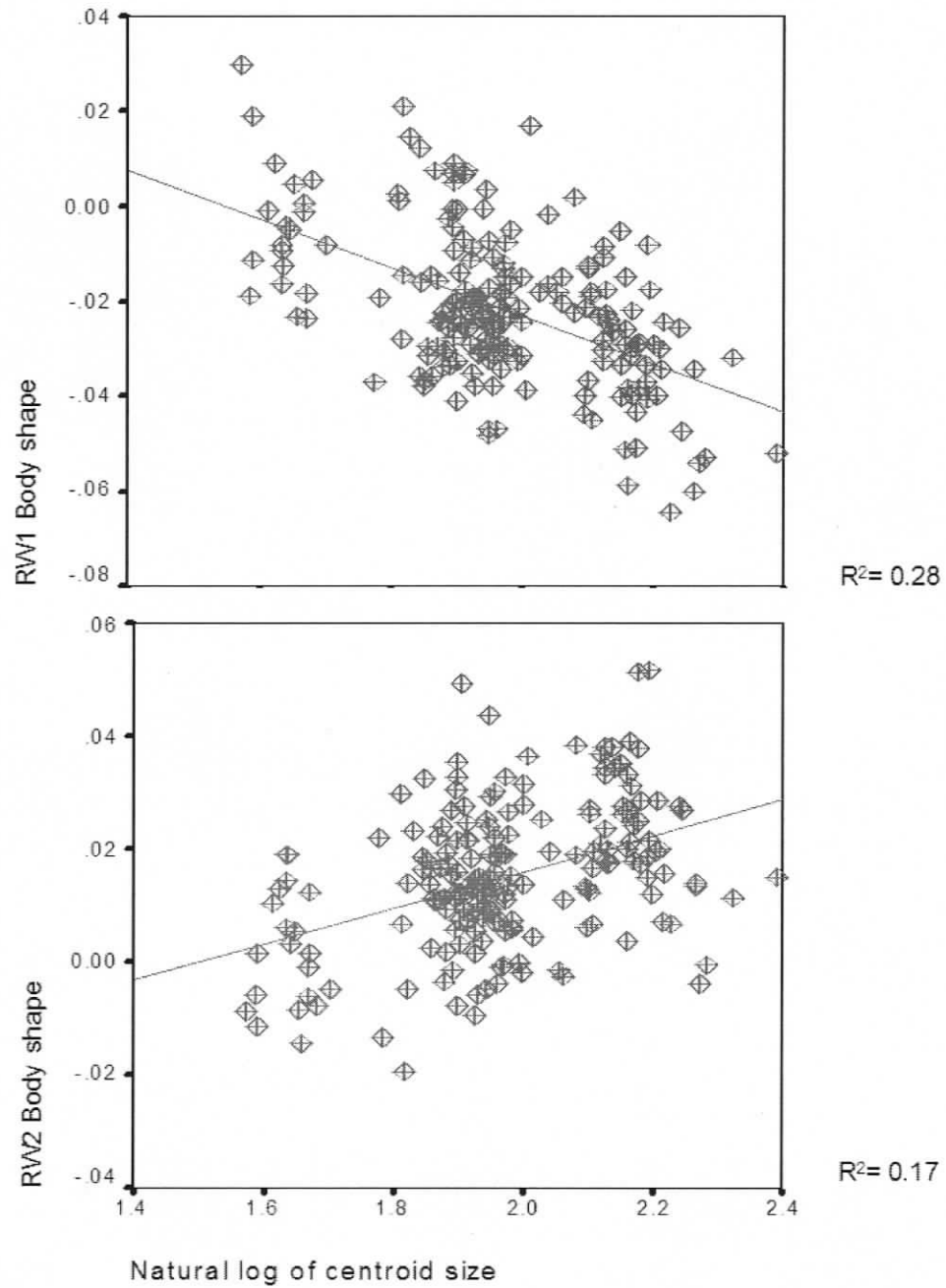


Figure 9. Relationship between RW scores and natural log of centroid size for Mayer Lake stickleback. RW1 accounts for 24.0% of total variation (top figure), and RW2 accounts for 16.0% of total variation (bottom).

Stickleback from Drizzle Lake exhibited slight ontogenetic shifts in body shape. Body shape quantified by canonical vectors is not statistically related to the natural log of centroid size, indicating shape is fairly conserved during ontogeny (CV1: $R^2 = 0.009$, $p = 0.32$; CV2: $R^2 = 0.001$, $p = 0.86$; figure 10). I detected a slight ontogenetic shift in body shape using relative warp scores in the regression (RW1: $R^2 = 0.011$, $p = 0.25$; RW2: $R^2 = 0.05$, $p = 0.015$). Adult stickleback from Drizzle Lake have relatively larger median fins compared to juveniles (figure 11).

3.3.3 Comparison of ontogenetic slope among 125 stickleback populations

I was able to quantify the ontogenetic changes in shape by examining the direction and significance level of slopes from the regression of each shape variable against the natural log of centroid size. Throughout the archipelago ($N = 125$) 52% of the population's develop thicker peduncles, posteriad and closely spaced dorsal spines, more anteriad pelvis and shorter median fins, as they grow larger (CV1+). Of this sub group, the ontogenetic slope of 15 populations (23.1%) is statistically significant ($p \leq 0.05$). The remaining 48% of the populations have narrower peduncles, anteriad and widely spaced dorsal spines, posteriad pelvis and longer dorsal and anal fins, compared to the juveniles (CV1-); 11 population's (18%) slope is statistically significant (lowlands: figures 12- 13; plateau: figure 14; mountain: figure 15). On the second canonical vector, 64% of the populations develop larger heads and deeper bodies throughout ontogeny (CV2+); results for 17 populations (21.3%) are significant. I determined that 36% of the populations possess smaller heads, and shallower bodies when they grow (CV2-), results for 14 populations (31%) are significant.

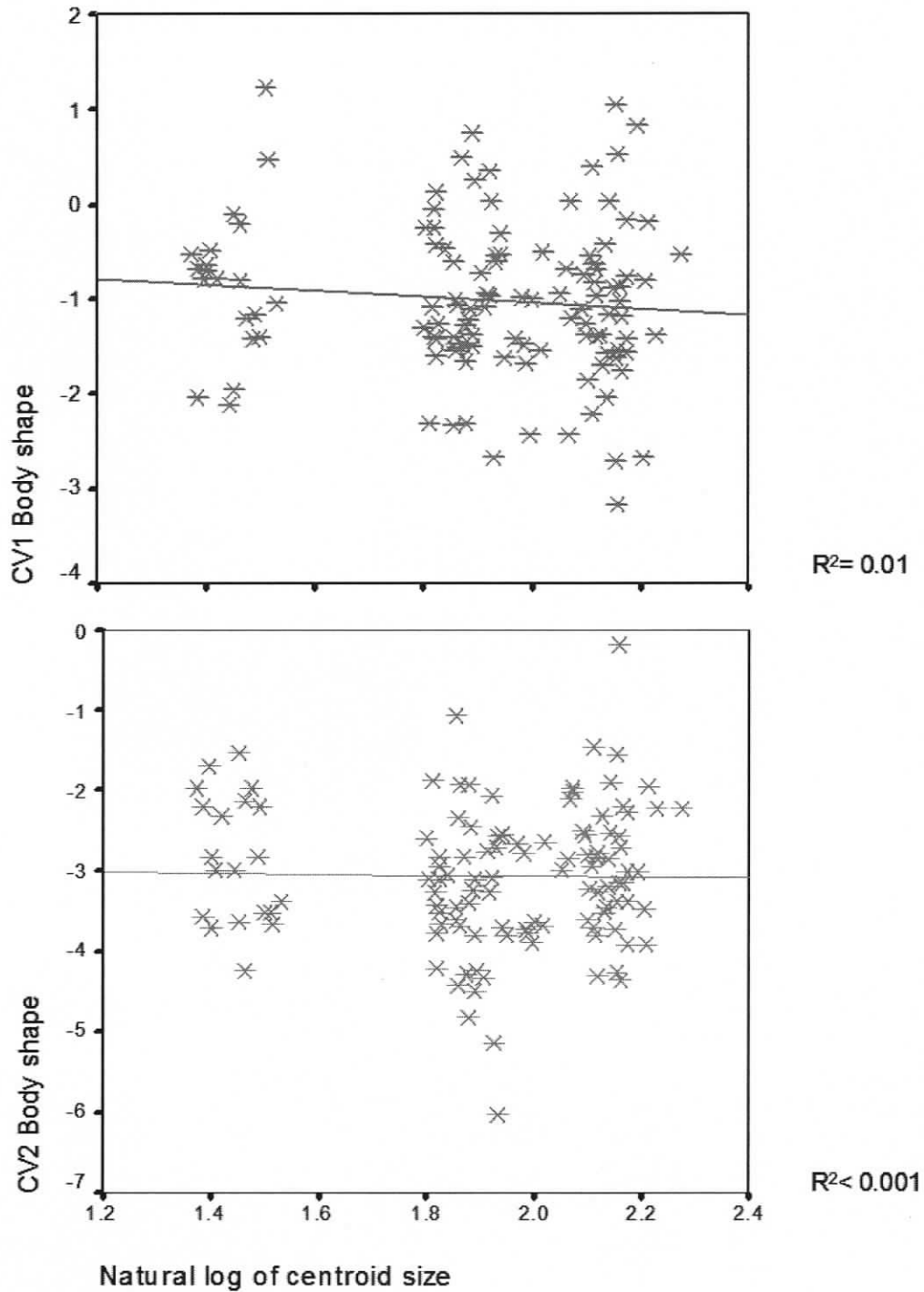


Figure 10. Relationship between CV scores and natural log of centroid size for Drizzle Lake stickleback. CV1 accounts for 33.4% of total variation (top figure), and CV2 accounts for 21.7% of total variation (bottom).

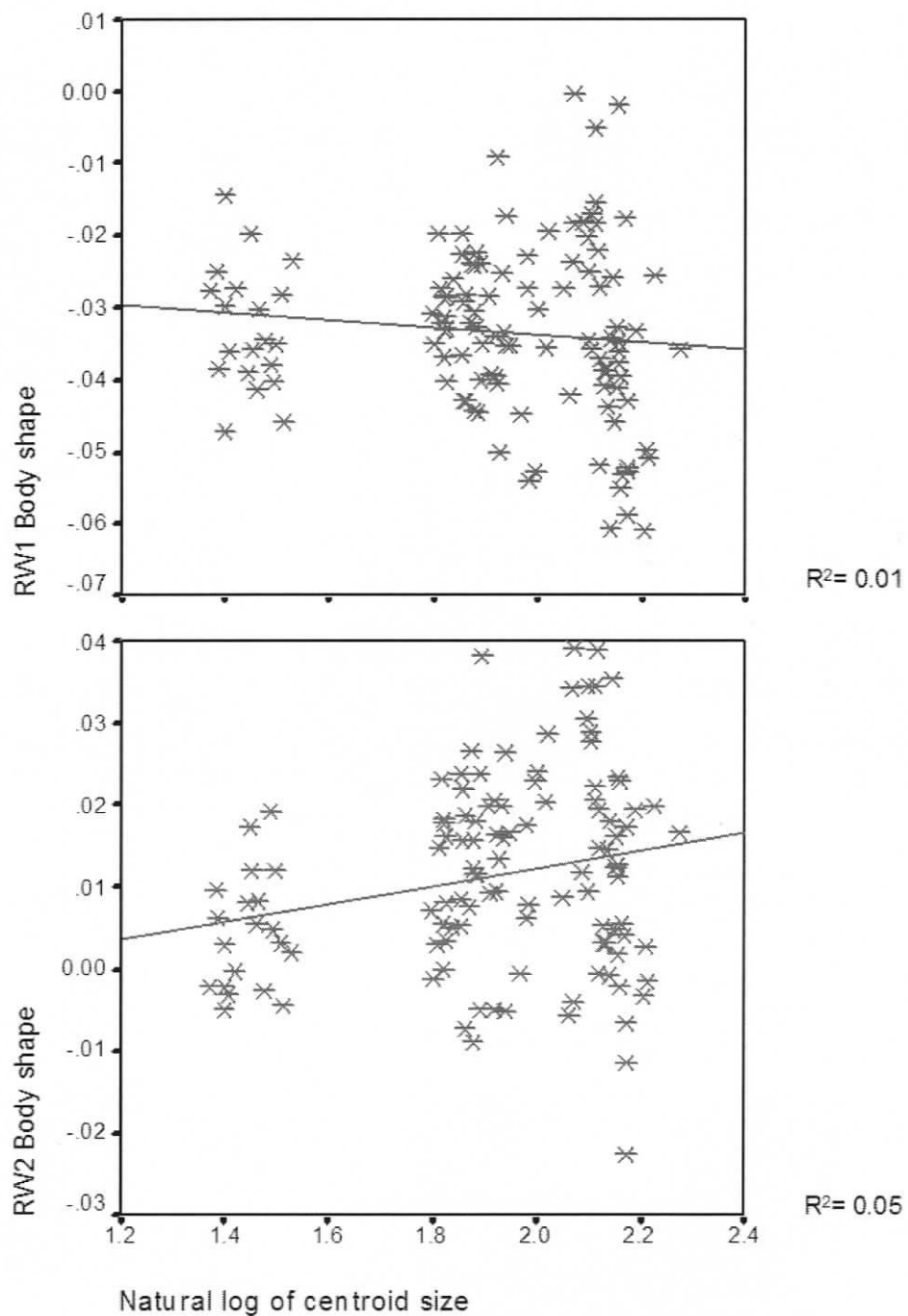


Figure 11. Relationship between RW scores and natural log of centroid size for Mayer Lake stickleback. RW1 accounts for 24.0% of total variation (top figure), and RW2 accounts for 16.0% of total variation (bottom).

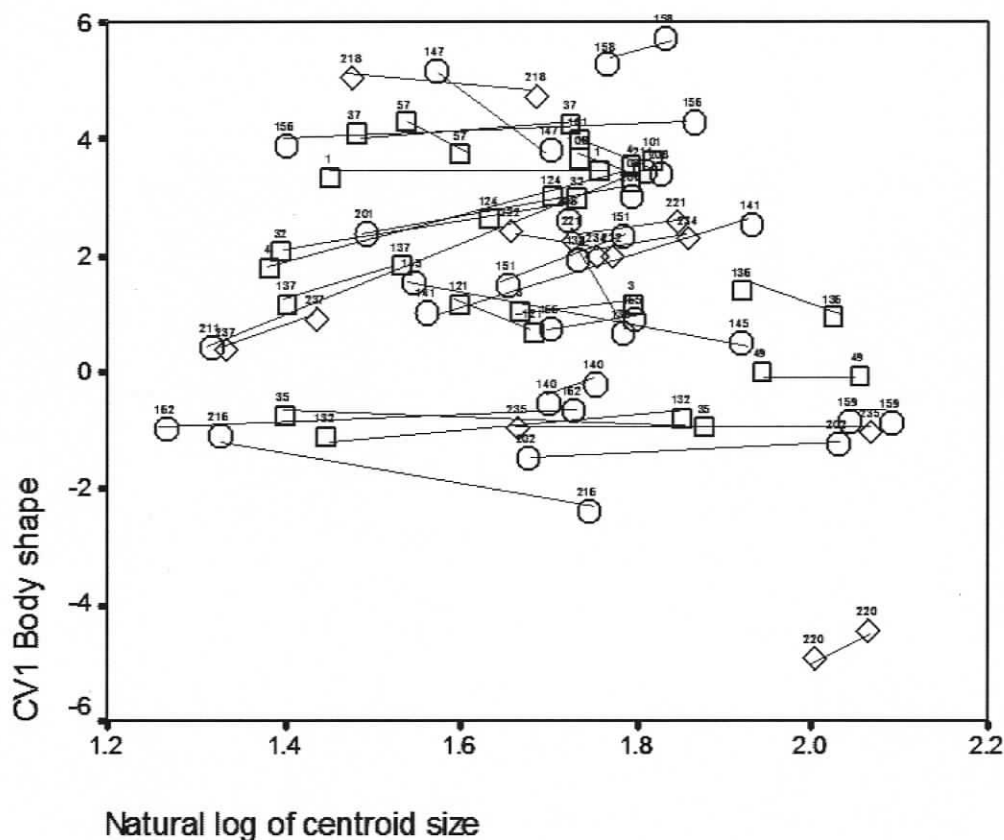


Figure 12. Ontogenetic slopes for 38 lowland populations. Body shape is quantified by CV1 (33.4% of total variation) for small and large stickleback. Site names are listed beside their corresponding number: 1= Anser, 3= Drizzle out, 4= Geike, 32= Grus, 35= Gold cr, 37= Cedar, 49= Gross, 57= Harelda upper, 101= Gosling, 108= Juno, 121= Florence cr pond, 124= Imber, 132= Cape Ball, 136= Bigfish, 137= Blue Danube, 138= Clearwater, 140= Cape Ball r, 141= Dam, 145= Down tree, 147= Cygnet, 151= Kumara, 155= Eriophore, 156= Heralda lower, 158= Heralda middle, 159= Hickey, 162= Kumdis, 201= Boulton, 202= Ain, 208= Blowdown, 211= Chown, 216= Delkatla, 218= Duck, 220= Entry pt, 221= Geike #2, 222= Labrador, 234= Branta, 235=Drizzle, 237= Elk survey cr.

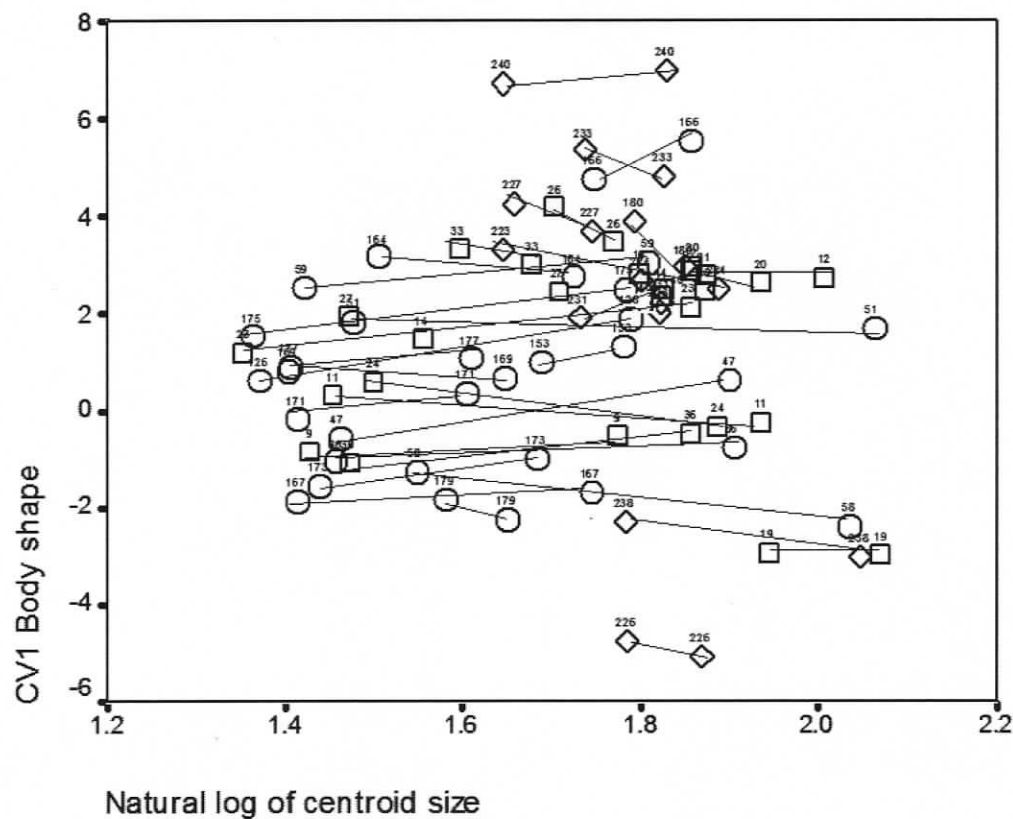


Figure 13. Ontogenetic slopes for 40 lowland populations. Body shape is quantified by CV1 (33.4% of total variation) for small and large stickleback. Site names are listed beside their corresponding number: 9= Ottern, 10= Parkes, 11= Pure, 12= Richter, 14= Slim, 19= Spence, 20= Spraint, 21= Mica, 23= Pontoon center, 24= Skonun, 26= Lumme, 27= Solstice, 33= Nuphar, 36= Woodpile, 40= Middle, 47= Stellata, 51= Laurel pond, 58= Vaccinium, 59= Midge, 86= Otter, 126= Oeanda, 153= Wiggins, 164= Lumme swp, 166= Mesa, 167= Molitors pd, 169= Sangan, 171= Sangan backwater, 173= Skaters, 175= Stump, 177= Survey cr, 179= Tlell est, 180= Watt, 223= Loon, 224= Loon cr, 226= Masset inlet, 227= Naked, 231= Silver, 233= Serendipity, 238= Mayer, 240= Rouge.

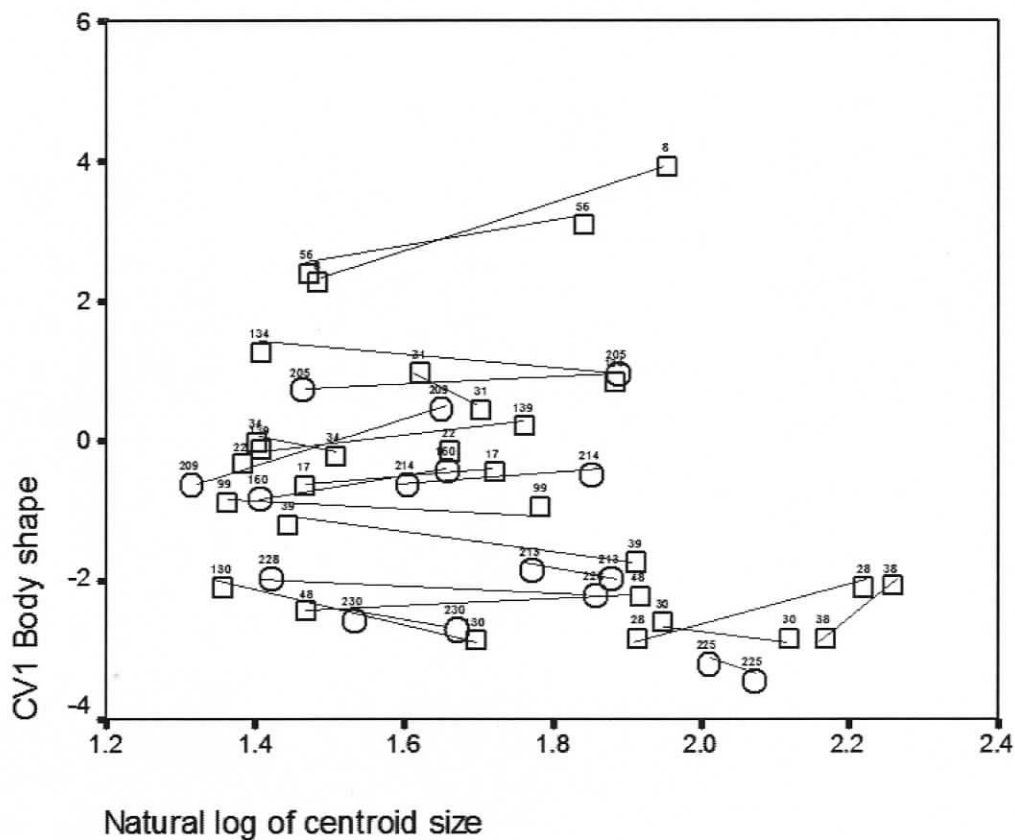


Figure 14. Ontogenetic slopes for 23 plateau populations. Body shape is quantified by CV1 (33.4% of total variation) for small and large stickleback. Site names are listed beside their corresponding number: 8= New Years, 17= Kiokathli, 22= Yakoun r, 28= Eden, 30= Coates, 31= Seal Inlet, 34= Blackwater cr, 38= Skidgate, 39= Marie, 48= Amber, 56= Cumshewa, 99= Menyanthes, 130= Yakoun lk, 134= Anderson south, 139= Desolate, 160= Krajina, 205= Anderson north, 209= Brent cr, 213= Copper river pd, 214= Debris, 225= Marion, 228= Peter, 230= Sheldon meadows.

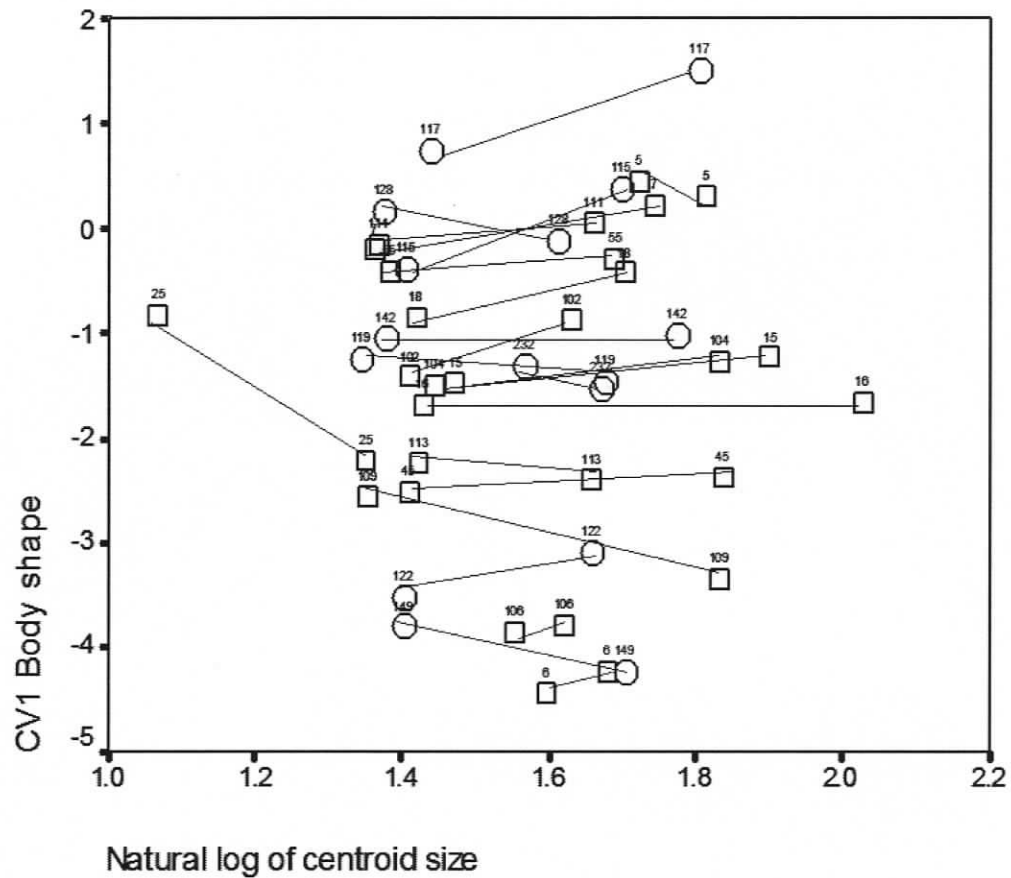


Figure 15. Ontogenetic slopes for 23 mountain populations. Body shape is quantified by CV1 (33.4% of total variation) for small and large stickleback. Site names are listed beside their corresponding number: 5= Gudal, 6= Inskip, 7= Lutea, 15= Stiu, 16= Escarpment, 18= Sundew, 25= Darwin, 45= Puffin, 55= Van Inlet, 102= Wright, 104= Lower Victoria, 106= Poque, 109= Mosquito, 111= Goski, 113= Fairfax, 115= Irridens, 117= Snub, 119= Dead toad cr, 122= Gowgaia, 128= White swan, 142= Dawson pd, 149= Gowgaia w, 232= Wegner.

Using body shape as quantified by relative warps, I determined that 38.4% of the populations have larger heads, posteriad dorsal spines, and deeper bodies throughout ontogeny (RW1+), 13 populations (27.1%) are statistically significant. The majority of populations, 55.7%, have smaller heads, anteriop dorsal spines, and shallow bodies as they get larger (RW1-), 19 populations (24.7%) are significant at the $p < 0.05$ level. In relation to the second relative warp vector, 48.8% of the populations develop longer dorsal and anal fins as they grow (RW2+), 15 populations (24.6%) are significant. I determined that 51.2% have proportionately smaller dorsal and anal fins as they grow to larger sizes (RW2-), 7 populations (10.9%) are statistically significant.

3.3.4 Ontogenetic slope and habitat volume/clarity

Analyzing all the populations on the archipelago together, I did not find a relationship between PC1_lk (volume/clarity) and the ontogenetic slope ($N = 70$; CV1: $R^2 = 0.01$, $p = 0.32$; CV2: $R^2 < 0.001$, $p = 0.89$; RW1: $R^2 = 0.02$, $p = 0.26$; RW2: $R^2 = 0.004$, $p = 0.11$). The lack of relationship between slope and PC1_lk was consistent when the data was partitioned into geographical area: lowlands ($N = 45$; figure 16; CV1: $R^2 = 0.001$, $p = 0.83$; CV2: $R^2 = 0.005$, $p = 0.63$; RW1: $R^2 = 0.02$, $p = 0.36$; RW2: $R^2 = 0.08$, $p = 0.05$) and plateau/mountain ($N = 25$; figure 17; CV1: $R^2 = 0.01$, $p = 0.58$; CV2: $R^2 = 0.01$, $p = 0.62$; RW1: $R^2 = 0.1$, $p = 0.13$; RW2: $R^2 < 0.001$, $p = 0.88$).

I did not find a relationship between PC1_lk (volume/clarity) and the direction and significance (sign significance) of ontogenetic trajectories for populations found throughout the archipelago ($N = 70$; CV1: $R^2 = 0.02$, $p = 0.22$; CV2: $R^2 = 0.03$, $p = 0.18$; RW1: $R^2 = 0.002$, $p = 0.73$; RW2: $R^2 = 0.004$, $p = 0.6$).

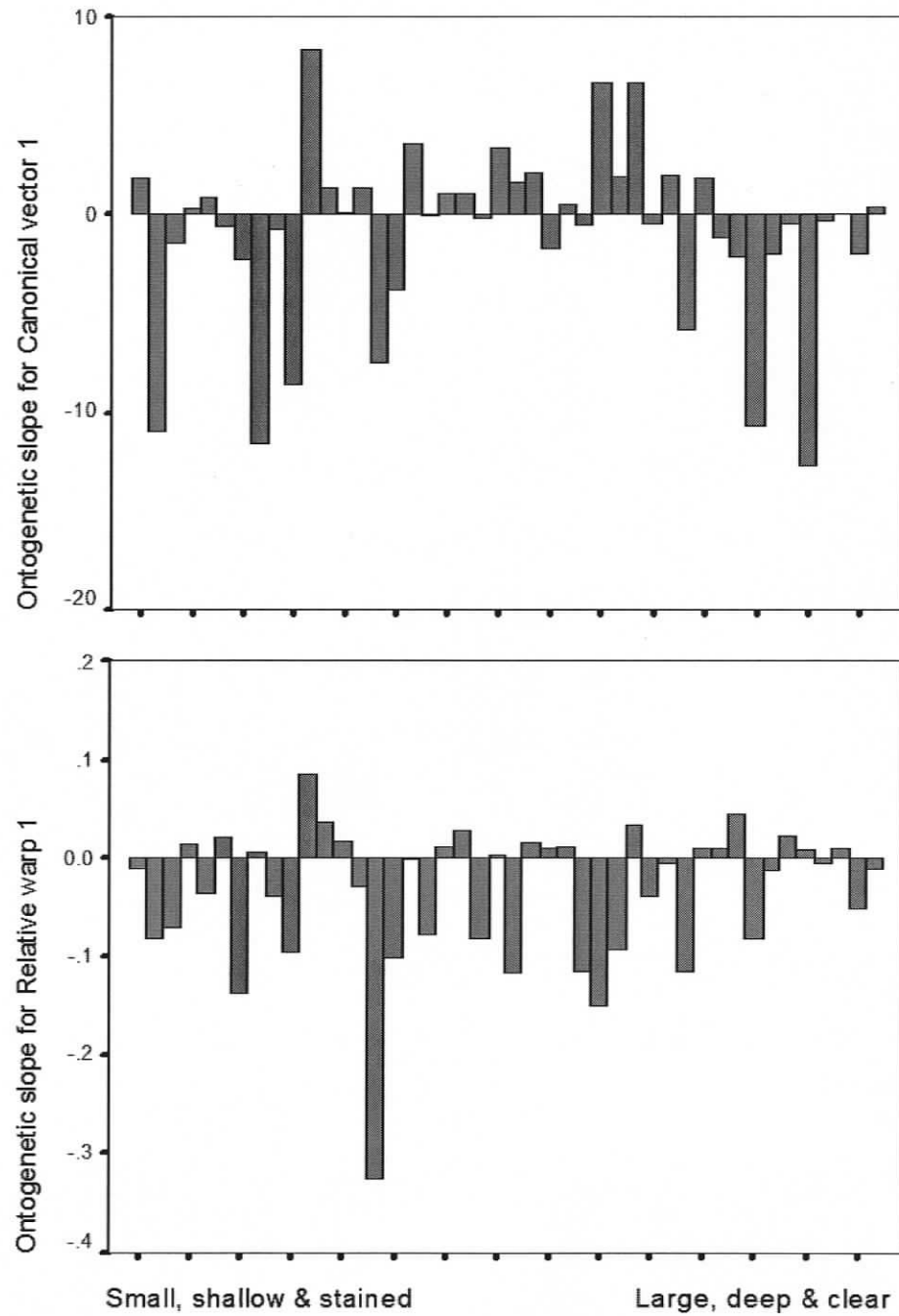


Figure 16. Relationship between ontogenetic slopes and lake volume/clarity for stickleback populations from the lowland region of Haida Gwaii. CV1 (33.4% of total variation) top figure, and RW1 (24.0% of total variation) bottom figure.

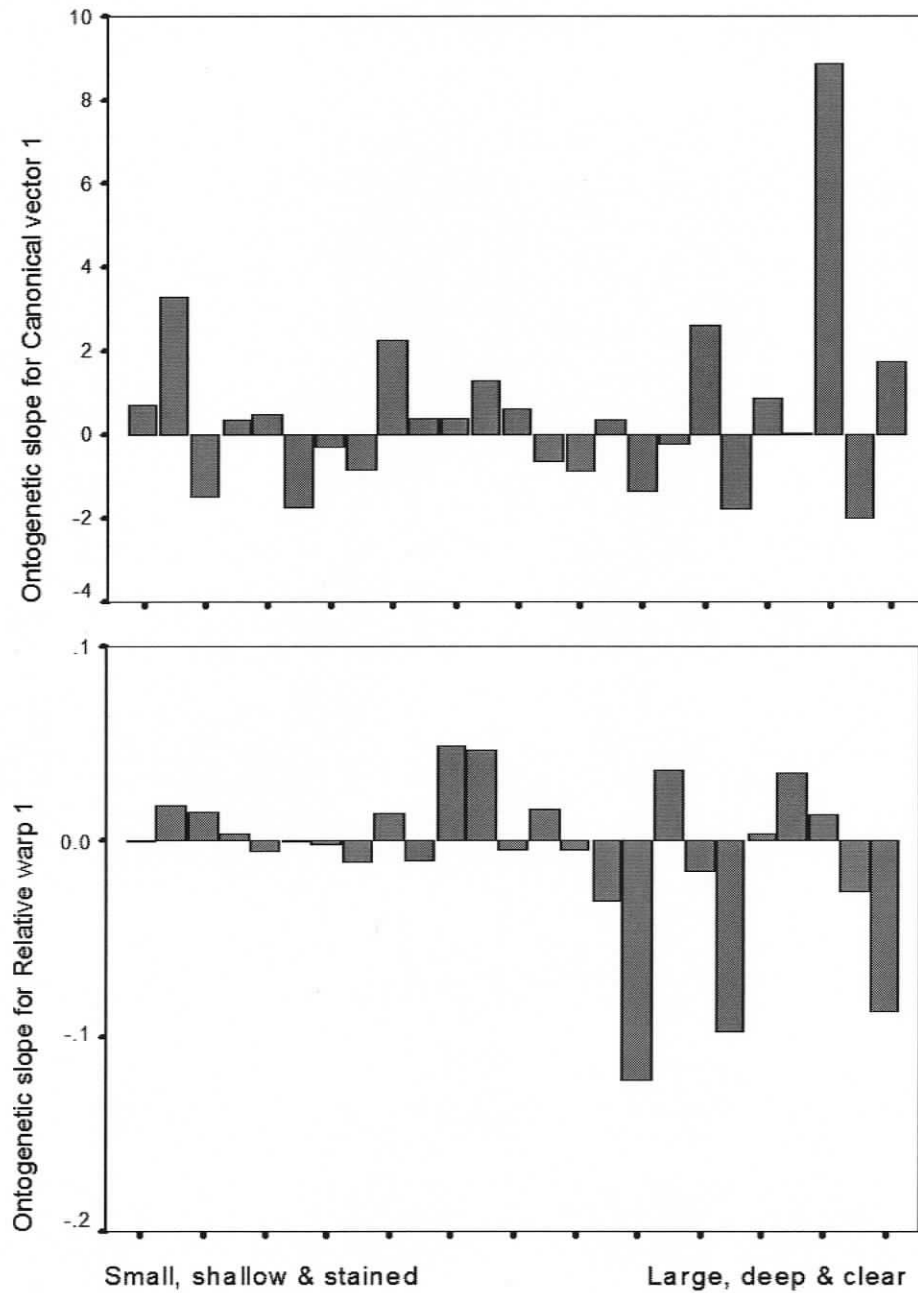


Figure 17. Relationship between ontogenetic slopes and lake volume/clarity for stickleback populations from the plateau/mountain region of Haida Gwaii. CV1 (33.4% of total variation) top figure, and RW1 (24.0% of total variation) bottom figure.

The same results were also found when I partitioned the data for geographical region: lowlands (N= 45; CV1: $R^2= 0.02$, $p= 0.36$; CV2: $R^2= 0.08$, $p= 0.07$; RW1: $R^2 < 0.001$, $p= 0.97$; RW2: $R^2= 0.006$, $p= 0.6$), and the plateau/mountain area (N= 25; CV1: $R^2 < 0.001$, $p= 0.98$; CV2: $R^2= 0.003$, $p= 0.81$; RW1: $R^2=0.09$, $p= 0.16$; RW2: $R^2= 0.04$, $p= 0.36$). The results were largely concordant when I regressed the sign inverse-variance against lake volume/clarity (PC1_lk). However, when partitioned by geographic region there were significant correlations in the lowlands between lake volume/clarity and both the sign inverse-variance of CV1 ($R^2= 0.15$, $p= 0.01$) and RW2 ($R^2= 0.17$, $p= 0.01$). These results were strongly influenced by two outliers of large sample size and small variance (Mayer Lake: $n= 185$ and Drizzle Lake: $n= 111$). When these populations were removed from the analysis, the associations became statistically non-significant (CV1: $R^2= 0.03$, $p= 0.23$; RW2: $R^2= 0.04$, $p= 0.21$).

Further analysis showed that, regardless of geographical region, there was no relationship between populations, whose ontogenetic slope was statistically significant, and volume/clarity (PC1_lk; CV1: N= 12, $R^2= 0.01$, $p= 0.72$; CV2: N= 18, $R^2= 0.02$, $p= 0.56$; RW1: N= 20, $R^2= 0.02$, $p= 0.61$; RW2: N= 14, $R^2= 0.04$, $p= 0.52$). Similarly, there was no relationship between populations, whose ontogenetic slope was non-significant ($p > 0.5$), and volume/clarity (CV1: N= 27, $R^2= 0.07$, $p= 0.19$; CV2: N= 22, $R^2= 0.1$, $p= 0.16$; RW1: N= 21, $R^2= 0.003$, $p= 0.8$; RW2: N= 30, $R^2= 0.02$, $p= 0.47$).

Regardless of significance level or geographical area, the direction of populations ontogenetic trajectories was not influenced by volume/clarity of the habitat (positive slope: CV1: N= 36, $R^2 < 0.001$, $p= 0.86$; CV2: N= 42, $R^2= 0.06$, $p= 0.12$; RW1: N= 31, $R^2= 0.002$, $p= 0.8$; RW2: N= 36, $R^2= 0.007$, $p= 0.63$ and negative slope: CV1: N= 34,

$R^2 = 0.06$, $p = 0.17$; CV2: $N = 28$, $R^2 = 0.008$, $p = 0.63$; RW1: $N = 39$, $R^2 = 0.06$, $p = 0.15$; RW2: $N = 34$, $R^2 = 0.07$, $p = 0.13$).

3.4 Discussion

There is considerable variation in ontogenetic shifts in body shape of Haida Gwaii threespine stickleback populations. Body shape of Mayer Lake stickleback changes significantly during ontogeny, while shape is relatively conserved during the growth of fish from Drizzle Lake. Throughout the archipelago and each geographical region, I did not find any correlations between highly variable population ontogenetic shifts in body shape and lake volume/clarity.

Geometric morphometrics are useful tools for investigation of ontogenetic shifts in body shape. One of the foundations of geometric morphometrics is the isometric scaling of the specimens prior to quantification of shape (Bookstein, 1989; Walker, 1997). Size and shape are two components of form. Size refers to the scale of a figure and shape refers to the geometry of a figure that is invariant to translations, rotations and scaling (Bookstein, 1991; Goodall, 1991). Isometric scaling does not distort shape, similar to reduction or magnification of a photocopy (Bookstein, 1989; Walker, 1997). This provides a powerful technique for investigations into changes in body shape during ontogeny, creating "size adjusted" shape variables that can be explored for spatial changes in body shape between different cohorts (Yeh, 2002).

Changes in size and shape during ontogeny result in changes in the swimming hydrodynamics and kinematics (Wakeling *et al.*, 1999). Mayer Lake is a large, deep, dystrophic lake (Reimchen, 1992c), with copepods as the primary food source in the open

water environment (Moodie, 1972a). Juvenile stickleback from Mayer Lake were found to be of intermediate morphology, and as they grow they develop narrower peduncles, more anterior and widely spaced dorsal spines, posterior pelvis, longer median fins, smaller heads and shallower bodies (CV1- and CV2-). Adult stickleback from this locality also have relatively small heads, more anterior dorsal spines, shallower bodies and longer median fins than the juveniles (RW1- and RW2+). The small size of the juveniles and comparative differences in shape suggest increased manoeuvrability (Webb, 1982; Walker, 1997) as an adaptation for foraging in the littoral areas of the lake. As stickleback grow, their diet rapidly becomes similar to that of the adults in the population (Wootton, 1984), indicating ontogenetic shifts in niche utilization. This adult body shape is effective for reducing components of drag by the laminarization of form which reduces wake formation (and increased drag) along the posterior regions of the stickleback (Aleev, 1977; Vogel, 1981; Walker, 1997). Copepods often have a patchy distribution in the open water environment (George, 1981; Pinel-Alloul *et al.*, 1988); therefore, adult stickleback would have to swim at a steady velocity for much longer periods of time in search of profitable food items (Walker, 1997).

The hydrodynamic constraints associated with the open water niche dictate that fish foraging in this environment should have adaptations to reduce components of drag associated with steady swimming (Aleev, 1977; Webb, 1982, 1984). The ontogenetic shifts in body-shape detected in the Mayer lake stickleback possibly represent size-specific adaptations to the hydrodynamic constraints associated with the Mayer Lake stickleback's cohorts realized niche.

In contrast Drizzle Lake fish had a relatively conserved body shape throughout ontogeny. This lake is also large, deep and dystrophic, and as such, adult Drizzle Lake stickleback are primarily open water foragers, similar in morphology to the Mayer Lake population. My results suggest that ontogenetic shift in this population is simple dorsoventral elongation with isometric growth of the characters I used to quantify their shape. Numerous studies have shown that ontogenetic changes in shape are often more complex than uniform elongation (Walker, 1993; Hood and Heins, 2000; Zelditch *et al.*, 2000), and often involve the integration of numerous phenotypic characters. However, after about 20 days post hatching, and at a standard length of about 11mm, young stickleback lose their larval characteristics and assume an adult-like shape (Wootton, 1976). Therefore, my results for Drizzle Lake are plausible, but should be interpreted with caution. I did not detect substantial changes in body shape during ontogeny, suggesting that, besides hydrodynamic constraints associated with size, there are other ecological factors selecting for similar body shape between juveniles and adults.

One of the differences between Drizzle and Mayer is the relative littoral area in each lake (Reimchen, 1992c). Relative littoral area can be defined as the ratio of littoral area (depth of the lake bottom less than the euphotic zone) to limnetic area (Walker, 1997). Drizzle Lake is more “kidney shaped” and has considerably less relative littoral area, than the long narrow topography of Mayer Lake. The conserved nature of the juvenile- adult body shape of the Drizzle Lake stickleback could be a result of the uniformity of habitat in the lake. Likewise, the body shape of the mullet, *Mugil saliens*, was shown to be conserved over a range of standard lengths comparable to those observed in my study, even though the Reynolds number nearly doubled (Aleev, 1977).

This suggests that body shape may be conserved once juvenile fish attain adult form, and the forces associated with movement through water are compensated for with adaptations other than shape.

Many species undergo extensive ontogenetic shifts correlated with food or habitat use, and individuals face different competitors and predators as they grow, creating complex ecological interactions in their natural habitat (Werner *et al.*, 1983a, 1983b; Werner and Gilliam, 1984). The lack of correlation between Haida Gwaii stickleback populations' ontogenetic shifts in shape and habitat volume/clarity suggests that each body of water containing stickleback has both unique and shared selective forces governing the ontogeny of body shape. In each lake there are different competitors and predators, at each stage during ontogeny, that stickleback interact with and these interactions are critically important in structuring these communities (Werner and Gilliam, 1984). It is likely that besides habitat volume/clarity, there are other differences in the selective landscape between lakes, which have lead to the observed differences in ontogenetic paths.

The paucity of the data may also be reflected in the variability of ontogenetic slopes and the lack of correlation between slope and habitat volume/clarity. Many of the lakes on Haida Gwaii can only be accessed by aircraft or on foot through dense vegetation. Consequently some samples are smaller than desirable and may contain a relatively uneven distribution of size classes (Moodie and Reimchen, 1976). However, the geometric procedures allowed me to detect slight changes in body shape in relation to body size. Therefore the slopes may represent shape variability within a population, rather than ontogenetic shifts, if the size differences were small.

Variability among maximum standard length, which has a genetic component (McPhail, 1977, 1993; Lavin and McPhail, 1985), and has been shown to be a discriminating character among populations (Reimchen *et al.*, 1985), may also confound the comparison of ontogenetic trajectories. Stickleback lose their larval characteristics at 11mm, but the lateral plates are not completely developed until the fish reaches 30mm (Hagen, 1973; Wootton, 1976). In a population whose maximum standard length is 40mm (i.e. Darwin Lake), the ontogenetic slope may suggest a unique developmental process or simply changes in the timing of development (Gould, 1977; Rice, 1997). I would expect these changes to be related to some aspect of lakes ecology but the limited time at each lake and the availability of equipment (Moodie and Reimchen, 1976), may have reduced the probability of detecting a correlation between physical characteristics of a habitat and rate of development.

There is a wide range of biophysical variation between stickleback habitats on Haida Gwaii (Moodie and Reimchen, 1976; Reimchen, 1980, 1983; Bergstrom and Reimchen, 2002). Growth patterns in stickleback vary between populations, suggesting that variation in food supply, and differences in physical parameters such as water temperature, may potentially influence the rate at which stickleback develop (Wootton, 1976, 1984; Giles, 1987; Reimchen and Nelson, 1987). These factors may also help explain the variability in ontogeny of body shape among the Haida Gwaii populations. These stickleback exhibit a range of phenotypic variation that encompasses the known morphologies in the circumboreal distribution of the species (Moodie and Reimchen, 1976; Reimchen *et al.*, 1985). Coupled with the variation in, and complexity of ecological interactions (Reimchen and Nosil, 2001c, 2002, 2004), it is not unexpected

that there is little correlation between ontogenetic slope and strictly physical characteristics of habitat.

Chapter 4 Sexual dimorphism of body shape

4.1 Introduction

Patterns of population variation play an important role in evolutionary diversification, and many insights into evolutionary processes have come from studies of within population variation over a broad geographical range (Badyaev *et al.*, 2000). One of the sources of intrapopulation variation is sexual dimorphism. Body shape is a phenotypically integrated trait (Strauss and Bookstein, 1982; Baumgartner *et al.*, 1988; Rohlf, 1990; Bookstein, 1991; Baumgartner, 1992; Walker, 1997; Walker and Bell, 2000; Klingenberg *et al.*, 2003) that often has ecological correlates (Irschick *et al.*, 1997; Losos *et al.*, 1997; Walker, 1997; Beuttell and Losos, 1999) and can be sexually dimorphic (Caldecutt and Adams, 1998; Butler and Losos, 2002).

Conspicuous sexual dimorphism is a widespread and often prominent feature of the natural world (Selander, 1966; Lande, 1980; Slatkin, 1984; Shine, 1989; Butler *et al.*, 2000; Butler and Losos, 2002). Sexual selection and natural selection based on sex-specific ecological differences are often cited as the primary reasons for the evolution and maintenance of sexual dimorphism (Darwin, 1874; Bonnet *et al.*, 1998; Bonnet *et al.*, 2001). Intrasexual competition for mates (often male vs. male contests) and intersexual mating preferences (usually females choosing males) can lead to the evolution of conspicuous characters that confer a mating advantage over individuals possessing the less conspicuous trait (Darwin, 1874). In short, characters evolved from purely sexual selection result from differential success in mate choice and mate competition (Howard *et al.*, 1998). Sexual dimorphism can also evolve through sex-specific selection, if each sex has distinctive roles in reproduction (such as maximizing fecundity), or differences in the

effectiveness of parental care, microhabitat use, in foraging behaviour and prey choice (Darwin, 1874; Howard *et al.*, 1998).

It may be short sighted to suggest that sexual, or sex-specific natural selection are the only factors involved during the evolution and maintenance of a sexual dimorphism. Purely ecological forces can lead to, constrain or amplify sexual dimorphism and lead to differences between optimal phenotypes of the sexes (Selander, 1966; Schoener, 1969; Lande, 1980; Slatkin, 1984; Shine, 1989; Reimchen and Nosil, 2001b, 2004). As the possible basis for ecological causation of sexual dimorphism, individual variation in resource use may reflect intrapopulation variation in a wide range of individual traits that determine resource-specific efficiency and preferences (Bolnick *et al.*, 2003). Provided there is genetic variation for a sexual dimorphism (Van Valen, 1965), divergent selection on the individual variation can lead to the evolution of optimal phenotypes between the sexes. However, to assign natural selection through ecological differences as the cause of a sexual dimorphism, requires observations and empirical data that males and females have different habits and employ the dimorphic character(s) adaptively in their distinct ecological settings (Lande, 1980).

Ecological causes of sexual dimorphism can be difficult to establish and evidence to answer the question of whether purely ecological forces are responsible is often hard to find (Slatkin, 1984). Therefore discerning the cause of a sexual dimorphism can be troublesome. For example, in the advanced stages of sexual dimorphism initiated by sexual selection, a secondary process of natural selection through ecological displacement (or vice versa) may occur, further driving phenotypic differentiation between the sexes (Lande, 1980). These complementary effects make it extremely

challenging to infer whether sexual selection, reproductive biology, ecological factors or a combination of them are responsible for the evolution of sexual dimorphism.

Regardless of the mechanism, differences in the expression of sexual dimorphism between related populations has been shown to vary in both intensity and direction (Badyaev *et al.*, 2000). This disparity may lead to inflated population variation for a phenotypic character, which may exaggerate observed ecological relationships between populations and their habitat (Endler, 1986) and lead to false correlations over a broad geographic range.

Long-term studies of phenotypic variation, over several episodes of selection on several traits in multiple populations, are especially valuable for examining mechanisms behind variation in sexual dimorphism (Badyaev *et al.*, 2000). Threespine stickleback populations on Haida Gwaii are ideal for an investigation of the possibility of geographical variation in sexual dimorphism in relation to ecological correlates. These populations inhabit geographically isolated, relatively pristine lakes and streams, which encompass the known range of natural environmental variation that can support stickleback (Moodie and Reimchen, 1976; Bergstrom and Reimchen, 2002).

Previous investigations of stickleback from this archipelago and throughout their holarctic distribution have detected sexual dimorphism for several morphological and behavioural features. Phenotypic and behavioural characters such as dentition (Caldecutt *et al.*, 2001), trophic structure (Caldecutt and Adams, 1998), lateral plate asymmetry (Reimchen and Nosil, 2001c), pelvis asymmetry (Reimchen, 1997; Reimchen and Nosil, 2001a), feeding behaviour (Bentzen and McPhail, 1984), number of dorsal spines (Reimchen, 1980), vertebral number (Reimchen and Nelson, 1987) and other standard

morphometric characters (Wootton, 1976; Reimchen *et al.*, 1985; Kristjansson *et al.*, 2002) have all produced evidence of sexual dimorphism, which could be correlated with both sexual selection and ecological factors.

This chapter is an investigation into the possibility of a sexual dimorphism of the phenotypically integrated character, body shape, in *Gasterosteus aculeatus* populations on the Haida Gwaii archipelago. I use geometric morphometrics to examine body shape of males and females among 125 localities to determine if there is sexual shape dimorphism, to quantify any difference between male and female shape and to test whether any observed differences are related to the volume and clarity of the habitat.

4.2 Methods

4.2.1 Body shape and female gravidity

Landmark data from sexually mature adult stickleback were used to generate partial warp scores and the uniform component of shape change (see chapter 2) for 2519 adult stickleback from 124 populations on Haida Gwaii (one sample consisted entirely of juveniles). A discriminant function and relative warp analysis on the partial warp scores, including the uniform component, were used to quantify body shape. Methods used to quantify body shapes at the extreme of each vector are described below. Prior to investigating potential differences in body shape between males ($n= 1303$) and females ($n= 777$), I calculated the maximum and mean differences between female and gravid females ($n= 439$) among the populations, to determine if it was justified to include them in my analysis.

Gravid females are readily identified by a distended abdomen, which has an effect on body shape. Two landmarks delimited the abdominal region of the stickleback (see

figure 5). Since the landmark demarcating the posterior tip of the pelvic girdle was previously removed (see chapter 2), I removed the landmark corresponding to the anterior insertion of anal fin, to obtain configurations without the effects of gravidity and quantified shape in the manner described above. I also calculated the differences between female and gravid females to compare with the differences calculated from the data with twelve landmarks.

4.2.2 Sexual dimorphism of body shape

I used population CV and RW z-scores (each shape variable for each population has a mean of zero and a standard deviation of one) to investigate the possibility of a sexual dimorphism of body shape. One drawback to this technique is that it does not allow one to accurately quantify shape, as the original CV or RW scores have all been adjusted. However, it should allow sexual dimorphism to be detected if it is in fact present. Prior to standardizing, my “adult only” population shape variables were tightly correlated to the “juvenile and adult” shape variables quantified in chapter 3 (CV1: $R=0.99$, $p<0.001$; CV2: $R=0.95$, $p<0.001$; RW1: $R=0.97$, $p<0.001$; RW2: $R=0.93$, $p<0.001$). Therefore I used the descriptions of shape at the extremes of each vector (chapter 3) to describe positive or negative deviations between the sexes. I used ANCOVA, to determine if there is an association between body shape (first two normalized canonical vectors and relative warps), sex and size. The natural log of centroid size was used, since it is tightly coupled to standard length ($R^2=0.99$, $p<0.001$) within all stickleback populations (see also Baumgartner *et al.*, 1988). The analysis was done for populations throughout the whole archipelago, and then separately for populations in each

geographical area to determine if there were consistent associations in each region. I also used Wilcoxon signed rank tests on the unstandardized mean CV and RW scores for 95 populations (samples with both sexes) to test if any differences in male and female shape are consistent throughout the archipelago, and each geographical region.

4.2.3 Shape dimorphism and physical habitat associations

I used Pearson correlation to examine the association between the degree of sexual-shape dimorphism (female mean shape- male mean shape) and a multivariate measure of lake type, for the whole archipelago and each geographical region. Shape was quantified from the first two canonical and relative warp vectors (CV1, CV2, RW1 and RW2), and habitat was quantified from a principal component analysis of lake physical data (see chapter 2 for complete description).

4.2.4 Sign tests on male and female morphometric measurements

I calculated a number of standard morphometric measurements from the data acquired using TPSDig (Rohlf, 2004) (see chapter 2). These morphometric measurements were standardized, to remove any effects of allometry using the equation:

$$\log Y'_{ij} = [\log Y_{ij} - (v_j (\log x_i - \log x))]$$

Where Y'_{ij} is the adjusted value of character j for individual i , Y_{ij} is the original value, v_j is the average allometric coefficient, x_i is the standard length of individual i and x is the mean standard length averaged over all individuals in the study (Reimchen *et al.*, 1985). I then conducted Wilcoxon signed rank tests on the data to determine the direction of any dimorphism.

4.3 Results

4.3.1 Body shape and female gravidity

I found substantial differences in body shape between females and gravid females throughout the Haida Gwaii archipelago. Examining shape quantified by CV1 (12 landmarks), females differed from gravid females by a maximum of 22.4%, but an average of 5.3% of the total range of variation among populations. I found a maximum of 52.3% and an average of 9.3% of the total range of CV2 scores (12 landmarks) among populations. Females differed in shape from gravid females by a maximum of 56.6% and an average of 8.9% of the total range of RW1 scores (12 landmarks) among populations. For aspects of shape quantified by RW2 (12 landmarks), females differed from gravid females by a maximum of 51.7% and an average of 12.5% of the total range of RW2 scores among populations. The results for the “abdomen” free data (11 landmarks) were similar (see table 6). Given these results I used shape quantified from 12 landmarks and excluded gravid females to test for sexual dimorphism.

4.3.2 Sexual dimorphism of body shape

Using all samples, I detected a slight sexual dimorphism for some aspects of body shape. I did not detect a sexual dimorphism in shape as quantified by the first two normalized canonical vectors (z-CV1, sex: $F_{1,2077}=0.01$, $p=0.93$; z-CV2, sex: $F_{1,2077}=0.25$, $p=0.62$). However, using relative warp analysis, I detected a dimorphism in shape between the sexes (z-RW1, sex: $F_{1,2077}=1103.9$, $p<0.001$), which was consistent within each geographical region (lowland populations: figures 18 and 19; plateau: figure 20; mountain: figure 21). There was also a body shape dimorphism detected with the second

Table 6 Comparison of percent differences in mean shape between females and gravid females from each population (N= 66) for data with 12 and 11 landmarks.

	Data with 12 landmarks		Data with 11 landmarks	
	Maximum difference (%)	Average difference (%)	Maximum difference (%)	Average difference (%)
CV1	22.4	5.3	53.4	18.0
CV2	52.3	9.3	61.5	19.6
RW1	56.6	8.9	55.8	9.2
RW2	51.7	12.5	31.9	7.4

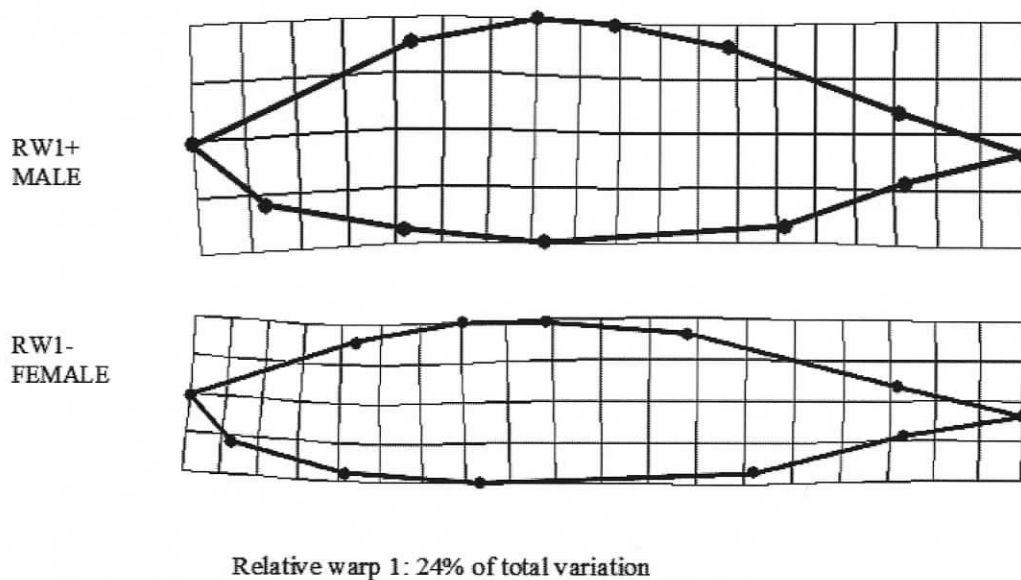
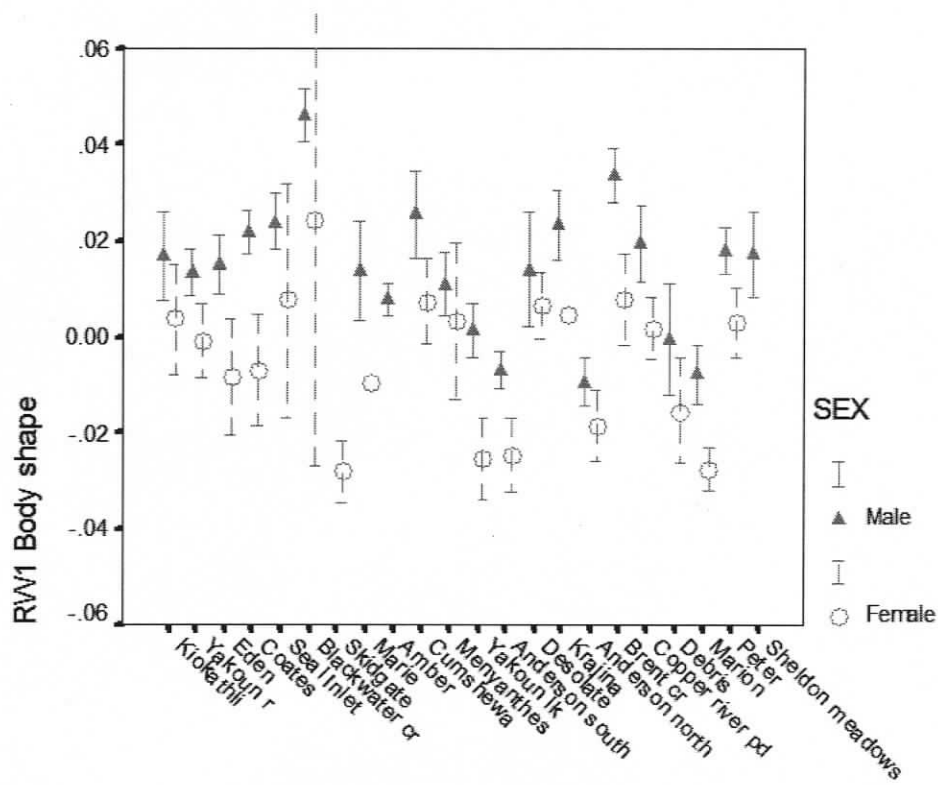


Figure 20. Error bar and thin plate spline plots of the general differences in body shape, as quantified by z-relative warp 1 scores, between male and female stickleback from the plateau region (N= 22) of Haida Gwaii. Spline plots represent the + and - difference at the extremes of the RW1 vector.

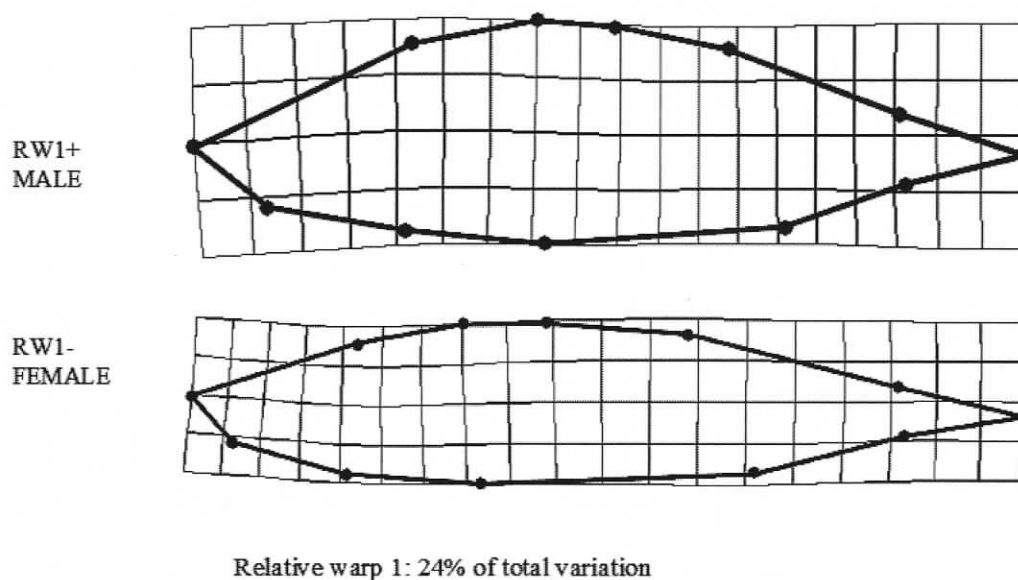
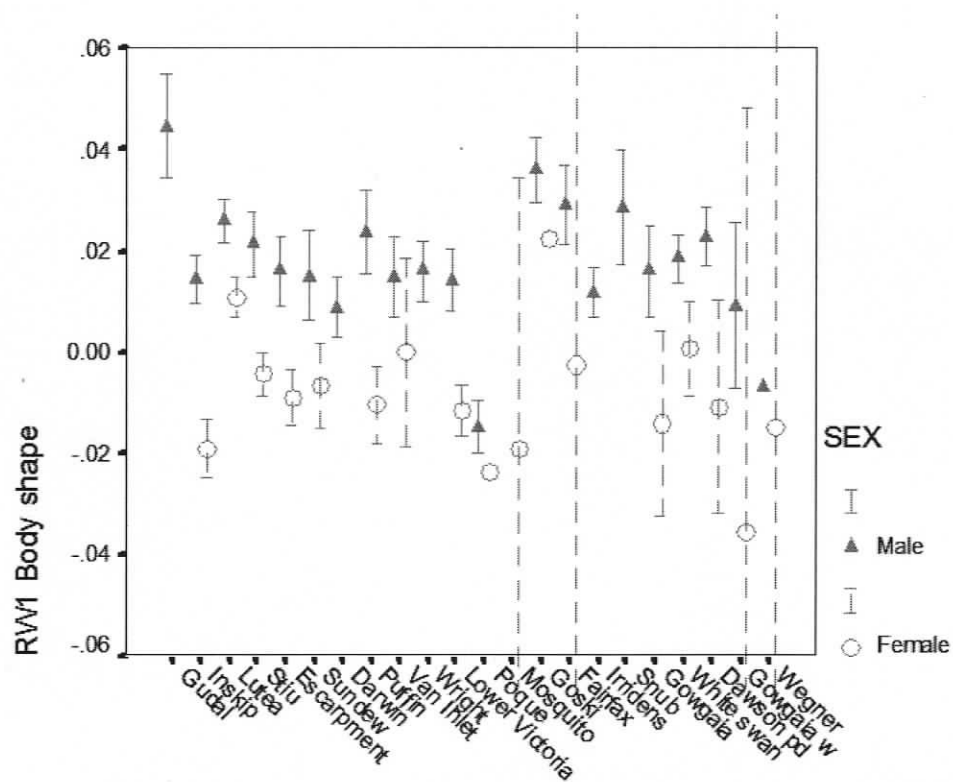


Figure 21. Error bar and thin plate spline plots of the general differences in body shape, as quantified by z-relative warp 1 scores, between male and female stickleback from the mountain region ($N=22$) of Haida Gwaii. Spline plots represent the + and - difference at the extremes of the RW1 vector.

relative warp (z-RW2, sex: $F_{1,2077}=785.9$, $p < 0.001$), which was consistent within each geographical area (lowland: figures 22 and 23; plateau: figure 24; mountain: figure 25). There were no statistical indications of any interaction effects suggesting that the detected dimorphisms are not more apparent with increasing body size among populations. My results suggest that compared to females, male sticklebacks have larger heads, posteriad dorsal spines, and deeper bodies (biased towards RW1+) with longer median fins (biased towards RW2+). Wilcoxon signed rank test confirms sexual dimorphism in shape as quantified by RW1 for 95 out of 124 populations of stickleback ($Z= 8.44$, $p < 0.001$) and RW2 ($Z= 8.33$, $p < 0.001$). The maximum difference in body shape between females and males, quantified by RW1, occurred in Gowgaia West at 4.5% of the total variation of population relative warp 1 scores. The maximum difference between males and females, for shape quantified by RW2, occurred in Midge at 4.6% of the total variation of population relative warp 2 scores.

4.3.3 Shape dimorphism and physical habitat associations

I did not find a relationship between body shape sexual dimorphism and lake volume/clarity (PC1_lk; $N= 54$; Pearson correlation CV1: $R= -0.003$, $p= 0.98$; CV2: $R= -0.07$, $p= 0.61$; RW1: $R= -0.1$, $p= 0.49$; RW2: $R= -0.06$, $p= 0.7$). Similar results were found using any shape dimorphism versus PC1_lk associations from lowland populations ($N= 35$; Pearson correlation CV1: $R= -0.04$, $p= 0.83$; CV2: $R= -0.17$, $p= 0.34$; RW1: $R= -0.19$, $p= 0.28$; RW2: $R= -0.26$, $p= 0.14$). I did not detect a relationship between lake volume/clarity and differences in male/female body shape for the plateau/mountain

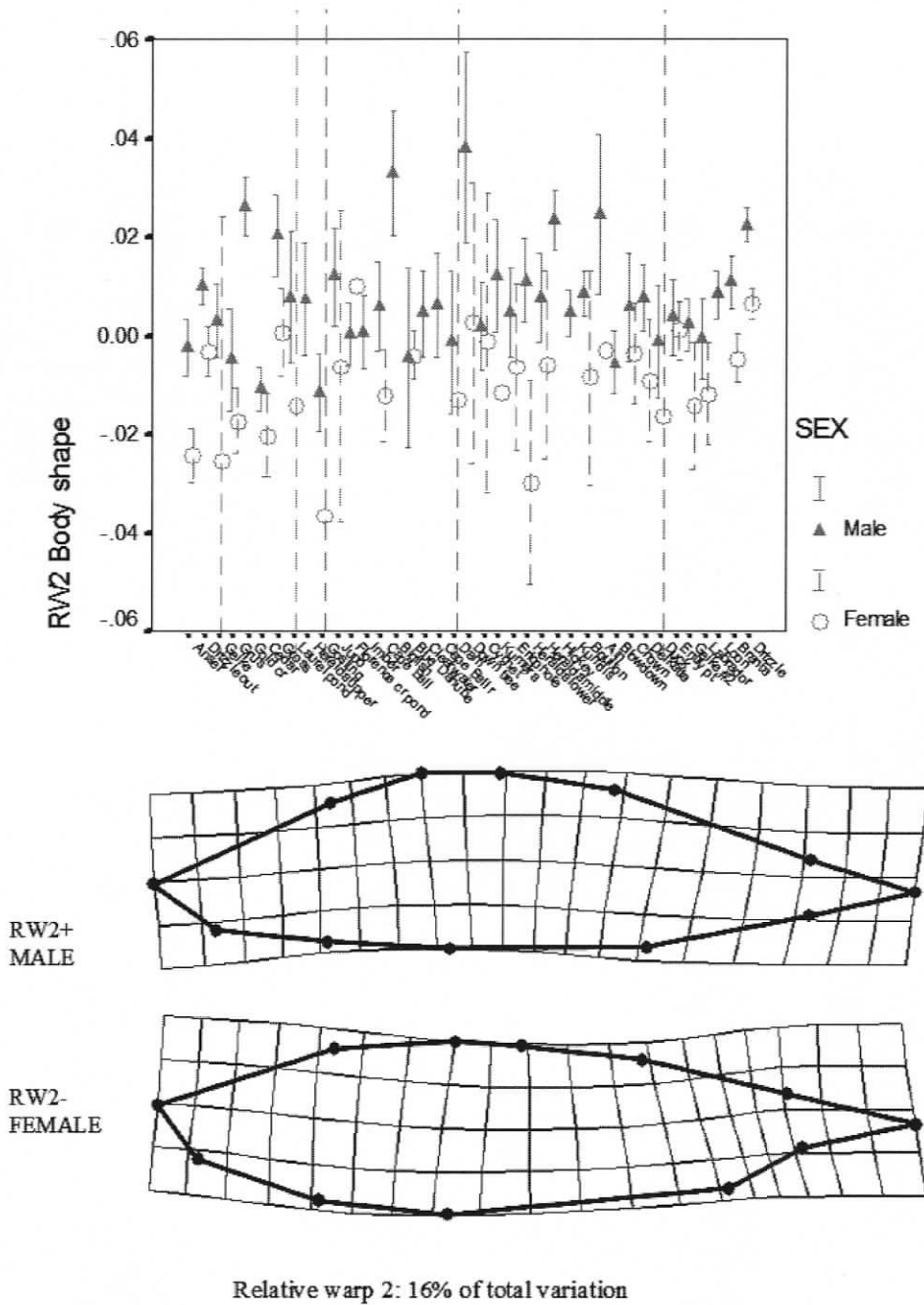
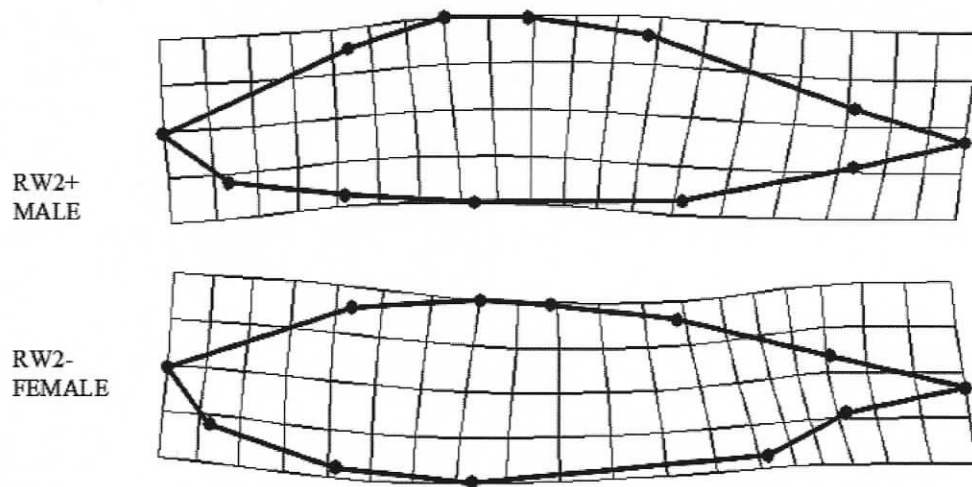
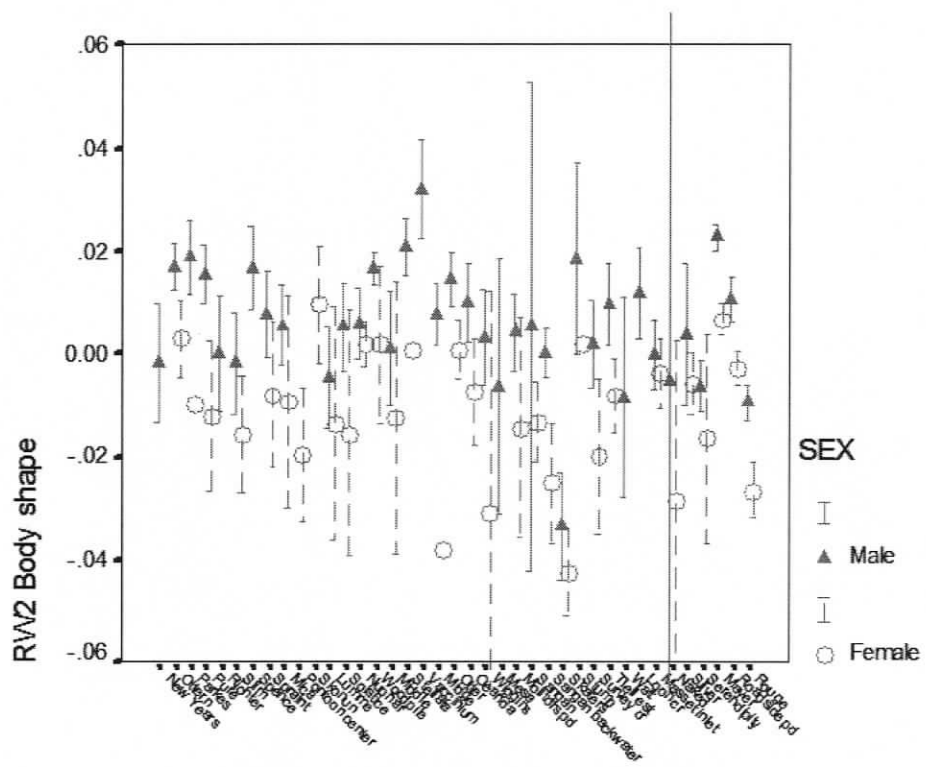


Figure 22. Error bar and thin plate spline plots of the general differences in body shape, as quantified by z-relative warp 2 scores, between male and female stickleback from an arbitrary subset of populations (samples 1-39) from the lowland region ($N=39$) of Haida Gwaii. Spline plots represent the $+$ and $-$ difference at the extremes of the RW2 vector.



Relative warp 2: 16% of total variation

Figure 23. Error bar and thin plate spline plots of the general differences in body shape, as quantified by z-relative warp 2 scores, between male and female stickleback from an arbitrary subset of populations (samples 40- 78) from the lowland region (N= 39) of Haida Gwaii. Spline plots represent the + and - difference at the extremes of the RW2 vector.

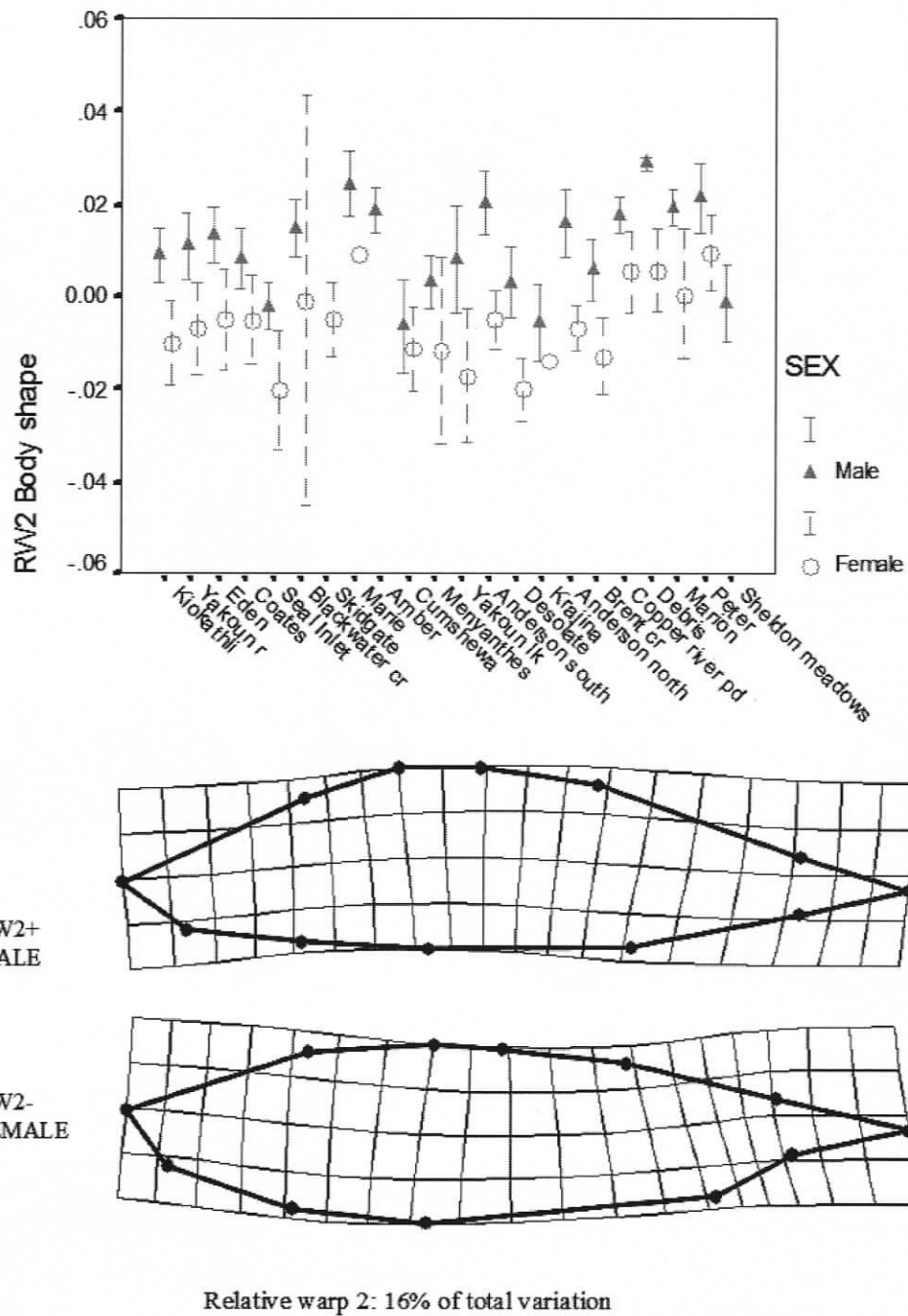


Figure 24. Error bar and thin plate spline plots of the general differences in body shape, as quantified by z-relative warp 2 scores, between male and female stickleback from the plateau region (N= 22) of Haida Gwaii. Spline plots represent the + and - difference at the extremes of the RW2 vector.

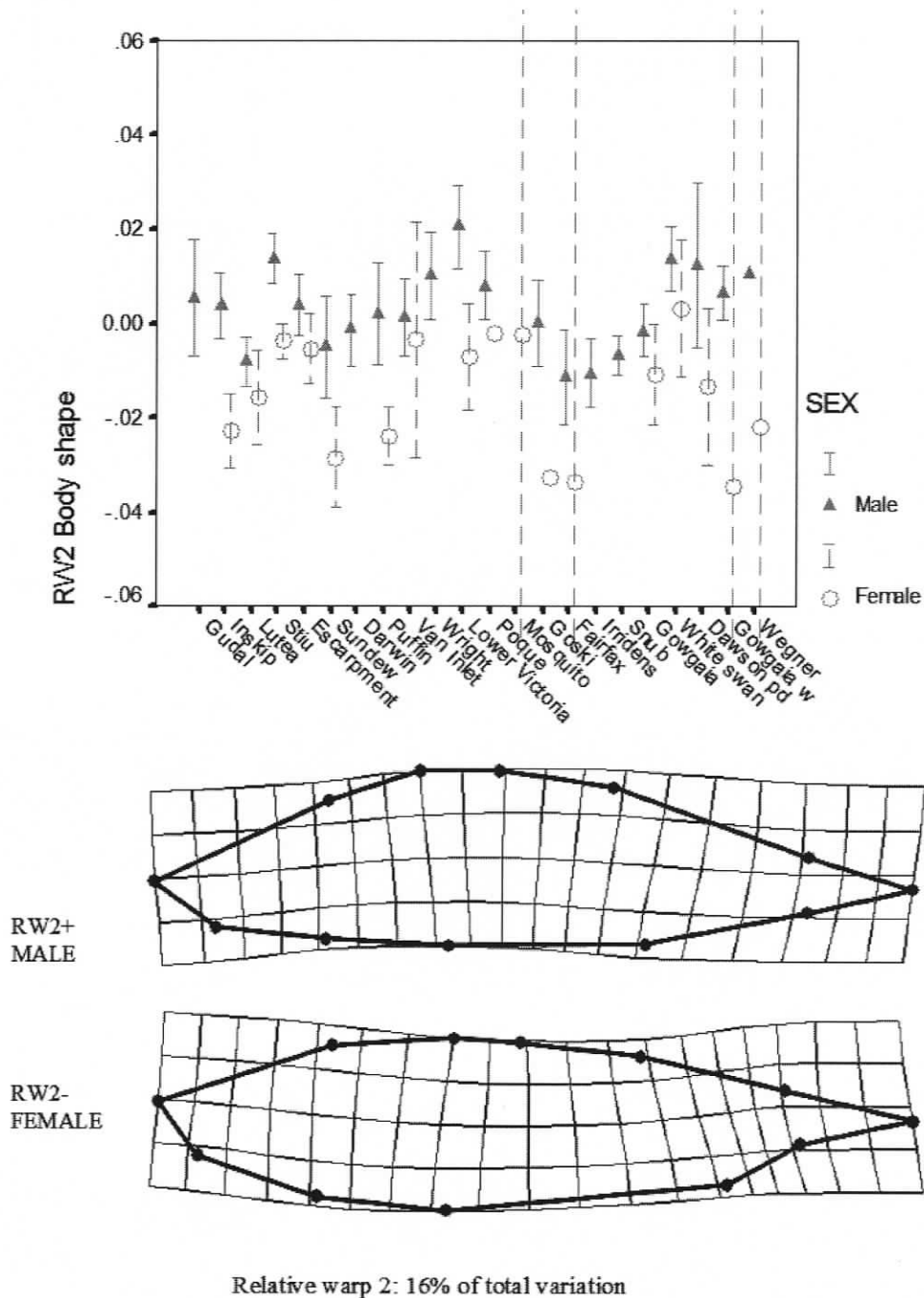


Figure 25. Error bar and thin plate spline plots of the general differences in body shape, as quantified by z-relative warp 2 scores, between male and female stickleback from the mountain region (N= 22) of Haida Gwaii. Spline plots represent the + and - difference at the extremes of the RW2 vector.

populations (N= 19; Pearson correlation CV1: R= -0.29, p= 0.24; CV2: R= -0.19, p= 0.43; RW1: R= 0.1, p= 0.7; RW2: R= 0.03, p= 0.9).

4.3.4 Sign tests on morphometric measurements

The Wilcoxon signed rank tests on mean morphometric measurements for each population indicate differences between males and females for a number of characters. These results suggest that although females are slightly larger in standard length, individual size adjusted shape traits in males are usually larger (table 7; figure 26). Male stickleback tend to have larger heads, which facilitate a larger gape; more posteriad position of dorsal spines; posteriad position of the pelvis; longer dorsal and anal fins and thicker caudal peduncles.

4.4 Discussion

Using two multivariate techniques to quantify lateral body shape, to investigate sexual dimorphism of body shape produced different results. There were no differences between the sexes when shape was quantified using discriminant function analysis. However, I found that stickleback from Haida Gwaii exhibit sexual dimorphism when body shape is quantified by relative warp analysis, which was confirmed when comparing standard morphological features. I did not find any statistical indication that shape differences between the sexes increase with increasing body size. The maximum differences in body shape between the sexes occurred in two populations (i.e., Gowgaia West and Midge Lake) of average size. The degree of dimorphism throughout the archipelago and in each geographical sub-region is not correlated with lake volume/clarity (PC1_lk). Nevertheless, throughout the archipelago, I consistently

Table 7. Wilcoxon signed rank test for sexual dimorphism of mean morphometric characters for 95 stickleback populations. Characters for each stickleback (except SL) were transformed using log-linear regression transformed to remove any effects of allometry. A positive difference indicates females have the larger trait (female trait – male trait).

Trait	Z	Difference	P value
SL	-2.26	+	0.02
Pelvis	-8.11	+	< 0.001
Pelvis position	-4.33	-	< 0.001
Head area	-8.37	-	< 0.001
Head length	-8.46	-	< 0.001
Head depth	-6.37	-	< 0.001
Gape	-8.46	-	< 0.001
Dorsal spine 1 (D1) position	-8.13	-	< 0.001
Distance between D1 and D2	-0.14	-	0.89
Body depth (anterior)	-1.31	-	0.19
Body depth (posterior)	-2.50	-	0.01
Dorsal fin length	-6.72	-	< 0.001
Anal fin length	-8.44	-	< 0.001
Caudal peduncle depth (anterior)	-5.42	+	< 0.001
Caudal peduncle depth (posterior)	-7.11	-	< 0.001
Caudal peduncle area	-3.41	-	< 0.001

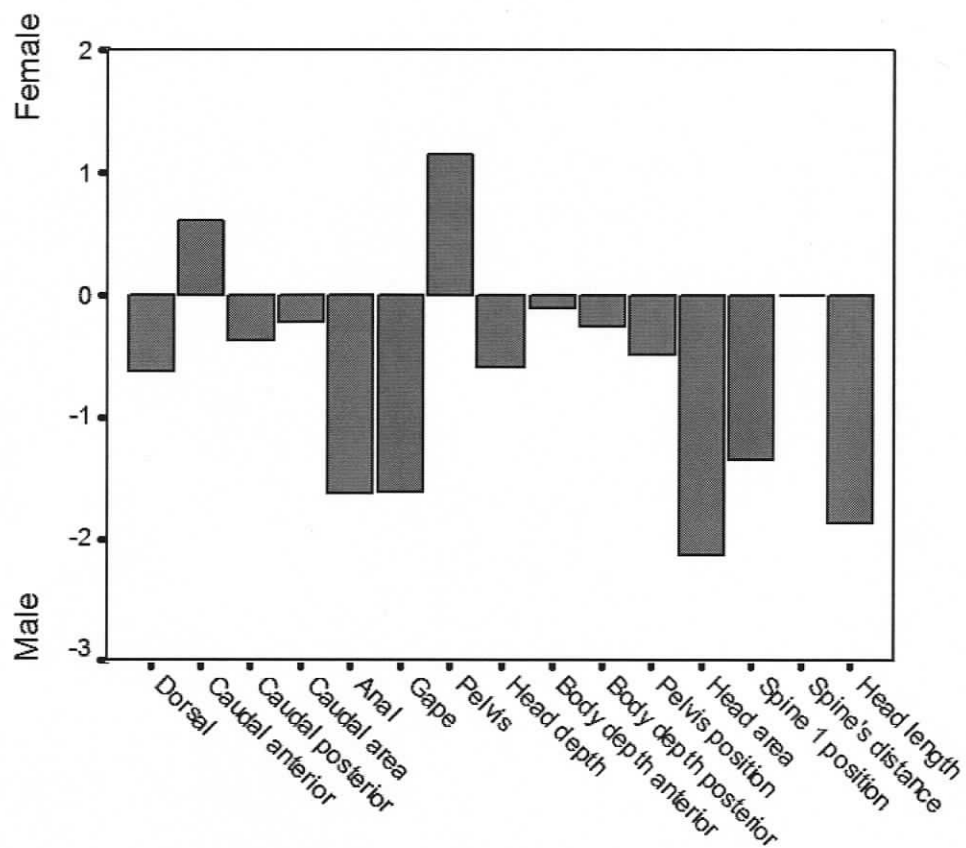


Figure 26. Bar graph showing the degree of differences (mm) between male and female stickleback for standard morphometric characters, calculated from pooled data of log linear regression transformed variables as female trait-male trait (i.e., female dorsal fin length-male dorsal fin length).

detected male stickleback having larger heads, posterior dorsal spines, and deeper bodies with shorter median fins than the females.

Although the differences in body shape were not substantial or correlated with habitat, they still represent the parallel evolution of similar adaptations to hydrodynamic constraints throughout the archipelago. One of the main agents of selection for body shape in aquatic organisms is drag (Aleev, 1977; Webb, 1982, 1984), the “difference between the dynamic pressure in the front of a swimming fish and the absence of the predicted counteractive pressure at the rear” (Vogel, 1981). Water flowing around a moving fish does not follow the contours of the body exactly, but instead separates from the body in the form of eddies and turbulence, creating pressure differences in the anterior and posterior of the body that provide resistance to movement (Aleev, 1977; Vogel, 1981; Walker, 1997). The similarity of shape between males and females and the consistent, but subtle differences between the sexes throughout the archipelago and within each geographical region suggests the evolution of body-shape optima.

I found that females, in comparison to males, had small heads, anterior dorsal spines, and shallower bodies. If the position of the dorsal spines places the center of inertia slightly more anterior in female stickleback, then this characteristic of female shape would enhance stability during motion (Aleev, 1977). The small head and shallower body may also be a form of laminarization of shape, a method to reduce the formation of turbulent eddies (and drag) along the posterior regions of the fish (Aleev, 1977; Vogel, 1981; Walker, 1997). In theory, a female stickleback gliding through the water column, would have water pass over its body, gradually decelerating in the rear with little to no separation (Vogel, 1981). The wedge like closure of the water behind the

stickleback would literally push the fish forward. Instead of being lost in the wake, “energy re-emerges as a forward directed pressure from the rear, which nearly counterbalances the difference in pressure in front of the fish” (Vogel, 1981). For a constant length and volume, a shallow-body fish will incur less drag during steady swimming than a deep bodied fish with a highly elliptic cross section (Walker, 1997). These hydrodynamic associations suggests that the female shape is an adaptation to the open water environment (Webb, 1984; Domenici and Blake, 1997; Walker, 1997), as the fish could glide through the water column with ease.

Males on the other hand, had proportionally large heads, posteriad dorsal spines, and deeper bodies. High acceleration performance requires a deep body and deep caudal peduncle to maximize thrust and overcome inertial resistance (Webb, 1982, 1984; Walker, 1997). If the posterior dorsal spine position, shifts the center of mass towards the rear of the fish, then this characteristic of male shape would allow greater manoeuvrability by increasing instability in the water column (Aleev, 1977). The body shape in males suggest an adaptation for rapid acceleration and manoeuvrability (Webb, 1984; Domenici and Blake, 1997), possibly in response to a subtle, sex specific selection regime (to increase performance of the zig-zag dance during courtship).

It seems plausible that the slight morphological divergence has ecological significance in adapting the sexes to different sub niches, where differential foraging behaviours within a common habitat (i.e. lake) is only one of several means reducing intersexual competition for food (Selander, 1966). The subtle body-shape differences between male and female stickleback were expected based on a single study of feeding behaviour, which suggests males are more efficient at feeding in the benthos, while

females are less efficient at eating larger benthic prey, but more efficient at feeding on organisms in the water column (Bentzen and McPhail, 1984; but see Kristjánsson *et al.*, 2002). The male's deep bodies, and posterior center of inertia increase manoeuvrability and therefore, should facilitate foraging in a structurally complex littoral zone (Hart and Gill, 1994). The female's streamlined bodies and anterior center of mass contribute to lower drag and greater stability during steady swimming, and should increase open water foraging performance (Webb, 1984; Walker, 1997). Given the patchy distribution of planktonic organisms (George, 1981; Pinel-Alloul *et al.*, 1988), female stickleback foraging in open water (Bentzen and McPhail, 1984) would swim at steady velocities for long distances, or cruise for much longer periods of time than males foraging in the littoral zone.

Sexual selection has often been implicated as the foundation of a sexual dimorphism. When sexual selection reaches its fullest development at sexual maturity, and if the dimorphic character functions mainly in one sex to confer a mating advantage on individuals with more extreme development of the character, sexual dimorphism will evolve (Lande, 1980; Slatkin, 1984). Selective forces of this nature shape sexes differently. Male body proportions face strong selection to increase attractiveness to females, to confer advantage in male-male interactions and to defend their nest (van Iersel, 1953). Female fitness is influenced by the ability to assimilate large amounts of nutrients necessary for vitellogenesis, and the ability to carry the eggs (Bonnet *et al.*, 2001).

Male stickleback have elaborate courtship behaviours which includes competition for nesting territory, defense of territory once established, a zig-zag dance to attract a

female, and defense of nest and fertilized eggs (van Iersel, 1953; Wootton, 1976, 1984; Bakker, 1994; Foster, 1994). In all of the territorial behaviours, males tend to rapidly accelerate or jump towards the intruder, and usually bite, bump and even fight (Bakker, 1994; Foster, 1994). The courtship dance involves numerous jumps toward and away from the female, which can be interpreted as aggression towards the female with the intention of enticing her into the nest (Wootton, 1976, 1984; Rowland, 1994). Undoubtedly, the more effective the male can perform these manoeuvres the higher absolute fitness it will have (Bakker, 1994), which suggests that male body shape is adapted for maneuverability and acceleration and has clearly been influenced by sexual selection.

The female stickleback's role in reproduction is the production of eggs, and spawning of the eggs in a male's nest (Wootton, 1976). Gravid females are easily distinguishable from males and other non-gravid females, as their abdomens swell as eggs mature. I excluded gravid females from the analysis of sexual dimorphism of body shape, and it may be inferred that the body shape of non-gravid females is an adaptation to expand their realized niche, securing different food resources and reducing competition with males (Slatkin, 1984). However, evolutionary theory predicts that particular body components should be differentially enlarged in the two sexes (Bonnet *et al.*, 1998), and I consistently found that females had larger pelvises which may help support developing eggs (Baker *et al.*, 1995).

Throughout the archipelago stickleback are preyed on by a wide number of vertebrate and invertebrate predators, which typically forage in different areas of lakes (Reimchen, 1994; Reimchen and Nosil, 2002, 2004). Although not mutually exclusive,

macro-invertebrates (e.g., odonate nymphs) and fish, when present, typically forage in the littoral zone, while if the lake is large enough, avian piscivores tend to forage in the deeper parts of the lake (see Reimchen 1994 for review). Undoubtedly, male and female stickleback encounter predation from each type of predator that is present. However, reproductively-active males encounter disproportionate predation from predatory fish and invertebrates, while females often encounter higher predation from birds (Reimchen and Nosil, 2004). Sexual dimorphism observed in body shape could represent selection from niche specific predation regimes. The deep male body shape, is not only an adaptation for littoral foraging and reproductive behavior, but could have evolved as a response to gape limited predators (Hoyle and Keast, 1987, 1988; Reimchen, 1991a, 1991b) and as a consequence of selection for burst performance (Taylor and McPhail, 1986), to evade predators by darting for cover. The shallower body shape of females possibly represents an adaptation to avoid detection by avian piscivores, allowing them to cruise the open water with minimal disturbance.

When the males and females are subject to different selection pressures in connection with their contrasting ecological roles, correlated evolutionary responses interfere with the separate adaptation of each sex (Lande, 1980). Nevertheless, under weak natural selection, the joint evolution of mean phenotypes of the two sexes continually increases the mean fitness in a population until an evolutionary equilibrium is attained. Provided there is genetic variation for sexual dimorphism of body shape, any correlated selective responses between the sexes do not prevent the evolution of both sexes to a ecologically optimum phenotype (Schoener, 1969; Lande, 1980).

My results suggest that there are numerous ecological forces acting in concert as agents of selection for the slight sexual dimorphism of body shape, which may represent phenotypic integration (Bell and Foster, 1994; Foster and Bell, 1994) at a finer scale (i.e., phenotypic response to subtle ecological differences between the sexes) than previously known. The slight dimorphism may have evolved through sexual selection but has been constrained and maintained by numerous ecological factors (Shine, 1989). The need to minimize resistance to movement in the dense aquatic medium while occupying divergent ecological niches has probably been the most important factor in constraining the observed dimorphism.

Chapter 5: Morphological variation in adult stickleback from Haida Gwaii

5.1 Introduction

Body shape is a major, but often overlooked component of an organism's phenotype. In aquatic organisms, forces associated with moving through the dense viscous medium can constrain shape, as is evident from the convergence of form in many diverse aquatic chordates (Aleev, 1977). Nevertheless, organisms differ in the niche space they occupy, and in aquatic species, the body shape optimum for ensuring survival changes accordingly (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 fish, 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; Sfakiotakis *et al.*, 1999). Most often, fish 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 fish with a body shape characteristic of sustained swimming have the ability use burst acceleration and *vice versa*. However shape adapted for maximum performance in one predominant mode of locomotion 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 that leads to a concomitant decrease in burst performance (McGuigan *et al.*, 2003).

Ecological interactions in different environments can have profound effects on the evolution of shape. The local environment provides the template on which evolution forges characteristic life history strategies (Southwood, 1977; Townsend and 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). However, a genetic component to body shape has been suggested (Walker, 1997; Guill *et al.*, 2003a; Langerhans and DeWitt, 2004b), through the diversification of 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.

Do organisms experiencing similar selection pressures evolve similar phenotypes, and can phenotypic integration be employed to explain observed phenomena? The populations of threespine stickleback from Haida Gwaii are excellent to address this question, as the current landscape is approximately 10 000 years old, and was initially colonized by marine stickleback (Moodie and Reimchen, 1976) from both the Euro-North

American and Japanese (Trans-North Pacific) lineages (O'Reilly *et al.*, 1993; Orti *et al.*, 1994; Deagle *et al.*, 1996). The Haida Gwaii populations inhabit geographically isolated, relatively pristine lakes and streams, which encompass a wide range of abiotic and biotic variation (Moodie and Reimchen, 1976; Reimchen, 1994; Bergstrom and Reimchen, 2002). In this chapter I examine associations between adult threespine stickleback body shape and ecological factors from a number of perspectives to try to infer mechanisms that select for body-shape differences among populations from Haida Gwaii.

5.2 Methods

5.2.1 Quantification of body shape in adult Haida Gwaii Threespine stickleback

I photographed 3808 individual stickleback, and captured the Cartesian coordinates of morphological landmarks from the digital images to quantify body shape from 125 populations on Haida Gwaii. In most samples there were distinct size classes (juvenile, sub adult and adult). I chose the largest stickleback from each sample for the analysis of adult body shape (N= 124, n= 2519). The program TPSRegr (Rohlf, 2000) was used to generate partial warp scores, which correspond to variation in body shape at different scales, and the uniform component of shape change (see chapter 2) . Discriminant function (CV) was used on the partial warp and uniform component scores to quantify multivariate measures of body shape among specimens throughout the archipelago. I included the uniform component 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 (CV1: R= 0.97, p< 0.001; CV2: R= 0.88, p< 0.001). Pearson

correlation was used to describe body shape along each shape vector from log linear transformed measures of standard morphometric variables.

I used all the largest fish from the samples for the shape analysis. Although the majority of populations had similar sex ratios, some of the populations had gravid females as well as divergent sex ratios, which could inflate shape differences among populations (see chapter 4). Consequently, a concurrent analysis was done on only the male stickleback from each lake and similar results were obtained (CV1: $R=0.99$, $p<0.001$; CV2: $R=0.99$, $p<0.001$). Univariate ANOVA was used to test the hypothesis that there is no difference in body shape among populations. I used these estimates to reveal any ecological associations of habitat with body shape.

5.2.2 Body shape and body size relationship

I used the natural log of centroid size (see chapter 2) as the measure of overall body size as it was tightly coupled to standard length ($R^2=0.99$, $p<0.001$). Pearson's correlation between population's mean centroid size and mean shape scores was used to determine if body shape quantified by canonical vectors was related to centroid size.

5.2.3 Body shape differences between lake–river pairs

I assessed the multivariate measures of shape to make a broad comparison between the ranges of body shapes between habitat types. As the modern marine stickleback's phenotype has apparently remained relatively unchanged for greater than ten million years (Bell, 1977, 1994; Bell and Foster, 1994; Walker and Bell, 2000), I used only one marine sample, the Entry Point population's body shape, as the less derived

shape. There were 5 brackish, 19 river and 99 lake stickleback populations used in the analysis. I compare the variation in shape between lake-river pair populations using paired sample t-tests and independent samples t-tests, but focus my analysis on the variation among lake populations.

5.2.4 Comparison with stickleback species pairs from southwestern British Columbia

In the Strait of Georgia in southwestern British Columbia there are lakes that contain pairs of coexisting benthic and limnetic biological species of threespine stickleback. The members of these pairs differ significantly in morphology, which has been interpreted as adaptation to benthic (littoral) and limnetic (open water) habitats of each lake (Larson, 1976; McPhail, 1984, 1992; Lavin and McPhail, 1985; Schluter and McPhail, 1992). I included benthics and limnetics from 3 populations (Enos, Paxton and Priest lakes) to quantify their body shapes and compare the percent differences in mean CV scores between ecotypes to the variation in shape among the Haida Gwaii populations.

5.2.5 Phenotypic plasticity in body shape

In 1992 a common garden experiment was started by Reimchen (*pers. com.*), as part of the long-term investigation of threespine stickleback from Haida Gwaii. The distinct giant melanistic stickleback from Mayer Lake, a lake that is large, deep and dystrophic with the full suite of vertebrate predators, were transplanted into its ecological opposite, a small shallow eutrophic pond, with no vertebrate predators. The biological and physical differences between Mayer Lake and the experimental pond span the range

of habitat types on Haida Gwaii. A few years later the experiment was replicated and the distinct giant melanistic stickleback were transplanted from Drizzle Lake into another experimental pond. Again the source lake and recipient pond were ecological opposites.

The tests for plasticity were conducted on two subsets of the data. To obtain the most accurate estimates of phenotypic plasticity in body shape, I selected only the experimental ponds first generations when they obtained adult size (55-75mm SL). I used the percent differences in population mean CV scores, between the experimental ponds and source populations and compared them against the range of CV scores throughout the archipelago. To make a broad assessment of evolutionary changes in body shape, I used the most recent samples collected from the experimental ponds (Mayer experimental pond 2002 and Drizzle pond 2003) and made the same comparison listed above for the first adult generations.

5.2.6 Body size, body shape and ecological associations

To determine if there was a relationship between body size or body shape and lake type, I used linear regression of population mean centroid size or CV scores against multivariate measures of lake physical data (table 8; see chapter 2). I ran all analysis for the whole archipelago. Previous work has shown lake types within each geographical region are more similar than lake types between regions (Reimchen, 1989, 1992c). I used ANCOVA to determine if any ecological relationships are consistent between regions.

Table 8. Principal component matrix of loading scores for physical characteristics of lake habitat, (N= 70) on Haida Gwaii. PC1_lk explains 38.9% of the variation among habitats and represents lake volume/clarity. PC2_lk explains 25.2% of the variation and represents the productivity of the lake. All of the variables except pH and T400 (% transmission at 400nm) were natural log transformed prior to the principal component analysis.

	Volume/clarity (PC1)	Productivity (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 marine	-0.23	-0.39

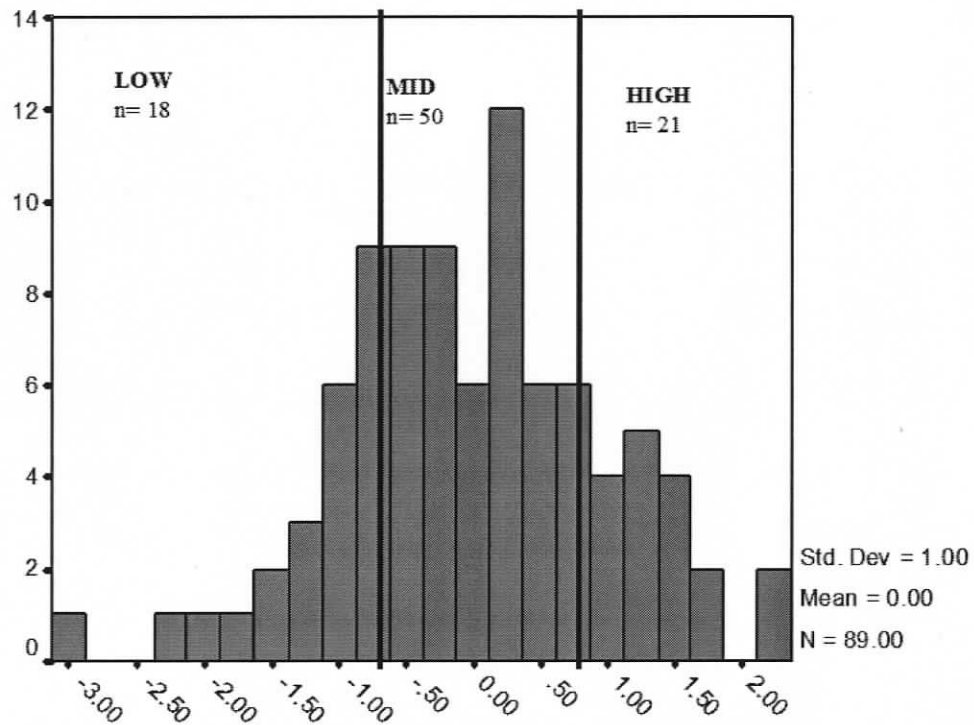
I also test whether size or body shape could be predicted by the predation regime of the habitat or population defensive condition. Predator state was previously defined as either salmonid, or bird/invertebrate (Reimchen, 1994), but neither of these categories is mutually exclusive. In previous work Reimchen (1983, 1992a, 2000) determined functional aspects of stickleback defensive armature, and provided a database of the threespine stickleback defensive apparatus. Fully plated stickleback usually have a full complement of 30-35 lateral plates, robust pelvis and spines. However the majority of populations on Haida Gwaii are low plated. To get an accurate measure of the variability in the defensive condition of low plated morphs, I ran a principal component analysis on Reimchen's data, which includes the population means of lateral plate number, ascending process height and the length of the left pelvic spine (table 9). I categorized the defensive condition factor score into high, medium and low condition. High and low defensive conditions are composed of the scores that fall above and below 0.75 standard deviations of the mean (figure 27). Using ANCOVA, I investigated the possibility of associations between body size or body shape, habitat and predator state and body size or shape, habitat and defensive condition.

5.2.7 Mitochondrial haplotype, shape and habitat associations in lowland populations

The presence of the Japanese (Trans-North Pacific) stickleback mitochondrial lineage has been detected, at varying frequencies, in 19 lowland populations (O'Reilly *et al.*, 1993; Deagle *et al.*, 1996). I tested if any body shape and habitat associations are

Table 9. Principal component matrix of loading scores for defensive characteristics of 83 stickleback populations on Haida Gwaii. PC1 explains 68.2% of the variation among defensive characters and represents “defensive condition”. Measurements were taken on the left side of each stickleback.

	Defensive condition (PC1)
Mean plate number	0.73
Length pelvic spine/ sl	0.90
Ascending process height/ depth	0.85



PC 1 Defensive adaptation (68.2% of total variation)

Figure 27. Distribution of PC1 on the defensive adaptations of low plated lake stickleback populations. The low morph represents stickleback populations with no lateral plates and a vestigial pelvic girdle, while those categorized as high, have an average of 12 plates and a robust pelvis.

consistent between when the Japanese haplotype is present ($n= 5$) or absent ($n= 32$) in lowland populations.

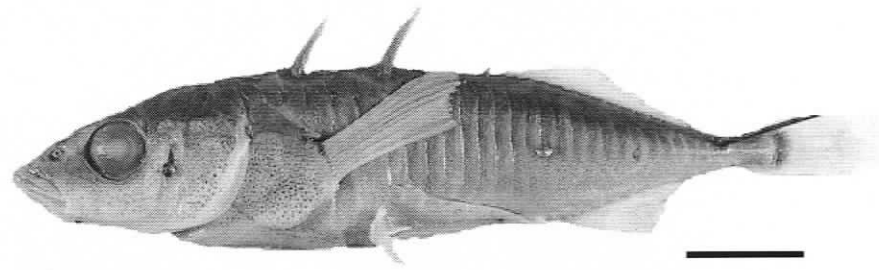
5.3 Results

5.3.1 Body shape variation among Haida Gwaii Threespine stickleback

Discriminant function analysis predicted group membership (i.e. stickleback sample) based on reliable mean differences of combinations of partial warp and uniform component scores 77.5% of the time, and produced canonical vectors describing differences in body shape among samples. Pearson correlations between population mean canonical vector scores and standard morphometric measurements (table 10) allow us to describe shape along each canonical vector. The first CV accounts for 33.4% of the total variation and contrasts the less derived marine stickleback (figure 28A), on the negative end of the vector with a small narrow peduncle, anterior and widely spaced dorsal spines, posterior pelvis and long dorsal and anal fins vs. more derived fish on the positive extent of the vector with thick peduncles, posterior and closely spaced dorsal spines, anterior pelvis and short dorsal and anal fins, represented by Rouge Lake (figure 28B). The second vector accounts for 21.7% of the total variation and contrasts derived stickleback with negative loadings characterized by small heads and shallow bodies, represented by Mayer Lake (figure 28C) vs. less derived fish on the positive extent of the vector with large heads and deeper bodies, represented by fish from Fairfax Lake (figure 28D). There were significant differences among population mean body shapes (CV1: $F_{124, 2394} = 155.5$, $p < 0.001$; CV2: $F_{124, 2394} = 100.7$, $p < 0.001$; figure 29).

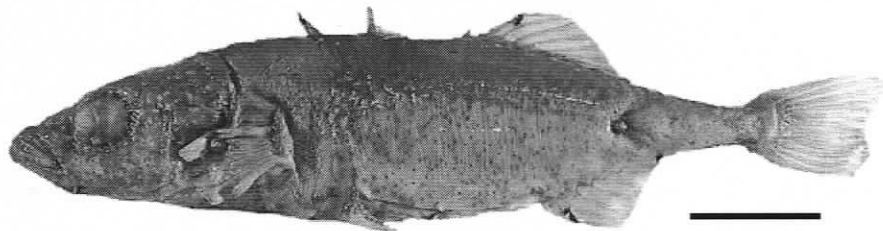
Table 10. Pearson correlations for CV1 and CV2 between log linear regression standardized morphometric measures calculated from inter-landmark distances for 124 populations. CV1 explains 33.4% of the variation among populations of adult stickleback from Haida Gwaii; CV2 explains 21.7% of the variation.

Character	CV1		CV2	
	Pearson	P value	Pearson	P value
	Correlation		Correlation	
Pelvis	-0.72	< 0.001	0.41	< 0.001
Pelvis position	-0.56	< 0.001	0.57	< 0.001
Head area	0.08	0.93	0.32	< 0.001
Head length	-0.28	0.001	0.30	0.001
Head depth	0.11	0.24	0.51	< 0.001
Gape	0.25	0.006	0.02	0.81
Dorsal spine 1 (D1) position	0.50	< 0.001	0.09	0.40
Distance between D1 and D2	-0.91	< 0.001	0.44	< 0.001
Body depth (anterior)	-0.20	0.02	0.38	< 0.001
Body depth (posterior)	-0.08	0.36	0.27	0.002
Dorsal fin length	-0.41	< 0.001	0.03	0.72
Anal fin length	-0.41	< 0.001	-0.34	< 0.001
Caudal peduncle depth (anterior)	0.09	0.30	0.60	0.52
Caudal peduncle depth (posterior)	0.66	< 0.001	-0.30	0.001
Caudal peduncle area	0.73	< 0.001	-0.45	< 0.001



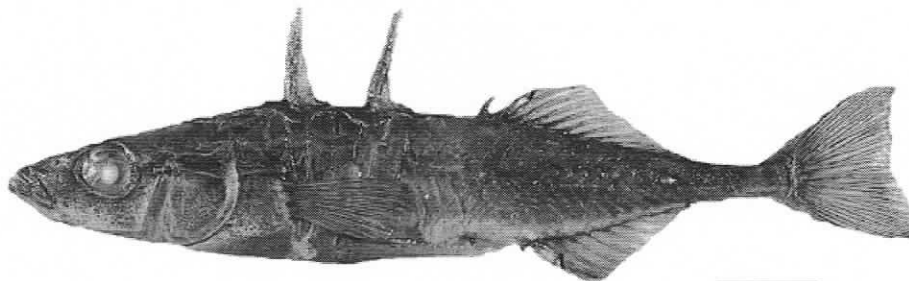
A: Entry Point

10 mm



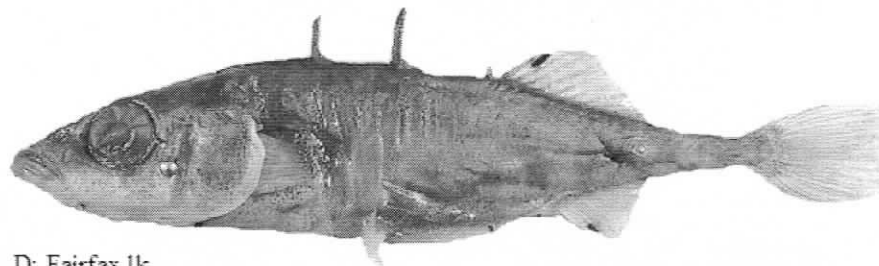
B: Rouge lk

10 mm



C: Mayer lk

10 mm



D: Fairfax lk

10 mm

Figure 28. Photos of representative stickleback.

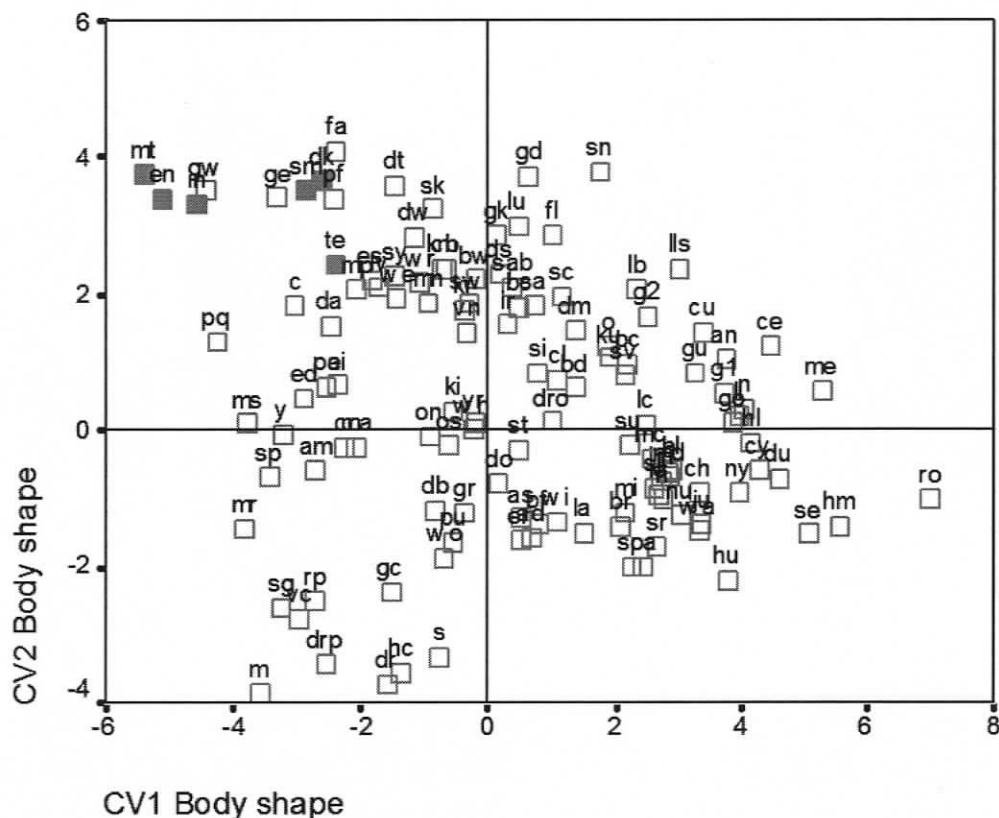


Figure 29. Canonical vector 1 (33.4% of total variation) and CV2 (21.7% of total variation) quantifying body shape of adult stickleback from Haida Gwaii.

Anadromous populations are denoted by solid squares. Legend for population names: 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 pd, 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= Heralda Lower, hm= Heralda 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= Ottern, 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.

5.3.2 Body shape and body size relationship

Overall the body-shape mean for each population was correlated with adult body size. Body shape of populations on the negative extreme of the first canonical vector were slightly larger than those on the positive extreme (CV1: $R = -0.19$, $p = 0.04$). Shape quantified by CV2 was also negatively correlated with centroid size (CV2: $R = -0.46$, $p < 0.001$), suggesting larger fish at the negative extremes of each canonical vector.

5.3.3 Body-shape differences between lake–river pairs

Overall, I found only marginal differences in body shape between lake-river stickleback pairs (CV1: $df=6$, $t = -0.8$, $p = 0.46$; CV2: $df=6$, $t = -2.14$, $p = 0.08$). However, when each lake-river pair was analyzed separately, 5 out of the 7 population pairs showed significant differences in shape quantified by CV1 and 6 of the 7 pairs had significant differences in body shape quantified by CV2 (table 11). In each significant case the lake populations had less derived negative loadings for CV1 and the more derived negative loadings for CV2, than their respective river populations. These populations of lake stickleback can be characterized with smaller heads, shallower bodies, narrower peduncles, anteriorly and widely spaced dorsal spines, posteriorly pelvis and long dorsal and anal fins when compared to their stream/river counterpart.

5.3.4 Comparison with stickleback species pairs from southwestern British Columbia

I examined the benthic/limnetic populations from three lakes on islands in the Strait of Georgia in southern British Columbia as a comparison to the body shape

Table 11. Independent sample t-test results of the body shape, quantified by CV1 and CV2, between 7 lake- river stickleback pairs on Haida Gwaii. Frequencies of mitochondrial DNA haplotypes (Euro-North American (ENA) or Japanese (JP)) are reported (Deagle *et al.*, 1996). Frequency of the haplotype is 1, unless otherwise noted.

Population	mtDNA	N	Mean	T	P value	Mean	t	P value
			CV1			CV2		
Drizzle lk	ENA	97	-1.6	-16.6	< 0.001	-3.7	-22.4	< 0.001
Drizzle outlet	ENA	41	1.0			0.13		
Mayer lk	ENA	145	-3.6	-5.1	< 0.001	-3.9	-3.1	0.002
Gold cr	ENA	8	-1.5			-2.4		
Skidegate lk	ENA	10	-3.2	-2.6	0.014	-2.6	-5.7	< 0.001
Copper r	/	19	-2.3			-0.27		
Loon lk	ENA	9	2.7	0.5	0.62	-0.96	-2.4	0.03
Loon cr	ENA	17	2.5			0.06		
Lumme lk	0.8 JP	20	4.0	1.5	0.14	0.19	-4.1	< 0.001
Lumme cr	/	5	3.0			2.4		
Skonun lk	ENA	10	-0.75	-4.1	0.001	-3.3	-15.6	< 0.001
Sangan r	ENA	12	0.72			1.8		
Yakoun lk	ENA	20	-3.2	-12.2	< 0.001	-0.06	-0.5	0.62
Yakoun r	ENA	20	-0.2			0.09		

variation among Haida Gwaii populations. There is considerable variation between benthic and limnetic stickleback from these southern populations (Baumgartner *et al.*, 1988; Schluter and McPhail, 1992; McPhail, 1994). The benthic stickleback (Enos: n= 20; Paxton: n= 22; Priest: n= 23) represent the extreme shape along the positive loadings of the first canonical vector, comparable to the shape of Rouge Lake stickleback, a derived shape from Haida Gwaii. They have thicker peduncles, posteriad and closely spaced dorsal spines, anterior pelvis position and short median fins. The limnetic samples (Enos: n= 13; Paxton: n= 21; Priest: n= 16) have an intermediate shape (i.e., average peduncle thickness, average dorsal spine position etc) relative to the Haida Gwaii stickleback populations, surrounding the grand mean shape of CV1. I found that the benthic samples differed among lakes by 6.5- 24.7% of the total range of CV1 scores for all samples, while the limnetics differed among lakes by 2.1- 7.5% the total range of variation of CV1. The benthic and limnetic samples differed by 38.5- 55.8% the total range of CV1 scores throughout the Haida Gwaii archipelago. I found limited variation between both benthic and limnetic samples along the second canonical vector. The benthics scored slightly above the grand mean, differing by 0.8- 7.3% of the total range of CV2 scores. The limnetics scored slightly below the grand mean, and differed by 4.2- 14.7% of the range of CV2 scores. The benthic and limnetic samples differed by 10.2- 19.8% the total range of CV2 scores among all Haida Gwaii samples (figure 30).

5.3.5 Phenotypic plasticity in body shape

In the first level of assessment, I found differences between body shape of the two source populations and the experimental transplant populations in ponds. Field-caught

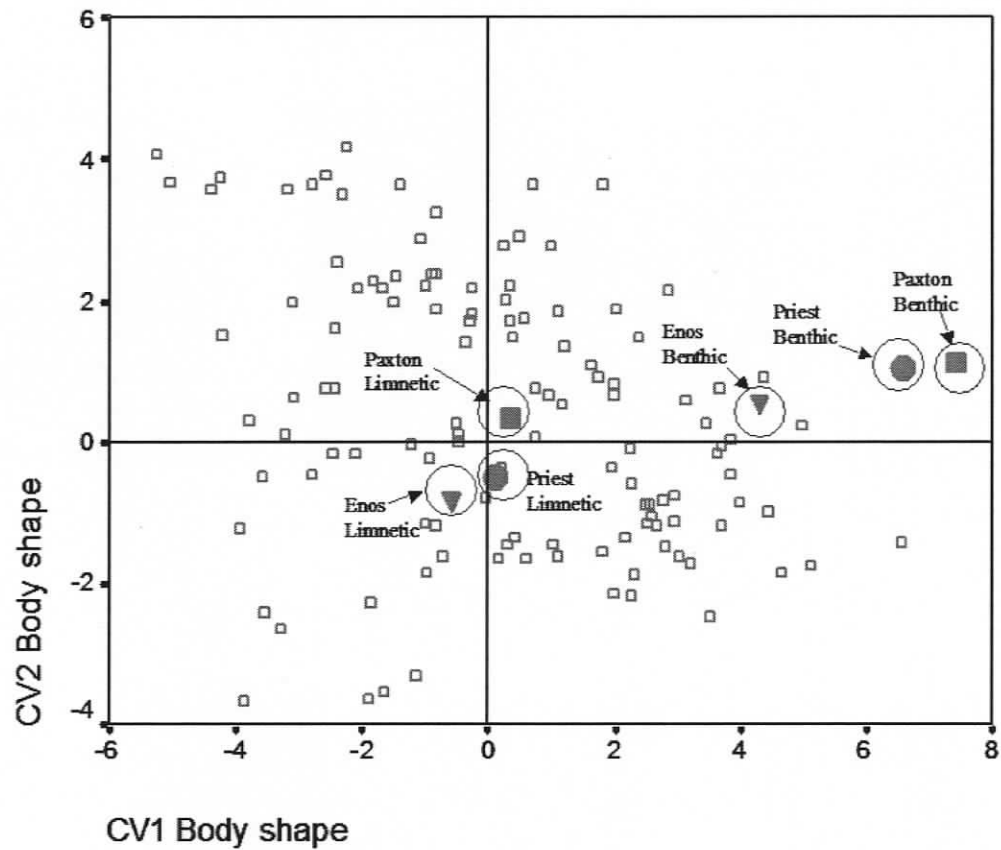


Figure 30. The position in morphospace, quantified by the first two canonical vectors (CV1: 33.4% and CV2: 21.7% of total variation), of the benthic/limnetic ecotypes from southwestern BC.

Drizzle Lake fish (n= 47) differed from the first generation from the Drizzle experimental pond (n= 44) by 8.0% of the variation of all Haida Gwaii population CV1 scores and 6.6% of all populations CV2 scores. Although the differences are marginal, stickleback from Drizzle experimental pond had higher negative loadings for CV1 and more positive loading for CV2. These differences characterize the Drizzle experimental pond stickleback as possessing smaller narrower peduncles, more anterior and widely spaced dorsal spines, posterior pelvis, longer median fins (CV1-), larger heads and deeper bodies (CV2+) compared to Drizzle Lake stickleback. The differences between Mayer Lake (n= 81) and the first generation from the Mayer experimental pond (n= 15) were 1.8% of the population range of CV1, and 3.8% of the population range of CV2 scores. The Mayer experimental pond had slightly more negative loadings for CV1 and positive loadings for CV2. Although the magnitude of the shape change differs slightly, the shape differences between the Mayer experimental pond and Mayer Lake are the same as those listed for Drizzle Lake and Drizzle experimental pond (figure 31A). These results suggest that on average there is < 6.0% of variation that can be attributed to phenotypic plasticity.

After a maximum of ten years (see methods) both recipient populations were still visibly indistinguishable from their respective source populations, and results comparable to the first generations were found. Drizzle Lake (n= 47) and Drizzle experimental pond 2003 (n= 26) differed in shape by 6.0% for the range of population CV1 scores, and 5.9% the range of CV2 scores. The shape differences for these populations are very similar to the differences found when comparing the first generation from Drizzle experimental pond. Mayer Lake (n= 81) and Mayer experimental pond 2002 (n= 30) differed by 8.5% of the range of CV1 scores and 20.2% the range of CV2 scores. After 10 years the Mayer

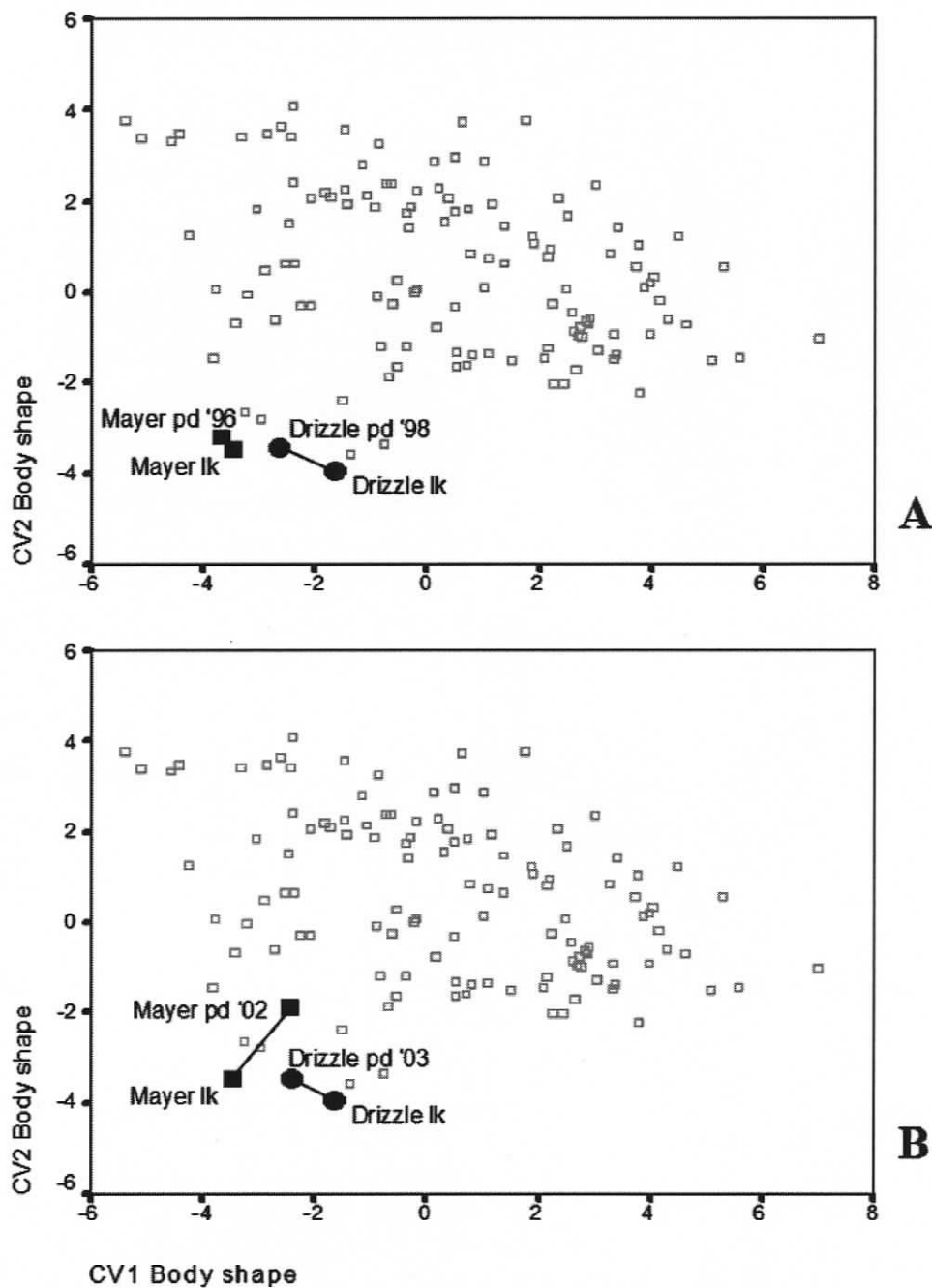


Figure 31 The difference in body shape between two experimental pond stickleback populations and their respective source populations. Figure A shows the differences between each source population and the first generation from the experimental ponds. Figure B shows the differences after a maximum of ten years.

experimental pond had more positive loadings for both CV1 and CV2, and can be characterized thicker peduncles, more posterior and closely spaced spines, anterior pelvic, shorter median fins (CV1+), larger heads and deeper bodies than the stickleback from Mayer Lake (figure 31B). When examining changes in shape over a maximum of 10 years, there is on average, < 11.0% of variation that can be attributed to phenotypic plasticity.

5.3.6.1 Body size and ecological associations

Body size can be predicted by physical and biological habitat characteristics. Smaller stickleback occur in small, shallow, stained ponds and larger stickleback in large, deep, clear lakes (PC1_lk: $R^2 = 0.08$, $p = 0.02$). Smaller stickleback are also found in habitats of high productivity and large stickleback are found in habitats of low productivity (PC2_lk: $R^2 = 0.15$, $p = 0.001$). A few highly productive lakes with small stickleback, and a few lakes of low productivity with large stickleback affect this association. The relationship between shape and volume/clarity was consistent in each geographical area (GEO_cd: $F_{1,67} = 7.3$, $p = 0.009$). Stickleback centroid size is larger when living in larger, deeper, clearer lakes in each region (figure 32). When body size, lake productivity and predation are examined, I found larger fish in habitats with salmonid predators compared to those populations primarily under bird/invertebrate predation (PRED_cd: $F_{1,67} = 9.8$, $p = 0.003$; figure 33). Body size can also be predicted by defensive condition. Populations with a large average size often have the high defensive

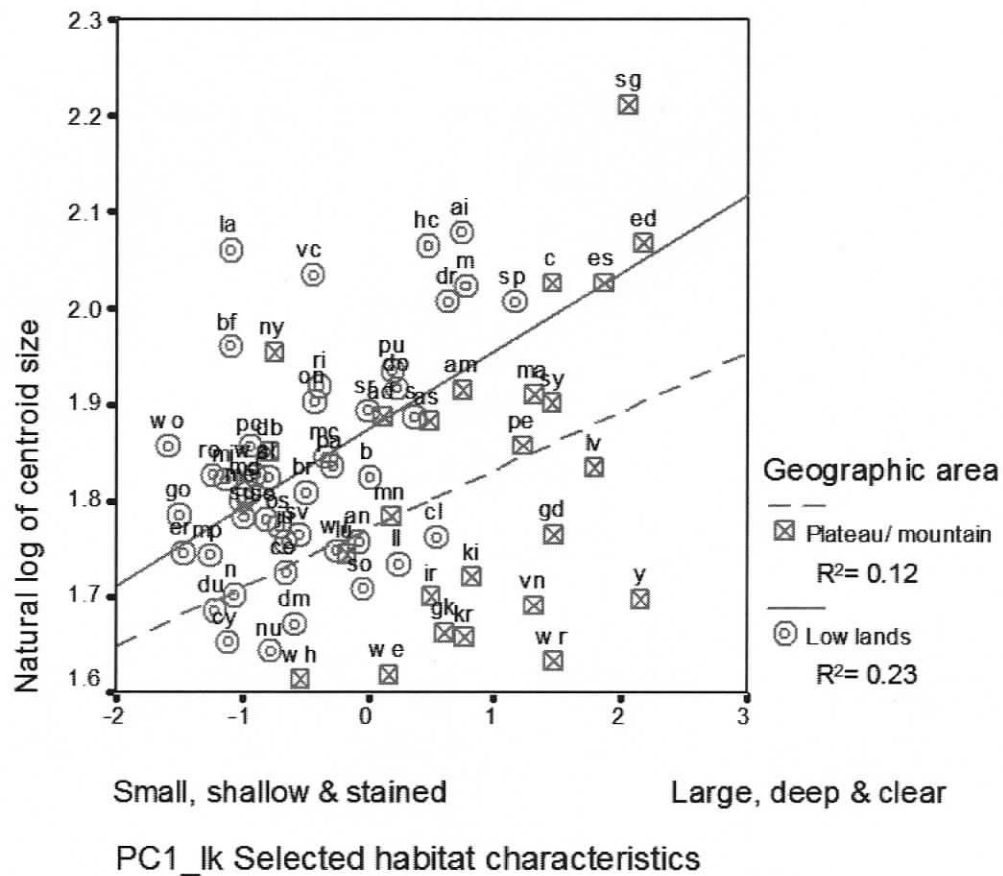


Figure 32. Relationship between centroid size, geographical area and lake volume/clarity (PC1_ik: 38.9% of total variation) for each geographical area. Lakes that are small, shallow and stained contain small stickleback, compared to large, deep clear lakes that have large stickleback. Legend for lake populations: ai= Ain, am= Amber, ad= Anderson North, as= Anderson South, an= Anser, bf= Bigfish, b= Boulton, br= Branta, ce= Cedar, cl= Clearwater, c= Coates, cy= Cygnet, dm= Dam, db= Debris, do= Down tree, dr= Drizzle, du= Duck, ed= Eden, er= Eriophore, es= Escarpment, gk= Goski, go= Gosling, gd= Gudal, hc= Hickey, ir= Irridens, ju= Juno, ki= Kiokathli, kr= Krajina, la= Laurel Pond, lv= Lower Victoria, ll= Lumme, lu= Lutea, ma= Marie, m= Mayer, mn= Menyanthes, me= Mesa, mc= Mica, mi= Middle, md= Midge, mp= Molitors Pond, n= Naked, ny= New Years, nu= Nuphar, on= Otter north, os= Ottern, pa= Parkes, pe= Peter, pc= Pontoon Center, pu= Pure, ri= Richter, ro= Rouge, se= Serendipity, sv= Silver, sg= Skidgate, s= Skonun, sl= Slim, so= Solstice, sp= Spence, sr= Spraint, sy= Stiu, su= Stump, vc= Vaccinium, vn= Van Inlet, wa= Watt, we= Wegner, wh= White Swan, wi= Wiggins, wo= Woodpile, wr= Wright, y= Yakoun.

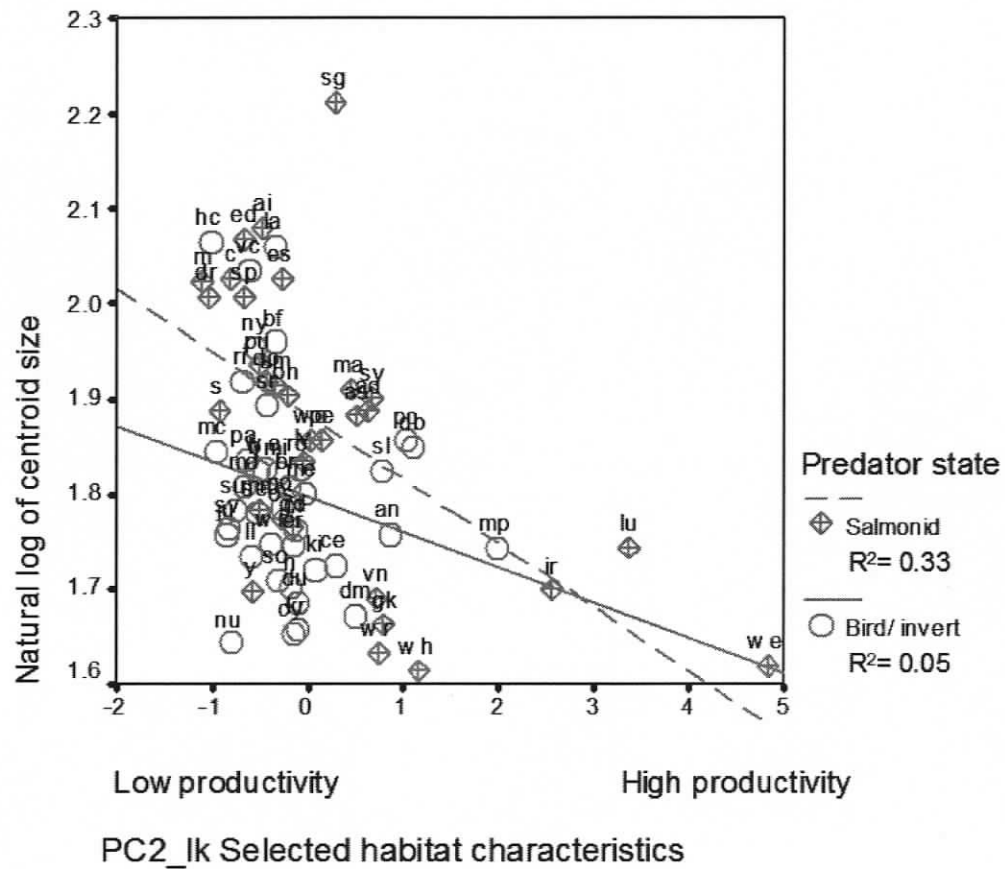


Figure 33. Relationship between centroid size, predator state and lake productivity (PC2_1k: 25.2 % of total variation). Populations subject to salmonid predation are larger, while populations subject to bird/invertebrate predation are smaller in similar habitats.

condition; mid-size populations have mid-level defensive adaptations and populations with small average size often have the low defensive condition (DEF_cd: $F_{2,61} = 4.2$, $p = 0.02$).

5.3.6.2 Body shape and ecological associations

Body shape quantified by the first canonical vector can be predicted by both physical and biological habitat characteristics. Fish with thicker peduncles, posteriorly positioned and closely spaced dorsal spines, anteriorly positioned pelvis and shorter dorsal and anal fins (high positive loadings of CV1) occur in small, shallow, stained ponds. Less divergent stickleback with high negative loadings have narrower peduncles, anteriorly positioned and widely spaced dorsal spines, posteriorly positioned pelvis and longer dorsal and anal fins occur in large, deep, clear lakes (PC1_lk: $R^2 = 0.46$, $p < 0.001$; figure 34). This relationship was consistent in each geographical region (GEO_cd: $F_{1,67} = 0.5$, $p = 0.48$). Body shape can also be predicted by the predation regime. Populations of stickleback with salmonid predators are intermediate in shape, while populations with predominantly bird/invertebrate predation have the derived shape characterized by positive loadings on the CV1 vector when found in small shallow stained ponds (PRED_cd: $F_{1,67} = 19.3$, $p < 0.001$; figure 35). An interaction effect between lake productivity and predator state indicates that as productivity increases, populations under predation from salmonids have shape characterized by the more derived positive loadings, and populations under bird/invertebrate predation have the less derived shape characterized by negative loadings along CV1 (PRED_cd*PC2_lk: $F_{1,66} = 4.4$, $p = 0.04$). The majority of lakes are of low

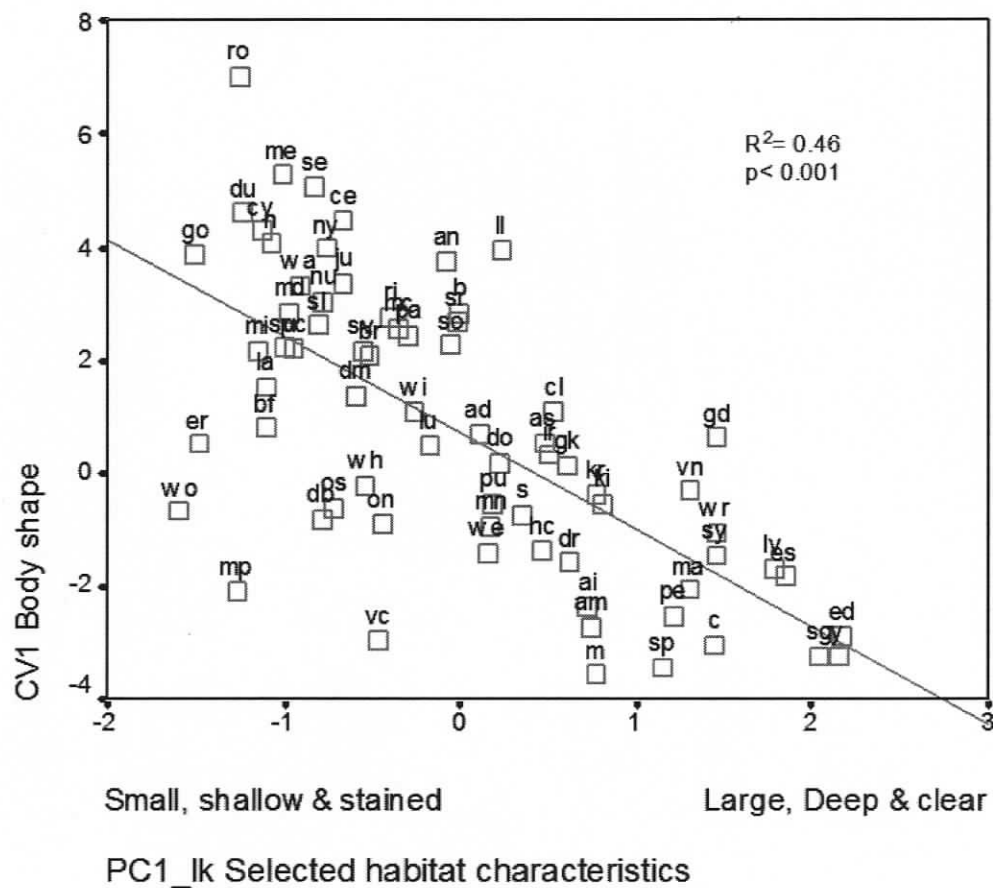


Figure 34. Relationship between body shape (CV1: 33.4% of total variation) and lake volume/clarity (PC1_lk: 38.9% of total variation). Lakes that are small, shallow and stained contain stickleback with derived body shape, compared to large, deep clear lakes which have less derived stickleback.

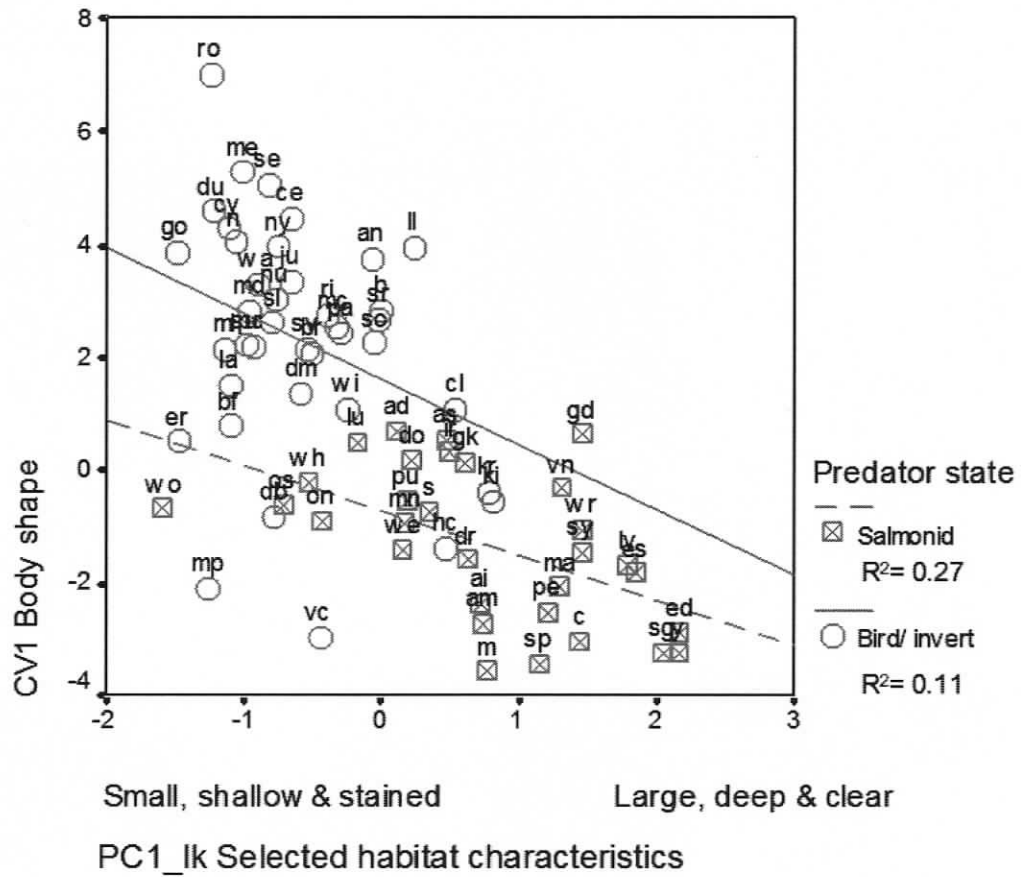


Figure 35. Relationship between body shape (CV1: 33.4% of total variation), predator state and lake volume/clarity (PC1_ik: 38.9% of total variation). Populations subject to either predation regime have less derived body shape in large, deep clear lakes.

to intermediate productivity, and the effects are driven primarily by a few highly productive lakes (figure 36). I also found that shape could be predicted by the population defensive condition. Stickleback from the low defensive category have the extreme expression of the derived shape quantified by positive loadings on CV1, mid level defenses also have positive loadings, and populations with the high defensive condition are intermediate in shape when living in small shallow stained ponds (DEF_cd: $F_{2,61} = 22.8$, $p < 0.001$; figure 37). Regardless of predation regime or defensive condition, populations in large deep lakes have the less derived shape characterized by negative loadings along the first canonical vector.

Shape quantified by the second canonical vector can also be predicted by some physical attributes of habitat. Stickleback with slightly smaller than average heads, and shallow bodies (slight negative loadings) are often in small, shallow, stained ponds and stickleback with (slight positive loadings) slightly larger heads and deeper bodies are in large deep clear lakes (PC1_lk: $R^2 = 0.08$, $p = 0.02$; figure 38). Although the relationship was significant, the majority of populations were intermediate in shape quantified by CV2. Stickleback with derived shape (high negative loadings) characterized by small heads and shallow bodies were found in habitats of low productivity and fish with less derived shape (high positive loadings) distinguished by large heads and deeper bodies are often in lakes of high productivity (PC2_lk: $R^2 = 0.25$, $p < 0.001$; figure 39). This relationship was observed when the data was partitioned by geographical area, but a significant interaction effect between productivity and geographical region suggests that the relationship is strongest in the lowlands (PC2_lk*GEO_cd: $F_{1,66} = 10.1$, $p = 0.002$; figure 40).

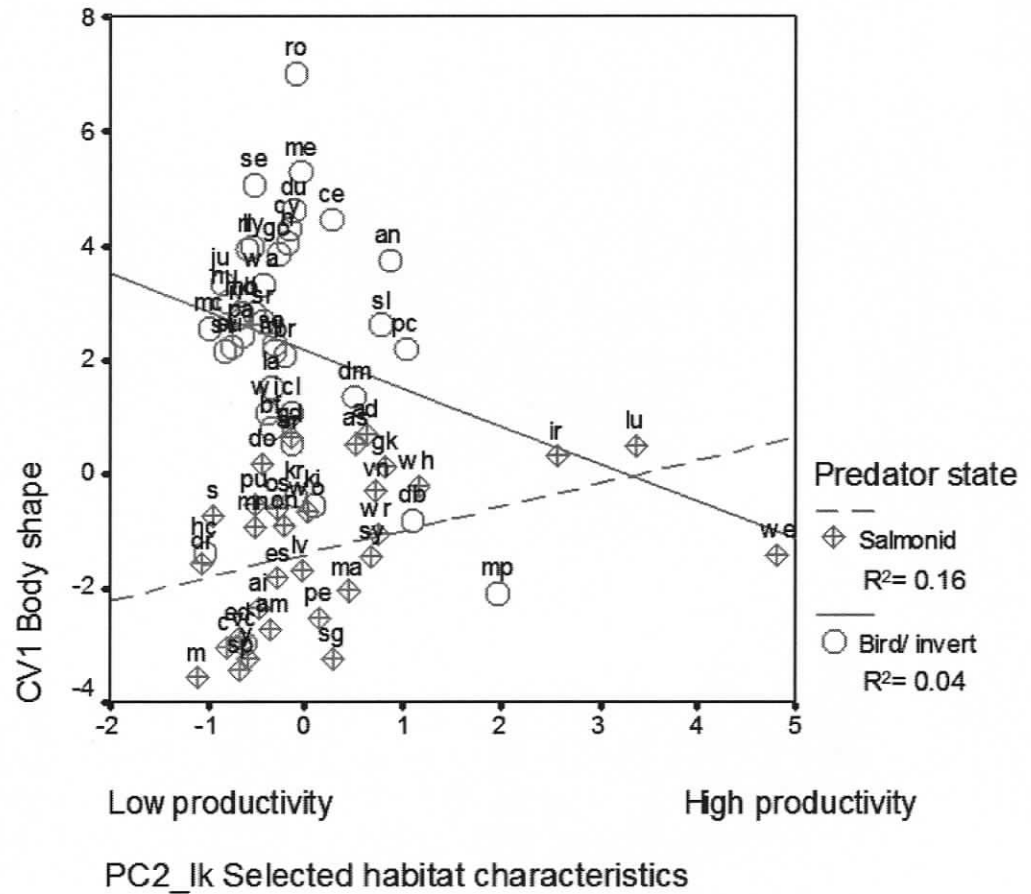


Figure 36. Relationship between body shape (CV1: 33.4% of total variation), predator state and lake productivity (PC2_1k: 25.2 % of total variation).

Populations subject to salmonid predation are more derived in body shape, while populations subject to bird/ invertebrate predation have less derived body shape in habitats of higher productivity.

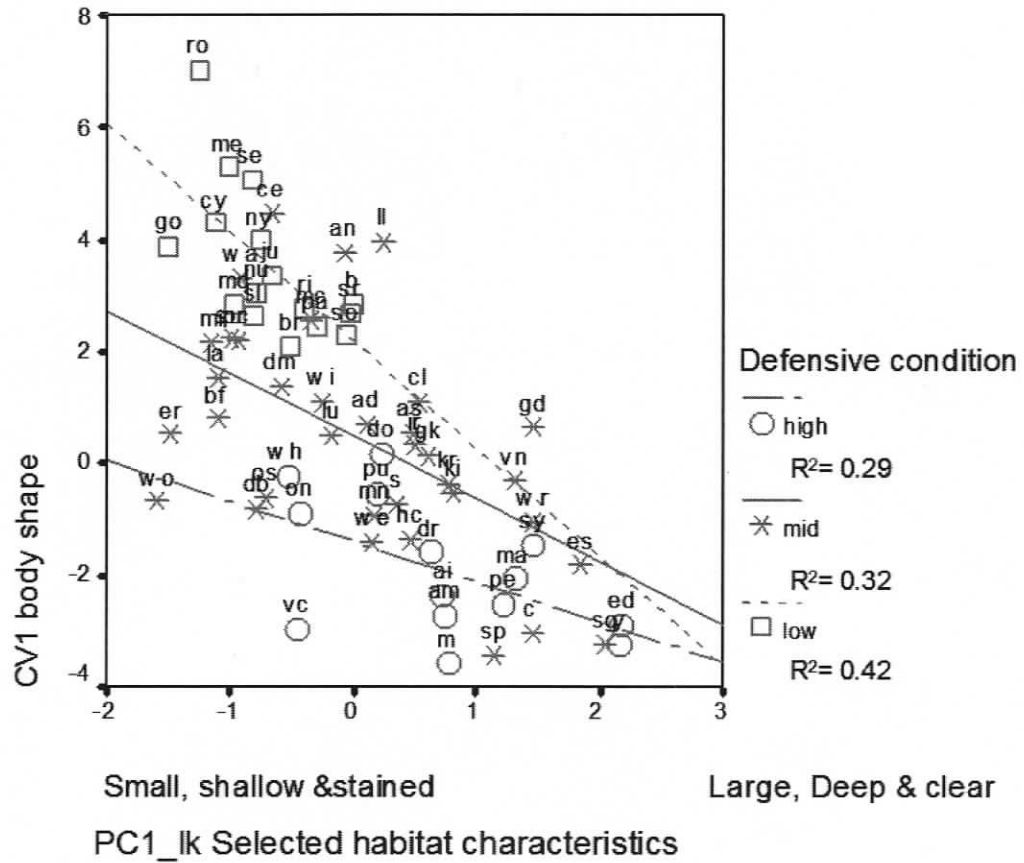


Figure 37. Relationship between body shape (CV1: 33.4% of total variation), defensive condition and lake volume/clarity (PC1_1k: 38.9% of total variation). Populations within each defensive category have less derived body shape in large, deep clear lakes.

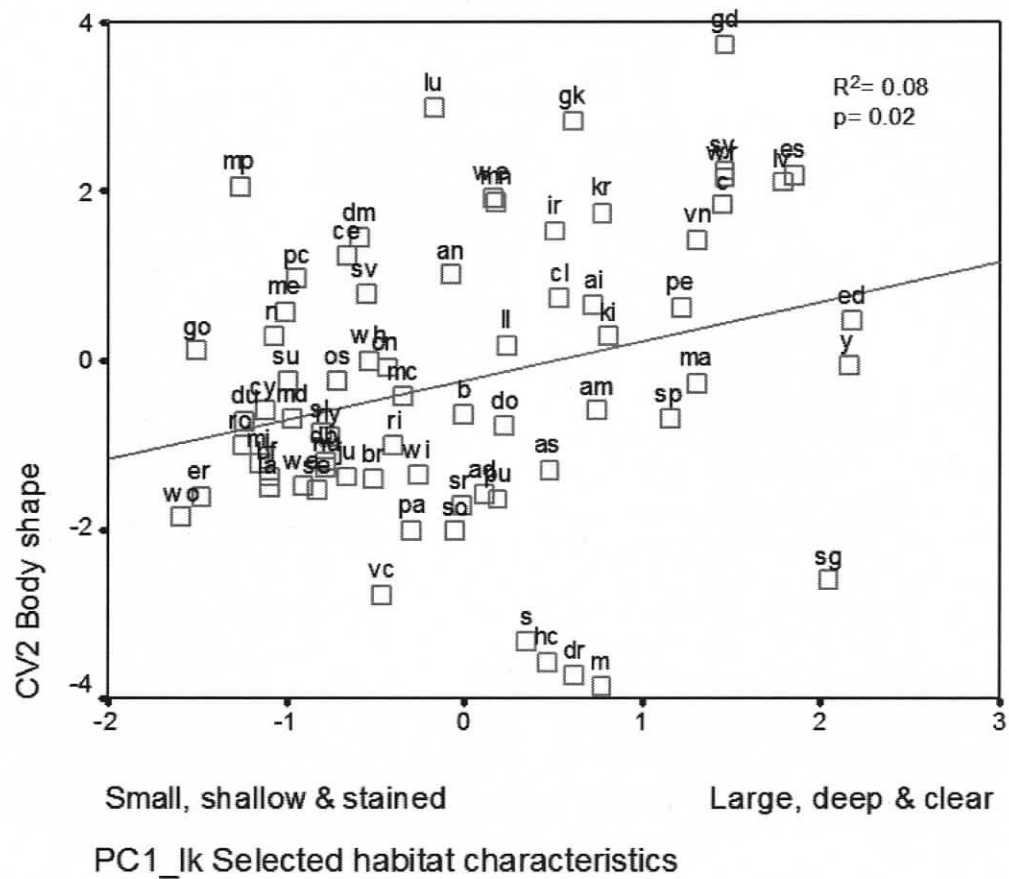


Figure 38. Relationship between body shape (CV2: 21.7% of total variation) and lake volume/clarity (PC1_1k: 38.9% of total variation). Lakes that are small, shallow and stained contain derived stickleback with small heads and elongate bodies, compared to large, deep clear lakes which have less derived stickleback with large heads and deeper bodies.

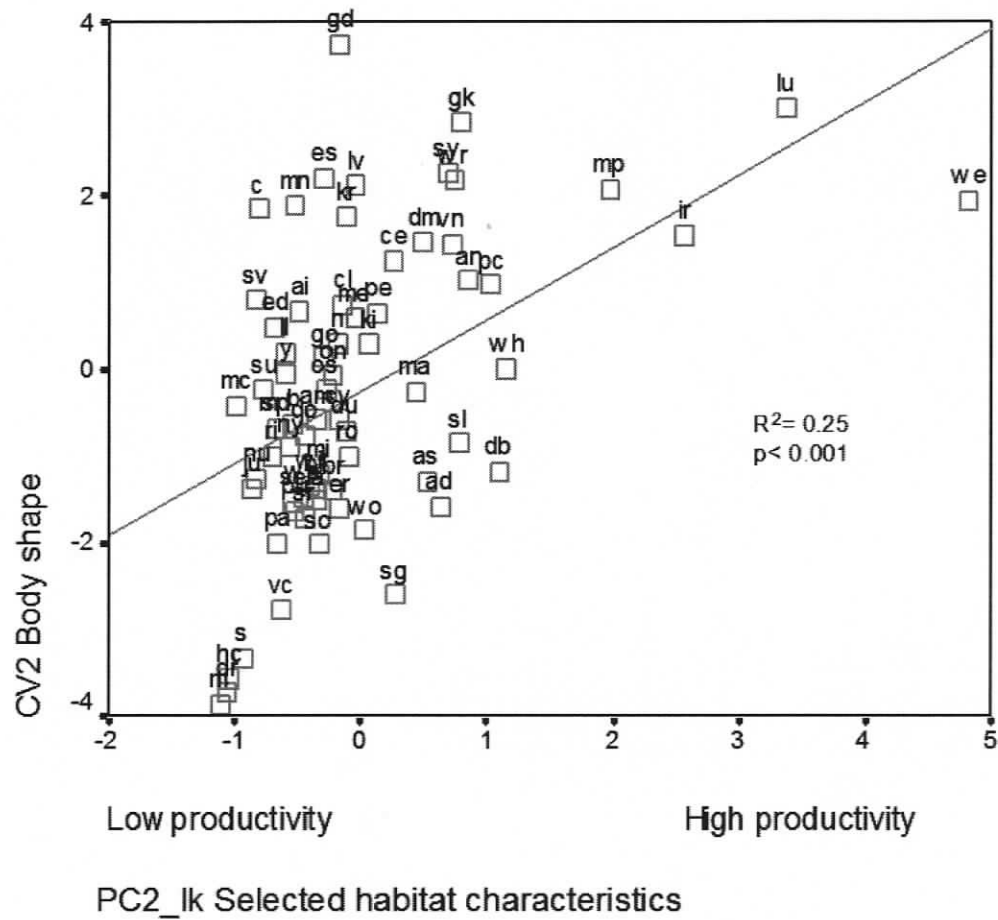


Figure 39. Relationship between body shape (CV2: 21.7% of total variation) and lake productivity (PC2_ik: 25.2 % of total variation). Lakes that are of low productivity have derived stickleback with small heads and elongate bodies compared to lakes of high productivity that support less derived stickleback with large heads and deeper bodies.

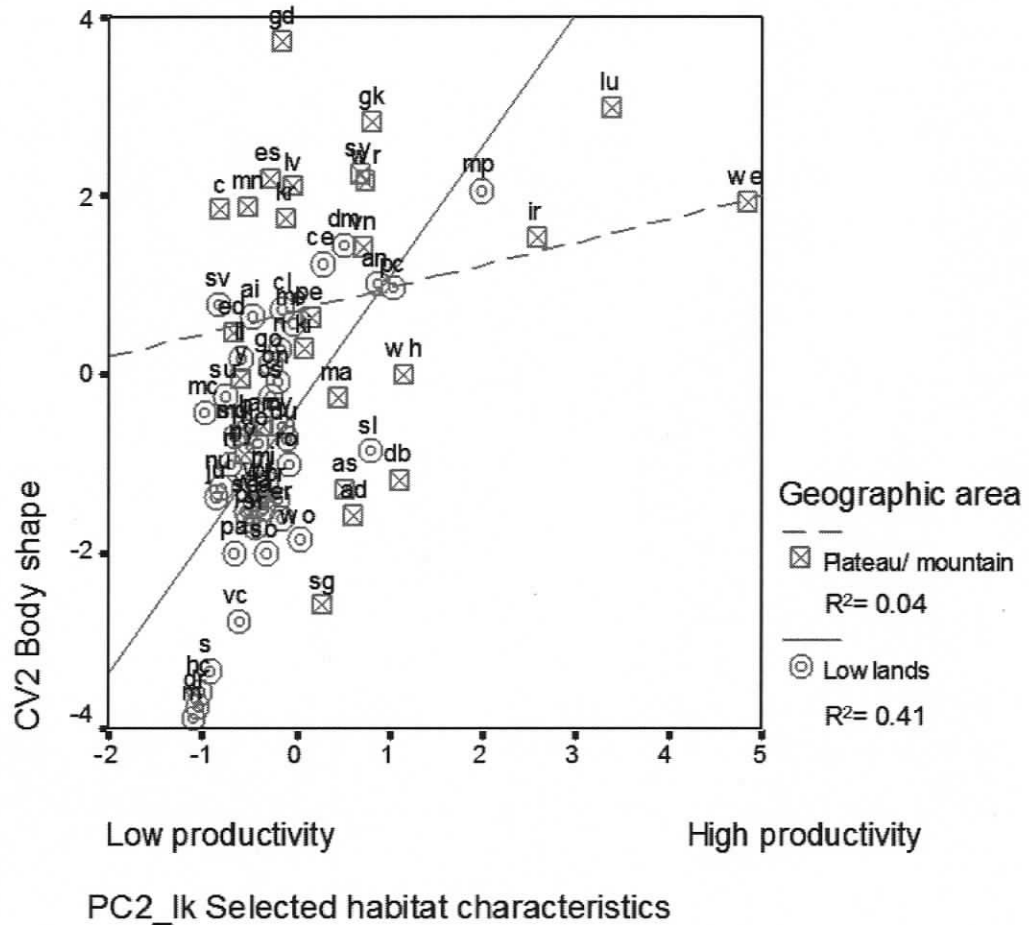


Figure 40. Relationship between body shape (CV2: 21.7% of total variation), geographic area and lake productivity (PC2_1k: 25.2 % of total variation). In each geographic region lakes that are of low productivity have derived stickleback with small heads and shallow bodies compared to lakes of high productivity that support less derived stickleback with large heads and deeper bodies. The relationship is strongest in the lowlands.

These aspects of shape cannot be predicted by predation regime, but can be predicted by defensive condition. Stickleback with the low defensive condition are intermediate in shape and populations with either the mid or high defensive condition are the more derived shape with negative loadings on CV2 when in small, shallow, stained ponds (DEF_cd: $F_{2,61} = 3.2$, $p = 0.05$). There was no statistical indication of an interaction effect (PC1_lk*DEF_cd: $F_{2,59} = 1.6$, $p = 0.2$). Therefore regardless of defensive condition stickleback populations are less derived, characterized by positive CV2 loadings when found in large deep clear lakes (figure 41).

5.3.7 Mitochondrial haplotype, shape and habitat associations in lowland populations

The ecomorphological relationships were confirmed in lowland populations with the Japanese (Trans-Pacific) mtDNA haplotype. Regardless of the phylogenetic composition of the population, I found derived fish (high positive loadings of CV1) with thicker peduncles, posterior positioned and closely spaced dorsal spines, anterior pelvis and shorter dorsal and anal fins, in small shallow stained ponds and less derived stickleback with high negative loadings (opposite characteristics) in large deep clear lakes (PC1_lk: $F_{1,34} = 19.9$, $p < 0.001$; figure 42). Shape quantified by CV2 showed an opposite relationship with volume/clarity, between each lineage (PC1_lk*mtDNA: $F_{1,33} = 5.0$, $p = 0.03$), suggesting that populations with the Japanese lineage present, had a more derived shape (smaller heads and shallower bodies; negative CV2 loadings) in smaller ponds, and a less derived shape (larger heads and deeper bodies; positive CV2 loadings) in larger lakes.

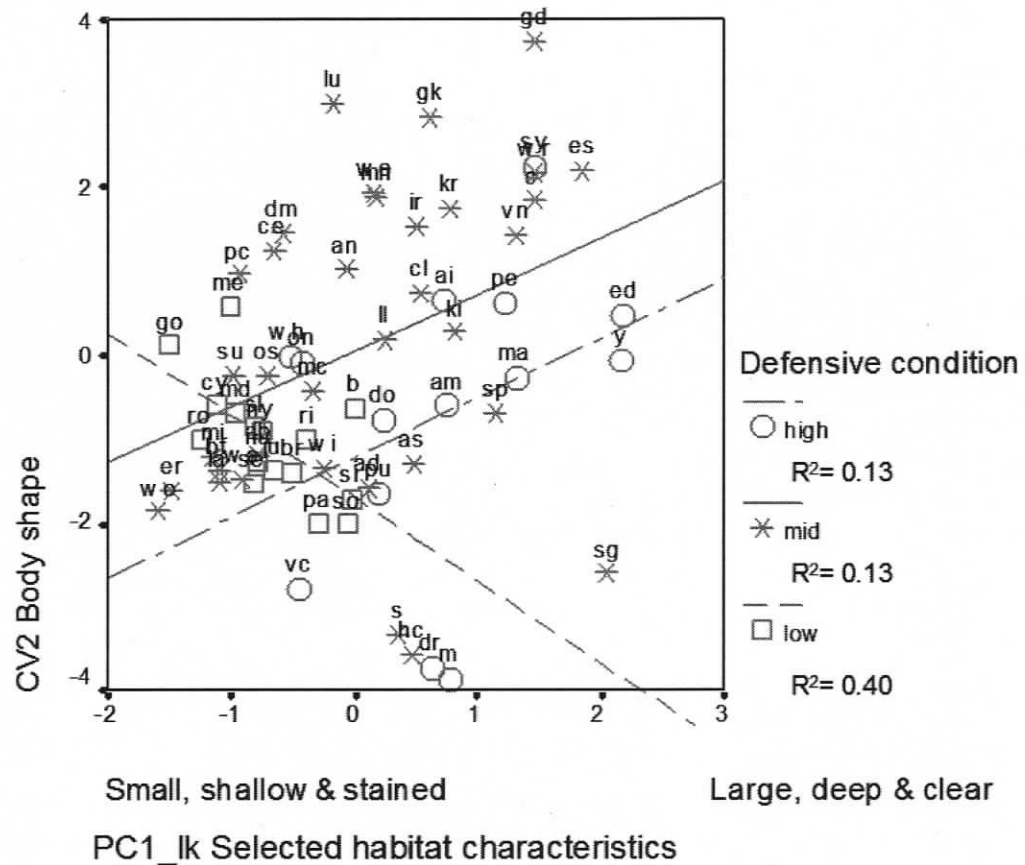


Figure 41. Relationship between body shape (CV2: 21.7% of total variation), defensive condition and lake volume/clarity (PC1_ik: 38.9% of total variation). Populations with high and mid level defensive adaptations have less derived body shape with large heads and deeper bodies in large, deep clear lakes. Populations with low-level defenses have derived shape with small heads and shallow elongate bodies in large, deep clear lakes.

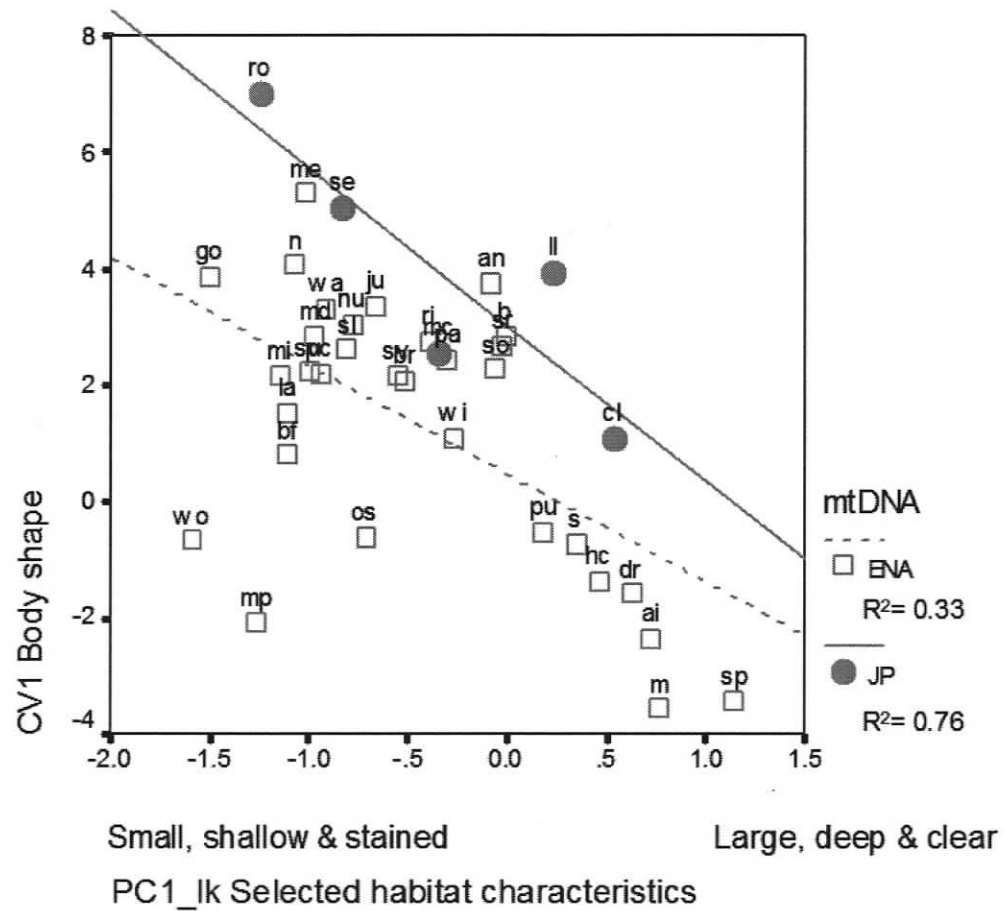


Figure 42. Relationship between body shape (CV1: 33.4% of total variation), mtDNA haplotype and lake volume/clarity (PC1_1k: 38.9% of total variation). Populations within each haplotype have less derived body shape in large, deep clear lakes.

Lowland populations that are monomorphic for the Euro-North American lineage were intermediate in shape in smaller ponds, but more derived (negative CV2 loadings) in larger lakes (figure 43). I also determined that populations with either lineage, had derived shape (high negative CV2 loadings) in habitats of low productivity and less derived shape (high positive CV2 loadings) in lakes of high productivity (PC2_1k: $F_{1,34} = 23.0$, $p < 0.001$), although the relationship was strongest in lowland populations that are monomorphic for Euro-North American stickleback (figure 44).

5.4 Discussion

The discriminant function analysis on partial warp scores and the uniform component, allowed me to quantify the body shape of Haida Gwaii stickleback populations. It has previously been established that the phenotype of the modern marine stickleback has remained relatively unchanged since the Miocene and are relatively homogenous geographically (Bell, 1977; Bell and Foster, 1994; Walker and Bell, 2000), and the trachurus body shape therefore represents the ancestral or less derived condition. My marine population (Entry Point) had extreme negative loadings for CV1 and high positive loadings for CV2 and can be characterized as having larger heads, deeper bodies, narrower peduncles, anterior and widely spaced dorsal spines, posterior pelvis and longer dorsal and anal fins. If the anterior position of the dorsal spines places the center of inertia forward in relation to the position of maximum depth, approximately

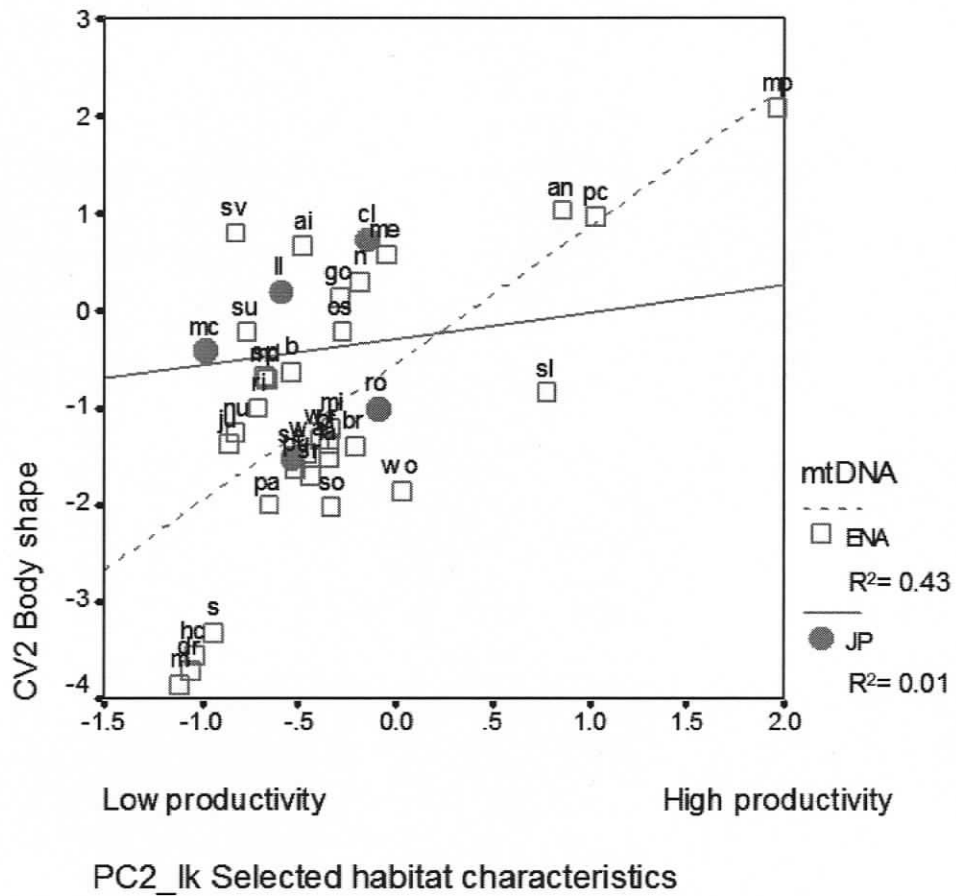


Figure 44. Relationship between body shape (CV2: 21.7% of total variation), mtDNA haplotype and lake productivity (PC2_Ik: 25.2 % of total variation). Populations within each haplotype are more less derived in lakes of high productivity.

one third of the stickleback standard length, then this would enhance stability during motion (Aleev, 1977). Combined, these attributes typify a stickleback with the most effective shape for reducing components of drag (Aleev, 1977; Vogel, 1981; Webb, 1982), which have presumably evolved in association with the migratory lifestyle of anadromous fish (Wootton, 1976; Taylor and McPhail, 1986; Walker, 1997). The freshwater stickleback populations in the northern hemisphere, including those on Haida Gwaii, were initially colonized by the marine form, and have diversified since the retreat of the Pleistocene glaciers, 10 000 years ago (Münzing, 1963; Hagen, 1967; McPhail and Lindsey, 1970; Moodie and Reimchen, 1976; Schluter and McPhail, 1992). The body shape of freshwater populations quantified by canonical vectors are scattered around two orthogonal axes adjacent to the marine form. The populations with the most derived body shape, quantified along the first canonical vector have thicker peduncles, posteriad and closely spaced dorsal spines, anterior pelvis and shorter dorsal and anal fins. If the posterior position of the dorsal spines place the center of inertia further back on the body in relation to the position of maximum depth, then it has been suggested that characteristics of this body form are adaptations for increased maneuverability (Aleev, 1977; Domenici and Blake, 1997; Walker, 1997). The second canonical vector allows us to quantify another derived stickleback morphology: freshwater stickleback with small heads, and shallow elongate bodies. This shape is also effective for reducing components of drag associated with the open water environment, by the laminarization of shape, which reduces wake formation (and drag) along the posterior regions of the stickleback (Aleev, 1977; Vogel, 1981; Walker, 1997).

Although there was variability in the body shape of each stream population, the differences between lake and stream stickleback were in the expected direction. Throughout their holarctic range, the majority lake-dwelling stickleback have body shape (slim bodies) and trophic adaptations for open water existence (smaller mouths and more and longer gill rakers) compared to stream-dwelling populations (Hagen and Gilbertson, 1972; Gross and Anderson, 1984; Taylor and McPhail, 1986; McPhail, 1994). In general, stream stickleback have larger heads, deeper bodies, thicker peduncles, posteriad and closely spaced dorsal spines, anterior pelvic and smaller median fins (dorsal and anal) in comparison to their lake counterpart.

Water velocity is likely to be an aspect of a stickleback's environment that exerts a selective force on body morphology (McGuigan *et al.*, 2003). The characteristics such as the deeper body and caudal area in stream fish could facilitate a greater proportion of red muscle mass and are an adaptation for sustained swimming (Swain and Holtby, 1989; McGuigan *et al.*, 2003; Hendry and Taylor, 2004). Increased body depth is thought to result in an increased cost of sustained swimming through drag (Webb, 1984). However, the cost of frictional drag associated with deeper bodies might be offset by the benefits of a deep body in terms of reduced recoil ("tail wagging the head") (Webb, 1982). The slightly more posterior center of inertia suggests that stream stickleback are better adapted for manoeuvring in a flowing environment (Aleev, 1977; Hendry and Taylor, 2004). The existence of similar patterns of variation in several aspects of body shape, for multiple pairs of lake-stream populations, is persuasive evidence that natural selection is responsible for the association between morphology and habitat (McPhail, 1994).

There are two evolutionary models that may explain the nature of the divergence in these lake-stream pairs. Most lakes on Haida Gwaii were initially colonized within the last 10 000 years (Moodie and Reimchen, 1976), indicating a relatively short time frame under which the divergence could occur. The historical model postulates that the lake and stream forms diverged separately in two habitats, and then came into secondary contact after the period of divergence (McPhail, 1994). The nature of the divergence, alternatively hypothesized by the parallel model, suggests the *in situ* evolution of the lake stream pairs (McPhail, 1994), when a single population colonizes a lake-stream system. The divergence in phenotype occurs as the response to differing selection pressures on either side of the ecotone, wherever the stream enters or leaves the lake (Reimchen *et al.*, 1985; McPhail, 1994; McKinnon *et al.*, 2004). Recent work by Hendry and Taylor (2004) suggests that limitation of gene flow between lake-stream stickleback allows some adaptive divergence, which then causes a reduction in gene flow, and further divergence until some equilibrium is reached and two distinct populations have evolved. The variable differences I found between each lake-stream pair independently and identical mitochondrial DNA from five of the lake-stream pairs (Deagle *et al.*, 1996), lend support to this hypothesis. There are different interpretations for the origin of the lake-stream pairs that only fine scale molecular analysis can resolve.

The investigation using the two experimental ponds provides insight into the nature of the divergence among Haida Gwaii stickleback populations. The differences in body shapes observed among populations in this study may reflect genetic adaptations, phenotypic plasticity or some combination of the two. A definition of phenotypic plasticity is a change in the expressed phenotype of a genotype as a function of the

environment (Scheiner, 1993; DeWitt and Scheiner, 2004). Explicitly, alternative alleles or their products react differently to the environment; those that are favourable persist while others perish. Cichlid fishes have a wide array of trophic morphological characters, which can be partially explained by phenotypic plasticity (Meyer, 1987; Klingenberg *et al.*, 2003). A slight alteration of growth rates, due to availability and abundance of prey types during development, can have dramatic effects on phenotype at various life stages and may also influence mature phenotypes (Liem, 1973; Alberch *et al.*, 1979; Meyer, 1987). These results suggests that plasticity may be a form of inertia against speciation (preserve gene frequencies) (Meyer, 1987), and if applicable, the stickleback species complex may be more phenotypically plastic, which may account for the relatively conserved nature of body shape observed in these populations.

Stickleback from the experimental ponds on Haida Gwaii were very similar in appearance and shape to their parental populations, but I detected 1-21 % of the variation in body shape among the Haida Gwaii stickleback populations that can be attributed to phenotypic plasticity. The experimental ponds were the ecological opposite of the parental populations' habitat (small, shallow, clear-water ponds, with insect predators vs. large, dystrophic lakes, with the full suite of predators) and any heterochronic changes similar in magnitude to those observed in cichlids (Meyer, 1987) would have been detected in the mature specimens I analyzed. I detected small differences between the first generations and their respective source populations that likely represent phenotypic plasticity, rather than genetic differentiation. The possibility exists that there were gene frequency changes within the first generation, that lead to this new phenotype. However, this seems unlikely given the similarity in body shape within each experimental

population after a maximum of 10 years. I presume these differences represent a phenotypically plastic response to the extreme shift in ecological habitats (source lakes to experimental ponds).

There were slight differences in the magnitude of shape change (reaction norms) between the two experimental populations and their parental populations. A reaction norm can be defined as the range of phenotypes expressed by a single genotype across an array of environmental conditions (Stearns *et al.*, 1991). The phenotypic response is a property of a genotype, summarizing the direction and amount of plasticity that it is able to express (Gotthard and Nylin, 1995). Mayer and Drizzle Lakes were originally colonized by stickleback of the Euro-North American lineage (Deagle *et al.*, 1996), but genomic differences between the two populations are unknown. Differences between these population's genotypes in the plastic response to heterogeneous environments (Gotthard and Nylin, 1995) may have contributed to the different magnitude of reaction norms detected in the first generations and the opposite reaction norms between experimental populations after a maximum of ten years. The observed phenotypic responses may also reflect selection or plasticity affected by very subtle ecological differences between experimental ponds. However, the differences in body shape, between each source and experimental pond were relatively small, and I do not have any direct ecological evidence to support this hypothesis.

Among stickleback populations of southwestern British Columbia, common garden and breeding experiments provide strong evidence for a genetic effect on major phenotypic traits (McPhail, 1977, 1984, 1992, 1993). Day *et al.* (1994) experimentally determined that gill raker number was non-labile, but there was moderate plasticity in

snout length and head depth. The observed plasticity was in the expected direction for increased feeding performance, however the magnitude of the plasticity was not enough to account for observed variation among the southern populations (Day *et al.*, 1994; Day and McPhail, 1996). I have determined that on average less than 11% of the variation in Haida Gwaii populations can be attributed to plasticity.

Shape divergence between benthic and limnetic species pairs from lakes in southern British Columbia, is considerably less than the variation observed throughout the Haida Gwaii archipelago. Within each of the species pairs I detected differences in body shape ranging from 10-56% of the range of variation among Haida Gwaii samples. The shape of benthic ecotypes, which were more similar to each other than to their sympatric limnetic counterparts, was adapted for manoeuvrability (Aleev, 1977; Domenici and Blake, 1997; Walker, 1997) and resembled stickleback populations from benthic habitats on Haida Gwaii (Reimchen *et al.*, 1985). The limnetics were also more similar to each other in body shape and were intermediate in shape (less divergent from the common ancestor) compared to the variation among Haida Gwaii populations. Previous work, using traditional morphometrics, has characterized each ecotype within these sympatric populations, and lends support to my results. The benthics are deep bodied, with few short gill rakers, wide mouths and short broad snouts, adaptations for foraging on or near the bottom. The limnetics are slim bodied, with numerous long gill rakers, narrow mouths and long slender snouts, all of which are adaptations for a planktivory (Bentzen and McPhail, 1984; Lavin and McPhail, 1985; Baumgartner *et al.*, 1988; McPhail, 1993, 1994). The maintenance of this example of parallel sympatric character displacement suggests that divergent selection through adaptation to alternate

environments within a single habitat is strong enough to maintain morphological differences (Moodie and Reimchen, 1976; Schluter and McPhail, 1992; Rundle *et al.*, 2000; Schluter, 2001).

The primary criterion for supporting a sympatric pair in a given area is the presence of two trophic habitats between which divergent selection could operate (McPhail, 1993). Given the mild climate in southern British Columbia, compared to Haida Gwaii, there may be more niche space in the southern lakes (as a function of greater productivity) which allow for the maintenance of these ecotypes (McPhail, 1993, 1994). Wider niches may permit the coexistence of two ecotypes through divergent selection on greater phenotypic variation (in the population as a whole), if the variation is controlled to a significant extent by the adaptive diversity of the niche (Van Valen, 1965). I compared the variance in body shape among the ecotypes of the southern populations, to the variance among the Haida Gwaii populations (data not reported), and did not find any significant differences between them. My results confirm the hypothesis that directional selection is responsible for the evolution of more derived body shape morphologies when stickleback colonize habitats with extreme selective landscapes.

Population mean body size of Haida Gwaii stickleback was correlated with physical and biological habitat characteristics. Smaller stickleback occur in small, shallow, stained ponds, and larger stickleback occur in large, deep, clear lakes. Differences in adult body size can be the product of a number of factors such as maximum lifespan (Reimchen, 1992a; Baker, 1994), habitat productivity (Giles, 1987; Wootton, 1994), and sampling error due to small sample size. Nevertheless, size has a genetic component (McPhail, 1977, 1993; Lavin and McPhail, 1985) and has been used

as a discriminating character among populations (Moodie and Reimchen, 1976; Reimchen *et al.*, 1985). Manoeuvrability has been defined as the minimum space required for a turn at a given speed and agility relating to the rate at which a turn can be initiated (Norberg and Rayner, 1987). My results suggest that smaller adult body size could be the result of selection for increased manoeuvrability in the littoral zone, and the large size an adaptation for increased performance in the open water environment (Webb, 1982; Walker, 1997).

I found small stickleback in habitats of higher productivity, and large stickleback in habitats of low productivity. This result is consistent with what has previously been reported for stickleback populations in western North America (Moodie and Reimchen, 1976; McPhail, 1977; Moodie, 1984; Reimchen, 1991b, 1992a), but the opposite for what has been reported in northern Europe (Giles, 1987; Wootton, 1994). The majority of stickleback populations throughout their holarctic distribution are typically 30-70mm standard length and live for 1-2 years (Wootton, 1976, 1984; Reimchen, 1992a; Baker, 1994). However, there are populations on the west coast of North America with exceptionally large, (occasionally >100mm SL) (Moodie, 1972a; Moodie and Reimchen, 1976; Bell, 1984) and long-lived (≥ 8 years) stickleback (Reimchen, 1992a). The physical differences between habitats on Haida Gwaii and in Europe, could explain the size-productivity relationships found in these areas. The large-bodied stickleback from Haida Gwaii are found in dystrophic lakes and do not appear to be a result of accelerated growth but rather prolonged sub-adult growth (Reimchen, 1992a). The near constant growth rates, and life history characteristics in the European populations, possibly reflect the

higher and less variable levels of habitat productivity, compared to the extreme habitats on Haida Gwaii (Giles, 1987; Baker, 1994; Wootton, 1994).

The high productivity lakes on Haida Gwaii are also of intermediate volume, and the small body size of the stickleback populations could be an adaptation to foraging in the littoral zone. Species diversity is lowest in lakes of low productivity, peaks at intermediate productivity, and then declines in lakes of high productivity (Abramsky and Rosenzweig, 1984; Chase and Leibold, 2002). The encounter rate of stickleback and their prey in these high-productivity, low-diversity habitats is influenced by the density and structural complexity of the vegetation in the areas they search for food, accounting for the small body size (Hart and Gill, 1994).

On Haida Gwaii, the atypically large-bodied stickleback live in lakes with low productivity (Moodie, 1972a; Moodie and Reimchen, 1976; Reimchen, 1992a, 1994). These lakes are also have a large volume and the populations feed primarily on copepods (Reimchen *unpublished data*), which often have a patchy distribution (George, 1981; Pinel-Alloul *et al.*, 1988). The large body size could be an adaptation to increased feeding performance in this open-water low productivity habitat with widespread patches of food.

Populations with salmonid predation have larger mean body sizes, and robust defensive adaptations compared to populations subject to bird/invertebrate predation. Large body size could be an adaptation for prolonged flight response (Moodie, 1972a; Walker, 1997) as well as working in combination with the defensive apparatus to provide refuge from gape limited predators (Hoogland *et al.*, 1957; Moodie, 1972b; Reimchen, 1988, 1990, 1991b). The small size and reduction in bony armor in populations subject to bird/invertebrate predation could be an adaptation to increase manoeuvrability, increasing

the probability of escape from these predators (Reimchen, 1980, 1992a, 1994; Webb, 1982; Bergstrom, 2002b).

Body shape was correlated with the multivariate measures of lake habitat. The associations were similar in both the lowland and plateau/mountain regions, and between divergent lineages in the lowland region, indicating the extensive parallel evolution of body shape through adaptation to the hydrodynamic landscape imposed by habitat. Stickleback predominantly characterized with (CV1-) smaller narrower peduncles, anteriorly and widely spaced dorsal spines, posteriorly pelvis, longer dorsal and anal fins reside in large, deep, clear lakes through to stickleback with (CV1+) thicker peduncles, posteriorly and closely spaced dorsal spines, anteriorly pelvis, smaller dorsal and anal fins which inhabit small, shallow, stained ponds. Zooplankton are the primary food source in large deep lakes (Hart and Gill, 1994), and their distribution is often patchy in the open water environment (George, 1981; Pinel-Alloul *et al.*, 1988). Therefore stickleback in such habitats would have to constantly swim to find food. The body shape of stickleback found in large, deep lakes, is conducive to lower pressure drag than deep bodies during steady swimming and should therefore increase open water foraging performance (Webb, 1984; Walker, 1997). Long median fins of the streamlined stickleback in the marine and open water habitats suggests stabilizing selection has maintained this less derived condition in freshwater stickleback that forage in open water (Walker, 1997). However, large fins would increase drag associated with frictional forces during steady swimming. Stickleback in these environments presumably minimize drag by cruising through the water column with their median fins collapsed (Walker, 1997). Copepods are not conspicuous or passive drifters, instead selection has favoured a transparent body

(Johnsen and Widder, 2001) and the ability to accelerate quickly once detected in order to escape predation (Browman *et al.*, 1989; Trager *et al.*, 1994; Lenz and Hartline, 1999).

The clarity of water often associated with large deep lakes would only confound the sticklebacks' ability to identify transparent food items from particles in the water column (Strickler *et al.*, 2005). The large median fins in these populations effectively increase caudal depth, without the additive affects of drag associated with a thick caudal peduncle (Walker, 1997), thus greatly increasing acceleration performance, facilitating capture of elusive prey.

Small shallow ponds differ in prey availability and are very heterogeneous compared to the uniformity of the open water environment (Hart and Gill, 1994). The larvae and pupae of the Chironomidae are the primary food source in littoral habitats (Wootton, 1994), and the rate at which stickleback encounter prey is strongly influenced by the density and structural complexity of vegetation through which they must search (Hart and Gill, 1994). Benthic prey are relatively sedentary and stickleback acceleration during strike is considerably less compared to the attack on open water food items (Walker, 1997). The thick peduncles and short median fins of populations found in small shallow ponds potentially compromise acceleration due to a substantial increase in inertial resistance with little increase in thrust (Walker, 1997). Therefore directional selection has favoured the derived body shape (CV1+), with a posterior center of inertia in populations found in these habitats as adaptations for increased manoeuvrability to facilitate feeding in the structurally complex, shallow ponds (Webb, 1982, 1984; Taylor and McPhail, 1986; Walker, 1997).

Although the association between shape and lake productivity supports the idea of less derived shapes in habitats similar to the ancestral marine environment (with regard to productivity), there are some populations whose shape counters this argument. The relationship suggests that stickleback with the more derived shape quantified by negative CV2 loadings (i.e., smaller heads and shallower elongate bodies) are found in lakes of low productivity, and the less derived attributes of shape quantified by positive CV2 loadings (i.e., larger heads, deeper bodies) characterizes fish in lakes of high productivity. This again suggests the possibility of a relationship between the hydrodynamic constraints on body shape associated with prey availability in variable habitats. Prey species diversity is lowest in lakes of low productivity (Abramsky and Rosenzweig, 1984; Chase and Leibold, 2002). Mayer and Drizzle are two large, deep, dystrophic lakes with unusually large bodied, long-lived stickleback inhabitants that feed primarily on copepods (Moodie, 1972a; Moodie and Reimchen, 1976; Reimchen, 1992a, 1994). I found that these populations have the more derived shape (CV2-), with small heads and elongate shallow bodies. It is possible that directional selection for increased feeding performance for a low abundance patchily distributed food source in open stained waters, has led to the evolution of this derived form. These lakes also have intense salmonid predation.

I categorized two basic predator assemblages, salmonid and bird/invertebrate. However, there are bird and invertebrate predators in most lakes, including those that contain salmonids. The absence of salmonids in lakes on Haida Gwaii was usually associated with poor drainage or extremes in water chemistry (Reimchen, 1994; but see Jones *et al.*, 2003). Even these localities may occasionally be visited by anadromous

salmonids during periods of high runoff (Reimchen, 1992c). Given the taxonomic complexity of predation regimes among each habitat type, it is difficult to accurately estimate predation intensity (Reimchen, 1994). Nevertheless, previous investigations have indicated an evolutionary response of defensive phenotypes to differential predation regimes (Hagen and Gilbertson, 1972; Moodie, 1972b; Moodie and Reimchen, 1976). I found body shape could be predicted by predation regime. Throughout Haida Gwaii, stickleback populations subject to salmonid predation typically were intermediate in shape in small shallow stained ponds, but had the characteristics of the less derived shape (streamlined; CV1-) in large deep clearer lakes. Populations subject to primarily bird/invertebrate predation often had the derived thick peduncles and posterior center of inertia (CV1+) in small shallow ponds, but also had the characteristics of a more streamlined shape (anterior center of inertia, narrower peduncle and longer median fins) in deeper lakes. This association is similar to what was observed in the Alaskan stickleback populations, where the presence of predatory fish species had an effect on fin length but not body depth, suggesting that deep bodies compromise acceleration performance (Walker, 1997).

The body shape/predator associations are consistent with pursuit efficiencies of the predation regime. Salmonids and piscivorous insects have a high pursuit efficiency, while birds have a low pursuit efficiency (Reimchen, 1994). The body shape of populations under salmonid predation supports the notion of increased probability of survival with improved fast start performance (Reimchen, 1992b; Bergstrom, 2002b). Increasing posterior body depth is expected in escape specialists, as the thrust generated during the c-start (burst acceleration) is proportional to the caudal depth (Walker, 1997).

However, stickleback from deep lakes forage in open water (Hart and Gill, 1994; Wootton, 1994), and if these populations were to evolve deep caudal bodies in response to selective predation, this would compromise open water foraging performance because of increased friction drag due to the large surface area (Walker, 1997). Therefore, natural selection favours stickleback with a narrow posterior area with long median fins. This can perhaps account for the extreme body shape of the Mayer and Drizzle lake stickleback. The derived shape of populations with primarily bird/invertebrate predators is expected of manoeuvring specialists (Walker, 1997). The ability to make rapid turns is paramount in avoiding detection and escaping from large predatory diving birds such as loons, which typically search for prey on a linear path (Reimchen and Douglas, 1980). Perhaps the posterior position of the dorsal spines, characteristic of the derived body shape would increase the probability of post capture escape from piscivorous insects (such as odonate nymphs), but there is only limited data to support this (Reimchen, 1980).

There was an interesting interaction effect between predator state and productivity. Populations with bird/invertebrate predation had the less derived shape (CV1+) in habitats of high productivity, while populations with salmonid predators were more intermediate in shape. An outlier, Mollitors Pond, influenced the results of bird/invertebrate populations. This ephemeral habitat with high productivity has the potential for introgression from anadromous stickleback, which may have influenced body shape in this sample. The intermediate shape of populations subject to salmonid predation in high productivity habitats highlights the potential trade off between the benefits of foraging and the risk of predation (see Vamosi, 2002). Predation experiments indicate that vegetation in littoral areas can provide a refuge from piscivorous fish, and predation

risk can affect both choice of feeding site and the amount and type of prey eaten (Werner *et al.*, 1983a, 1983b; Ibrahim and Huntingford, 1989). The interaction may also reflect competition for resources when salmonids are present (Walker, 1997; but see Rundle *et al.*, 2003). The intermediate shape of these populations suggests an optimal phenotype for both foraging and predator evasion (Reimchen, 1994; Vamosi 2002).

A major theme in the literature has been the effects of differential predation regimes and the evolution of the stickleback defensive apparatus (Hagen and Gilbertson, 1972; Moodie, 1972b; Moodie and Reimchen, 1976; Gross, 1978; Reimchen, 1980, 1988, 1992b, 1994, 2000; Reimchen *et al.*, 1985). The anterior lateral plates (which are present in the majority of low plated morphs) often overlap and buttress the spines, providing major structural support and increase the surface area over which forces on the spines are distributed during manipulation by predators (Reimchen, 1983). I categorized each population's defensive condition, but in reality the state of the defensive apparatus represents a continuum from small spines and no plates without structural overlap to populations with large spines and a full complement of lateral plates with a high degree of overlap.

Body shape could be predicted by the population defensive condition, but the associations between shape, defences and habitat were variable. Throughout the archipelago and geographical sub-regions, populations with the less derived body shape (CV1-) consistently had a high defensive condition, and those populations with the more derived shape (CV1+) consistently had a low defensive condition. I took precautions to create shape variables that were independent of the defensive condition, by removing the landmark that delimited the posterior position of the pelvic girdle. Body shape with this

landmark included was very tightly correlated to the defensive principal component. Nevertheless, body shape and defensive condition appear to be intrinsically linked. My results support the notion that selection favours the low defensive condition in structurally complex littoral habitats, as the body shape of the low morphs (CV1+) is adapted for increased manoeuvrability and flexibility, which is suitable to evade predators (Reimchen, 1980, 1994; Webb, 1982, 1984; Taylor and McPhail, 1986; Walker, 1997). The body shape of stickleback populations with the high defensive condition found in large deep lakes and lakes of high productivity can be partially explained by a few alternate hypotheses.

The association between defensive condition and habitat productivity that I detected is consistent with the role of calcium levels and the evolution of defensive phenotypes (Giles, 1983). The low defensive morphs (in relation to the high ancestral condition) may be favoured in fresh water, because of increased costs of ion uptake to mineralize bone. The streamlined shape of my high defensive condition (low morphs with robust defenses) fish is an adaptation to the open water environment, and without considering effects of variable predation regimes, a possible explanation is that the high defensive condition is a product of the high calcium levels and productivity often found in large deep lakes. However, low calcium levels in lakes on Haida Gwaii are often associated with low pH and smaller size (Reimchen, 1992c), attributes which have been linked to an increase in invertebrate predation, and a decrease in avian piscivore and salmonid predation (Reimchen, 1994). The functional interpretation is that the low defensive morph (extreme lateral plate, pelvis and spine reduction) often associated with sticklebacks in habitats of low productivity is an adaptation to minimize the frictional

contact for grappling predators (odonate nymphs) (Reimchen, 1980). This hypothesis is further supported by experimental evidence suggesting a selective advantage for benthic stickleback with marked armor reduction against predation from backswimmers (Notonectidae) in calcium rich Paxton Lake in southwestern BC (Vamosi, 2002).

Populations subject to predatory fish usually have the high defensive condition (Hagen and Gilbertson, 1972; Moodie and Reimchen, 1976; Gross, 1978), and the less derived shape (CV1-) may reduce pressure drag while swimming. This shape combined with long median fins may increase fast-start performance, facilitating escape from predators (Bergstrom, 2002b). By erecting and locking the spines, stickleback populations with less derived, deeper bodies (CV2+) could have their effective diameter increased by up to 230% (Reimchen, 1991a), increasing predator-manipulation time (Hoyle and Keast, 1987, 1988) and assisting escape after capture. Piscivorous fish tend to ingest prey headfirst (Sanford and Harris, 1967; Reimchen and Douglas, 1984; Reimchen, 1991a), and the anterior position of the spines (CV1-), could puncture the mouth parts of the predator (Hoogland *et al.*, 1957) and increase the incidence of escape during handling by the predator (Walker and Bell, 2000). The less derived body shape of populations with high level defenses suggests that stabilizing selection in these habitats have maintained the ancestral condition (Walker and Bell, 2000).

The ecomorphological relationships were consistent between defensive categories, and replicated in each geographical region. For example, stickleback from each defensive category had significant differences in body shape but showed the same relationship (similar slopes) with the habitat covariate. High defensive condition populations were still streamlined in small, shallow habitats, but were more streamlined

in large, deep habitats (higher negative CV1 loadings). Low defensive condition stickleback had the derived shape in small, shallow habitats but were less derived (i.e.: more streamlined) in larger, deeper habitats. These associations strengthen the notion that the hydrodynamic constraints imposed by characteristics of habitat govern the selection for optimal body shape (Walker, 1997).

There are two divergent haplotypes (Euro-North American and Japanese), of *Gasterosteus aculeatus* in the northern hemisphere (Orti *et al.*, 1994). The majority of populations of stickleback on Haida Gwaii are monomorphic for the Euro-North American lineage, except for 19 populations in the northeastern corner of Graham Island (lowlands) and one population on the west coast of Moresby Island (plateau/mountain). These lakes contain stickleback of the Japanese lineage at varying frequencies, which may account for some of the diversification among populations (Deagle *et al.*, 1996). However, marine stickleback from both lineages are morphologically similar (fully plated trachurus morph (Taniguchi *et al.*, 1990), indicating that the Japanese and Euro-North American ancestor(s) to the Haida Gwaii populations were completely armoured (Deagle *et al.*, 1996). In freshwater habitats on Haida Gwaii, monomorphic populations (with either haplotype) and those with both haplotypes present exhibit similar ecological associations ranging from maintenance of fully plated morphology to reduction in lateral plates, in numerous allopatric populations (Deagle *et al.*, 1996). The ecological association with body shape between the presence or absence of the Japanese haplotype, mirrored those detected in each geographical region and between each defensive condition; more derived shape in small shallow ponds or habitats of low productivity, less derived shape in large deep lakes or habitats of high productivity. My results reinforce

the notion that the hydrodynamic landscape is the agent of selection for the evolution of body shape in stickleback populations. I did not have direct DNA evidence for many of the specific fish used in this analysis, and genomic differences among populations are unknown; therefore I cannot speculate as to the fine scale body-shape differences among haplotypes, which only molecular data can resolve. Nevertheless, current data on the distribution of each haplotype, and the morphological variability in each suggest that derived traits, such as armour reduction (Reimchen, 1980; Giles, 1983; Bell *et al.*, 1993) and derived body shape morphology has arisen independently in populations descended from both the Euro-North American and Japanese lineages, indicating that natural selection (not ancestry) has the predominant role in shaping these populations.

The ecomorphological relationships exhibited by the Haida Gwaii threespine stickleback are consistent with results from previous studies, that link body shape adaptations to multiple aspects of ecology in Alaskan stickleback populations (Walker, 1997; Walker and Bell, 2000). The body-shape associations detected in this study are also consistent with differences based on flow regimes for populations of Coho salmon (Swain and Holtby, 1989), Rainbow fish (McGuigan *et al.*, 2003), Darters (Guill *et al.*, 2003a), characids and cichlids (Langerhans *et al.*, 2003), and with those reported for feeding habits in serranid fishes (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 guppies (Langerhans *et al.*, 2004a; Langerhans and DeWitt, 2004b), as well as carp (Brönmark and Miner, 1992). The body shape of Haida Gwaii stickleback populations from open water habitats consistently possessed adaptations that theoretically lead to a reduction drag while foraging (Aleev, 1977;

Vogel, 1981; Webb, 1982). Populations from streams and littoral habitats consistently exhibited a more derived shape for increased manoeuvrability (Reimchen, 1980, 1994; Webb, 1982, 1984; Taylor and McPhail, 1986; Walker, 1997). Those populations under salmonid predation typically had large median fins to increase burst performance and robust armor for defense against gape-limited predators (Reimchen, 1988, 1990, 1991b), while stickleback with bird/invertebrate predators often had derived shape and reduced armor as possible adaptations to increase manoeuvrability, reduce added mass and optimize darting to cover (Webb, 1982; Reimchen, 1994). Shape was tightly coupled to defensive condition, which is expected, as body armor is a significant component of stickleback morphology. The associations were consistent in each geographical region and provide evidence for parallel evolution governed by the hydrodynamic selective landscape of the habitat.

The results of this study demonstrate the predictability of natural selection; shape evolving in response to the demands of subtle differences in the hydrodynamic landscape and predation regime of a given habitat. The racemic phylogeny of stickleback indicates that since the Miocene, marine stickleback have repeatedly colonized unstable, ephemeral, freshwater habitats, differentiated phenotypically as can be observed throughout their modern distribution, and then after a brief period (in geologic time) gone extinct (Bell, 1988; Bell and Foster, 1994). Palaeoecological and palaeolimnological data indicates that populations of the closely related stickleback, *Gasterosteus doryssus*, under a similar selection regime that can be observed today, exhibited the same rapid reduction (~ 7 800years) in bony armor after colonizing an ancient lake (Truckee Lake formation, Nevada, USA) from the marine environment (Bell, 1994). If the association I detected

between defensive condition and shape is as ancient, then similar studies of body shape may advance the idea of phenotypic integration, reflecting interactive responses of multiple characters to specific selection regimes (Bell and Foster, 1994; Foster and Bell, 1994). I have reported the evolution of body shape for 124 populations of threespine stickleback on Haida Gwaii, and interpret it as selection for optimal morphologies in response to volume/clarity, productivity, and predation regimes of habitat. Associations demonstrating body shape/habitat relationships are not new; body form is suited to the ecological niche space observed in actinopterygian groups (Webb, 1982), and the body shape of open water predators such as chondrichthyans, cetaceans and fossil ichthyosaurs indicate that body shape has been highly conserved over long sequences of geological time (Howell, 1930). This study demonstrated the efficacy of hydrodynamic processes on the evolution of aquatic vertebrate form and emphasizes the deterministic processes of evolutionary change.

Chapter 6: General discussion

The consequence of natural selection is the evolution of organisms with phenotypes that function more efficiently in certain environmental situations than did those of their predecessors (Savage, 1969). Through genealogical descent, all organisms resemble their recent ancestors, regardless of the environments they may colonize (Schwenk and Wagner, 2001). If the new environment is dissimilar from that of the ancestor, the colonists are subjected to novel environmental demands to which they must adapt. Adaptation can be characterized by the acquisition of advantageous features, through the evolutionary process of natural selection. Selection operates on the available genetic variation to produce a gene pool that interacts more efficiently with the environment (Darwin, 1859), implying that derived features provide a functional advantage over ancestral traits. Typically, evolutionary change has been quantified through measurements of a few discrete traits. However, there are typically dozens of traits that are related to the fitness of an organism, and gene expression must be adjusted for multiple traits so that phenotypic integration (interactive response of multiple characters to specific selection regimes) is maintained within the new environment (Schlichting, 1989; Bell and Foster, 1994). An organism's body shape is an example of a trait that reflects phenotypic integration, as shape is often quantified by numerous correlated characters (Strauss and Bookstein, 1982; Baumgartner *et al.*, 1988; Rohlf, 1990; Bookstein, 1991; Baumgartner, 1992; Walker, 1997; Walker and Bell, 2000; Klingenberg *et al.*, 2003).

In this thesis I have investigated variation in ontogenetic shifts, sexual dimorphism, and population diversification of body shape in relation to ecological factors among insular populations of freshwater threespine stickleback from the Haida Gwaii archipelago. Throughout stickleback's Holarctic and broad ecological distribution, the fragmentation of the gene pool into many thousands of isolated (or nearly isolated) demes has allowed for an extraordinary diversification of phenotype (Bell and Foster, 1994). The populations of stickleback on Haida Gwaii are unparalleled, as they exhibit a range of phenotypic variation (in size, longevity, trophic adaptations, nuptial colour display and defensive armature) that extend the limits among this species (Moodie and Reimchen, 1976; Reimchen, 1980, 1983, 1989, 1992a; Reimchen *et al.*, 1985). Many of the lakes in British Columbia were colonized by the ancestral marine phenotype, and most have been isolated from one another since the final drop in sea level over 10 000 years ago (Moodie and Reimchen, 1976; Schluter and McPhail, 1992). Some of the localities have high rates of predation from birds, fish and or invertebrates, and the variability in the physical characteristics of the lakes encompass the range of physical variation that can support temperate fresh water fish populations (Moodie and Reimchen, 1976; Reimchen, 1980, 1983; Bergstrom and Reimchen, 2002). In essence, each population represents a natural experiment that may be used to illuminate mechanisms for divergence.

The ontogenetic shifts in body shape in populations of stickleback from two widely studied lakes on Haida Gwaii (Mayer and Drizzle) were examined to elucidate ecological determinants of body-size (age class) and shape. I also quantified the ontogenetic trajectories for 125 populations of stickleback and investigated possible relationships with the volume/clarity of habitat (PC1_{lk}). For stickleback from Mayer

Lake I found statistically significant relationships between all multivariate measures of body shape and size indicating that adults possess relatively smaller heads, more anterior dorsal spines, shallower bodies and larger median fins than the juveniles. On the other hand, Drizzle Lake stickleback exhibited only slight ontogenetic shifts in body shape, suggesting that the only relative difference in body shape between juveniles and adults is in the length of the median fins (adults have larger fins). Among populations throughout the archipelago and within each geographical sub-region, ontogenetic shifts in body shape are variable (half the populations slope is positive at varying degrees of significance and vice versa). I did not detect any associations between ontogenetic slope and habitat volume/clarity, suggesting that that each body of water containing stickleback has both unique and shared selective forces (predation; intra/interspecific competition; food availability), and subtle differences in the selective landscape among lakes are driving the observed differences in ontogenetic paths. The paucity of the data may have caused the variability of ontogenetic slopes and lack of correlation between slope and habitat volume/clarity, as some stickleback samples had a relatively uneven distribution of size classes (Moodie and Reimchen, 1976).

Many species pass through ecologically distinct stages as they grow, and the variety of resources used by each cohort effectively expands the total niche width for the species (Polis, 1984; Olson, 1996). This notion has led to speculation that differences in resource utilization between cohorts have the same ecological basis as that between competing species. Under reduced interspecific competition, there is selective pressure for age groups to diverge in resource use (Van Valen, 1965; Polis, 1984), which may be the underlying mechanism for ontogenetic niche shifts. The variable ontogenetic shifts in

body shape detected in the Haida Gwaii stickleback populations may represent the optimal phenotype for juveniles in a given habitat. The variation observed in ontogenetic slopes may be influenced by the timing of ontogenetic niche shifts, which change in response to biotic and abiotic environmental conditions (Olson, 1996) and may be adaptive responses to variable ambient conditions (Werner and Gilliam, 1984).

Organisms in poor growth conditions in one niche advance to the next ontogenetic niche, while those in good conditions delay shifting to the next niche (Werner and Gilliam, 1984; Rowe and Ludwig, 1991). The extent of ontogenetic niche shifts may also be influenced by both abiotic factors and by interaction with other species (Olson, 1996). Small changes in early growth rates can lead to dramatic changes in the timing of the ontogenetic niche shift and likely effect patterns of body shape observed among lakes.

Recent work on the stickleback ontogenetic niche indicates that differences in habitat use may not only depend on individual size, or juvenile size relative to adult predators, but may also depend on the degree of development or expression of defensive structures (Sillett and Foster, 2000). Adult stickleback are known to cannibalize eggs and small juveniles (Wootton, 1976; Foster, 1988; Foster and Baker, 1995). Juvenile stickleback lacking pelvic spines use cover provided by vegetation when the risk of conspecific predation is high, while those that are not vulnerable (populations with pelvic spines) are not restricted to cover and can forage in open areas of the habitat (Sillett and Foster, 2000). Analysis of the juvenile stickleback diet suggests that asymmetric pelvic morphs are more benthic than symmetric pelvic juveniles, reducing the probability of eating pelagic copepods, the primary host of the stickleback parasite *Schistocephalus solidus* (Reimchen and Nosil, 2001a, 2001c). These results indicate that not only is there

niche partitioning between cohorts, but also within cohorts between phenotypes, highlighting the potential complexity of ecological interactions among species within different lakes (Reimchen, 1990).

The possibility of a sexual dimorphism of body shape and of associations between observed differences in shape and habitat volume/clarity, were investigated to determine if there is a shape polymorphism that may be explained by ecological factors. I detected sexual dimorphism in shape quantified by one multivariate technique (relative warp analysis), which was confirmed by a sign test on standard morphometric measurements. My results suggest the sexual shape dimorphism is evident throughout the archipelago and within each geographical sub region. However, in lowland populations sexual dimorphism becomes more apparent with size. Regardless, my results suggest that male stickleback have larger heads, more posteriad dorsal spines, and deeper bodies with shorter median fins than the females. I did not detect a relationship between the observed body shape dimorphism and lake volume/clarity, however it is possible that the morphological divergence between the sexes has ecological significance in adapting each sex to a different sub-niche. The differences in shape suggest that male shape is suited for increased maneuverability adapted to improve male/male competitive interactions, guard their nests, increase attractiveness to females and to facilitate foraging in the structurally complex littoral zone, while female shape could be an adaptation for increased foraging performance and protection against predators in the open water environment.

There are numerous mechanisms that may partially explain the evolution of sexual dimorphism of body shape. Sexual selection has often been thought of as the foundation from which a dimorphism between the sexes develops (Lande, 1980; Slatkin,

1984). Male body shape that may confer a competitive advantage or make certain males more attractive to females, thus increasing an individual's absolute fitness, facilitates the evolution of a dimorphism. Conversely, if there is variation in female shape, and if aspects of this variation allow a higher reproductive output, individual females with this phenotype are at a selective advantage (through greater absolute fitness). Selection will act on the variation between the sexes, selecting for shapes that confer higher absolute fitness, and dimorphism will evolve. Ecological causes of sexual dimorphism of body shape, may contribute to the evolution of a dimorphism if there are differences between the sexes in niche utilization (Lande, 1980; Slatkin, 1984). If males and females are subjected to alternative selective pressures (in alternative niches) and provided there is genetic variation for a sexual dimorphism, the evolution of optimal phenotypes between the sexes can occur.

The hypothesis of ecological causation relies on the existence of ecological differences between males and females (Shine, 1991). Given what is known about differential feeding efficiency between the sexes of stickleback (Bentzen and McPhail, 1984), I have suggested that the body shape of females is possibly an adaptation to increase foraging performance in the open water environment, while male shape represents an adaptation to increased performance in the structurally complex littoral regions of the habitat. Ecological divergence leading to sexual dimorphism can occur through competition between the sexes for a limiting resource or because of intrinsic ecological differences related to reproductive biology (Shine, 1991) but it requires open niche space or underutilization of resources and relaxation of interspecific competition (Smith and Skulason, 1996) in recently colonized habitats. The possibility of niche

expansion in the absence of interspecific competition is achieved by increased between-individual variation in resource use (Van Valen, 1965) and can reflect intrapopulation variation in a wide range of individual traits such as body shape that determine resource-specific efficiency and preference (Bolnick *et al.*, 2003). If there are intrinsic differences between the sexes in resource use and efficiency, natural selection can act on the available intrapopulation variation (between the sexes), and dimorphism of shape may evolve (Reimchen and Nosil, 2001a, 2001b). Sexual dimorphism may evolve slowly, and can occur through the process of ecological character release. Recent work on stickleback from the Dewdney-Banks archipelago off the mid-coast of British Columbia, suggests that ecological character release can occur when there is more available niche space in a given habitat, providing evidence that site specific ecological pressures can effect the evolution of sexual dimorphism (Nosil and Reimchen, 2005).

The evolution of sexual dimorphism through the process of ecological character release has been observed in diverse taxa. Morphological divergence in numerous snake genera originated as differences in body size or ecology due to reproductive biology, with the subsequent sexual dimorphism through independent adaptations to foraging (consistent with ecological character release) (Shine, 1991). On islands throughout the world, recently introduced allopatric populations (100-200 generations) of the Indian mongoose, *Herpestes javanicus*, were more sexually dimorphic and exhibited variation consistent with ecological release from competition with congeners (Simberloff *et al.*, 2000). The dimorphism was detected in *H. javanicus* canine teeth and is possibly due to differences in diets between males and females, which is consistent with competitive character displacement and release. Future studies on the evolution of sexually

dimorphic, phenotypically integrated traits, such as body shape, in closely related allopatric populations might be enhanced by the investigation of ecological character release through increased ecological opportunity.

Throughout the archipelago there are significant differences among populations of adult stickleback body shape, that likely represent adaptive divergence rather than ontogenetic differences among populations or extreme differences in body shape among the sexes. Discriminant function analysis allowed me to quantify derived freshwater population's body shape along two orthogonal axes relative to the marine form. Along the first shape vector, derived populations have thicker caudal peduncles and a posterior center of inertia, as possible adaptations for increased burst performance and manoeuvrability. The derived populations along the second shape vector are characterized as having small heads and shallow bodies, as adaptations to increased performance in the open water environment.

It is possible that phenotypic plasticity of body shape among populations contributes to the diversification of form when stickleback colonize ecologically different habitats from the ancestral marine environment. I detected different reaction norms between the two experimental populations, which may reflect genomic differences between the two source populations, or subtle ecological differences between experimental ponds. Nevertheless, the differences in body shape I detected (attributable to phenotypic plasticity) between each source and experimental pond (on average 11% of the variation among samples) are not large enough to account for the variation among populations and suggest that natural selection is changing allele frequencies governing the derivation of body-shape morphology throughout the archipelago.

The differences in body shape between stickleback species pairs on Haida Gwaii (lake-river) and in lakes from southern British Columbia (benthic-limnetic) reflect local adaptation rather than historical relationships. Most of the lake-river populations on Haida Gwaii that I analyzed are monomorphic for the Euro-North American mitochondrial lineage (Deagle *et al.*, 1996), yet I detected significant body-shape differences among most pairs. Stream stickleback, in comparison to their lake counterpart, have a body shape that is adapted for increased manoeuvrability in the flowing water environment, supporting the notion of local adaptation to differential selection regimes, rather than phylogenetic effects (Reimchen *et al.*, 1985; McPhail, 1994; Hendry *et al.*, 2002). The benthic ecotypes from the species pairs in lakes from southern British Columbia were similar in body shape to the populations of benthic stickleback from Haida Gwaii, while the limnetics were intermediate (less divergent when compared to the ancestral marine) in body-shape morphology. The similarity between body shape among the southern ecotypes and Haida Gwaii populations, suggests that directional selection is responsible for the evolution of more derived body-shape morphologies when stickleback colonize habitats with extreme selective landscapes. This type of diversification has been found in stickleback populations elsewhere (e.g., California by Baumgartner, 1992; Alaska by Walker, 1997) and in several species of boreal salmonids (see Bell and Andrews, 1997).

The associations detected between morphology and both physical and biological characteristics of each habitat suggest that the local selective landscape is responsible for this variation. I consistently found that populations subject to primarily bird/invertebrate predation are small, have body shape adapted for manoeuvrability and often occur in

small, shallow, stained ponds. Conversely, stickleback populations with salmonid predators have larger body-size and body shape adapted for sustained swimming and burst acceleration in large, deep habitats. Regardless of defensive condition, body shape was related to lake volume/clarity, with body shape remaining more streamlined (less derived) in populations from larger deeper lakes. These relationships were detected in each geographical region separately and provide evidence for parallel evolution, as stickleback shape and size are adapted to the specific biophysical attributes of each habitat in largely isolated lakes.

Phenotypic diversification can occur through processes other than natural selection. Founder effect (Mayr, 1942) can lead to differences in geographically isolated populations and influences how organism evolve in isolated habitats. Stochastic processes such as founder effect, population bottlenecks or genetic drift can lead to fixation of mal-adaptive or neutral traits, producing populations with characters that may not represent the optimal phenotype for a given habitat (Dobzhansky *et al.*, 1977). If divergence of stickleback phenotypes on Haida Gwaii were a result of these stochastic processes alone, distribution of body-shape morphologies would be randomly distributed throughout the archipelago. The possibility that these processes have occurred in Haida Gwaii stickleback populations cannot be ruled out, but the overwhelming degree of repeatability and replication of body shape within each geographical region among defensive conditions, predation regimes, mitochondrial DNA haplotypes and in similar habitats, suggest that natural selection has played the predominant role in shaping these populations (Reimchen, 1994; Bergstrom, 2002a). Further, numerous introductions of other species to islands around the world provide no evidence of substantial

morphological change to suggest stochastic processes, such as founder effect, as the mechanism of evolutionary divergence (Grant and Grant, 1996).

I have shown how body shape is associated with ecological factors, and inferred that shape evolves in response to the factors. The specific attributes of predation regime, stream versus lake habitat, lake volume/clarity and habitat productivity leads to selection for enhanced hydrodynamic performance, which suggests an optimal phenotype for a specific niche. Comparisons between closely related radiations of other taxa in different environments provide a unique window into understanding how both abiotic and biotic factors shape evolution (Irschick *et al.*, 1997; Losos *et al.*, 1997; Beuttell and Losos, 1999). The majority of mainland *Anolis* lizard species exhibit morphological characteristics unlike any Caribbean island anoles. Among islands, the same set of ecomorphs (distantly related species similar in ecology, morphology and behaviour) has evolved on each island and each ecomorph class represents distinct entities in morphological space (Beuttell and Losos, 1999). This suggests that the factors that caused the anole species to converge repeatedly in the West Indies are not present in mainland regions and that the environment can strongly influence the direction of morphological divergence (Irschick *et al.*, 1997; Losos *et al.*, 1997; Beuttell and Losos, 1999). Darwin's finches of the Galápagos archipelago represent another classic example of adaptive radiation. The various specimens from any one island do not form a continuously graded series (thick to thin billed for example), but instead form discrete groups, each having a distinct appearance that is correlated with environmental differences (Lack, 1947; Grant *et al.*, 1976). The finches' food supply on the Galápagos archipelago differs among each island along with differences in beak size, suggesting that

morphological differences are the result of selection pressures acting on genetically variable traits (Grant and Grant, 1982; Schluter and Grant, 1984). One strength of this study is that it involves the diversification of body shape in 125 stickleback populations, with predictable phenotypes derived independently from common ancestors, providing insight into the mechanisms that drive divergence among allopatric groups.

The evidence presented in this thesis indicates how in closely related allopatric populations body shape diversification is correlated with the hydrodynamic landscape. I have suggested that body shape evolves in response to the ontogenetic niche (variable ontogenetic slopes in alternate habitats), sexually dimorphic niches (subtle differences in body shape between males and females, conforming to what is known about differences in feeding efficiency and habitat use between the sexes), differential predation regimes (shape adaptations to evade capture and facilitate post capture escape), and foraging opportunities (littoral versus limnetic prey distribution). The body shape of stickleback populations on Haida Gwaii has evolved to suit the functional demands of the environment, which range from habitats such as streams and small shallow ponds, which contain stickleback with more derived shapes, though large deep lakes which are similar in hydrodynamic constraints to the ancestral marine and contain stickleback with less derived shapes. The associations observed were consistent between two independent geographical regions (lowland versus plateau/mountain) and numerous lakes within regions that were colonized independently and have a long history of independent evolution, confirming parallel evolution of stickleback body form on the Haida Gwaii archipelago. My results suggest that other examples of adaptive radiations may be

enhanced by the inclusion of body-shape ecology, by providing an alternative window through which the diversification of form can be studied.

This research focuses on the evolutionary implications of body shape, yet exemplifies the larger role adaptation plays in the distribution of phenotypes (Bergstrom, 2002a). The repeated parallel relationships between geographical regions and mitochondrial haplotypes indicate the importance of adaptive processes over stochastic factors and the effects of ancestry for evolutionary divergence. Similar phenotypes in diverse taxonomic groups evolve through drastically different developmental mechanisms, suggesting they have been moulded independently by selection to suit a similar function (Bergstrom, 2002a). The convergence of body shape observed in open water predators such as chondrichthyans, cetaceans and fossil ichthyosaurs (Howell, 1930) provide evidence that the numerous hydrodynamic constraints associated with similar niche space are of fundamental importance in the evolution of optimal phenotypes of aquatic organisms.

Given the post-glacial history of the Haida Gwaii archipelago (Moodie and Reimchen, 1976) and the speed with which adaptive differences between stickleback populations evolve (Bell *et al.*, 1985; Bell, 2001; Kristjánsson *et al.*, 2002; Bell *et al.*, 2004), each freshwater population represents an independent natural experiment with which to address the relative importance of stochasticity versus local adaptation. My results suggest that phenotype can be predicted by the biophysical attributes of the habitat. I repeatedly found that populations in small, shallow, stained ponds primarily subject to bird/invertebrate predation have small body size and body shape adapted for maneuverability and populations in large, deep habitats primarily subject to salmonid

predators have larger body size and body shape adapted for sustained swimming and burst acceleration. My results reinforce confidence in local adaptation as the cause of adaptive radiation. Since body shape is a highly integrated, multivariate phenotype my results also provide insight into the application of phenotypic integration to explain the diversification of form.

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