

## Morphometrics of Trophic Osteology in the Threespine Stickleback, *Gasterosteus aculeatus*

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**Landmark-based geometric morphometrics is used to detect interpopulation variation in skull morphology among samples from four populations representing anadromous, stream, and lacustrine planktivorous and benthic-feeding populations of threespine stickleback (*Gasterosteus aculeatus*) and between sexes within each population. Morphological differences among all four populations and between males and females are highly significant, although sexual dimorphism is primarily due to differences between the sexes in the stream population. The female stream and lacustrine benthic-feeding phenotypes are qualitatively and statistically indistinguishable. The anadromous phenotype is intermediate between lacustrine benthic and planktivorous phenotypes. Differences between samples are interpreted with reference to ecomorphological and functional morphological models.**

**T**HE threespine stickleback, *Gasterosteus aculeatus*, is widely distributed throughout coastal boreal and temperate Holarctic habitats. Because of its highly fragmented distribution in coastal marine, brackish, and freshwater habitats, it forms a phenotypically diverse species complex. Freshwater populations have been derived independently from anadromous ancestors many times (reviewed in Bell and Foster, 1994), and fossil evidence plus geographic homogeneity among extant populations suggest that anadromous threespine stickleback have been morphologically conservative during the known 10-million-year history of the species complex (Bell, 1994). Thus, extant freshwater populations may be considered to have a common anadromous ancestor of known phenotype.

Extreme phenotypic diversity of freshwater populations is a prominent feature of threespine stickleback biology. Much of this diversity involves modification of trophic morphology, a common feature of adaptive radiation in teleosts (Witte, 1984; Schluter and McPhail, 1993). Divergent trophic characters in *G. aculeatus* include gillraker number and length, snout length and width, and eye diameter (Gross and Anderson, 1984; Schluter and McPhail, 1992; McPhail, 1994). Although not strictly a trophic character, eye diameter is a limiting factor for locating planktonic prey (Meer and Anker, 1984; Walton et al. 1994). Variation in trophic

morphology of the threespine stickleback is genetically controlled (Hagen, 1973; Lavin and McPhail, 1985; Schluter, 1996), and, because populations are independently derived from a common ancestor, differences in trophic morphology are due to local selection and not ancestry (Bell and Foster, 1994).

Trophic morphology is highly correlated with habitat characteristics, such as lake size and depth and prey-species composition (Schluter and McPhail, 1992; Hart and Gill, 1994; Walker, 1997). Thus, variation in trophic morphology is probably primarily due to selection for greater foraging efficiency in different habitats (Benzten and McPhail, 1984; Schluter and McPhail, 1992; Schluter, 1993). Freshwater fishes are often polymorphic along an ecotypic and morphological continuum ranging from "benthic" to "limnetic" (Malmquist, 1992; Curtis et al., 1995; Robinson et al., 1996). Lacustrine threespine stickleback follow this pattern with limnetics more often observed in open water and tending to eat prey from the water column (plankton). Benthics spend more time in shallow, littoral areas (J. A. Walker, pers. comm.) and eat mostly larger, bottom-dwelling prey (Lavin and McPhail, 1985; Schluter and McPhail, 1992; Hart and Gill, 1994). Morphologically, limnetics are characterized by relatively shallow bodies, long snouts, large eyes, and longer and more numerous gillrakers, whereas benthics tend to have deeper bodies, shorter

snouts, smaller eyes, and fewer, shorter gillrakers (e.g., Lavin and McPhail, 1985; McPhail, 1994; Walker, 1997). Most lakes inhabited by threespine stickleback contain a single phenotypically intermediate population some of which are more benthic or limnetic than others (see Schluter and McPhail, 1992), but solitary extreme phenotypes do occur both in Alaska (Walker, 1997) and in British Columbia (Foster et al., 1992).

Threespine stickleback also display intrapopulation variation (sexual dimorphism) in trophic morphology and behavior. To test for sexual dimorphism in stickleback feeding habits, Bentzen and McPhail (1984) measured the feeding efficiency of limnetics and benthics of each sex. Benthic females were less efficient than males in feeding on large prey from the substratum, whereas limnetic females made no attempt to eat benthic prey. These results could be a reflection of the propensity for male stickleback to remain near the substratum while nesting. Based on this limited evidence that males tend to be more benthic in trophic habits than females, one would predict corresponding dimorphism in trophic morphology. A study of stickleback tooth configuration (WJC, unpubl. data), however, suggests the converse of this expectation. The premaxillary teeth of threespine stickleback are arranged in distinct series and tend to be more numerous in limnetics than in benthics. Qualitatively, limnetics have teeth arranged in straighter rows with more uniform spacing. Tooth number is also highly sexually dimorphic, and fish can be sexed accurately by observing dentition alone. Males from both benthic and limnetic populations tend to have more teeth, which are in straighter, more uniform rows than in females from the same population. Thus, males' dentition appears more limnetic in character than that of females, and this apparent contradiction calls for further investigation of the relationship among sex, feeding habits, and trophic morphology. Such a study of sexual trophic morphology dimorphism has the advantage that confounding factors such as geographical distribution and evolutionary history are minimized, allowing the causation of morphological variation to be more confidently interpreted (Shine, 1989).

Most previous research on interpopulation variation of threespine stickleback trophic morphology has used ratios between linear distance measures or counts of meristic characters (e.g., Hagen, 1967; Gross and Anderson, 1984; Lavin and McPhail, 1985). Geometric morphometrics, however, may provide a more powerful and sensitive tool to investigate diversification of tro-

phic morphology. Because specific ratios and indices need not be chosen in advance, shape variation involving characters not expected to be of interest can be detected. It is possible not only to determine whether individuals from two populations differ in shape but also to visualize where and how morphological differences occur (e.g., Rohlf et al., 1996; Adams and Funk, 1997).

Many structures directly related to food acquisition and processing (e.g., jaws, opercular bones, buccal cavity, orbits, suspensorial bones) are widely distributed over the teleost skull. Geometric morphometric comparisons of skull shape using such structures as landmarks should thus capture variation in trophic morphology. Because threespine stickleback trophic morphology is correlated with habitat, one would expect to find variation in skull shape among populations that differ in habitat and prey type (Liem, 1993).

The use of skull shape is desirable not only because of its suitability for geometric morphometrics but also because it allows one to pose specific predictions based on trophic ecology and functional models (e.g., Anker, 1978). Thus, due to prey type and habitat divergence, one would expect differences in skull morphology between benthic and planktonic feeders. One such difference would be orbit size, which should be greater in a limnetic stickleback to accommodate a larger eye and increase resolving power for detecting small planktonic prey (Meer and Anker, 1984; Hart and Gill, 1994; Walton et al., 1994).

In this paper, we use geometric morphometrics to compare skull shape in threespine stickleback using four populations from different habitats. Walker (1995, 1997) analyzed body shape variation in 40 populations using geometric morphometrics, but his data only superficially covered cranial morphology. Therefore, we have set out to detail variation in cranial shape as a means of better understanding the morphological specifics of trophic adaptation. We chose our samples to represent the full range of habitats used by threespine stickleback: one from an anadromous population, one from a freshwater stream, and two from freshwater lakes (benthic and limnetic). The anadromous population represents the putative common ancestor of the three freshwater populations (see Bell and Foster, 1994). We test mean skull configuration for statistically significant differences among populations and between sexes within populations. For all significant comparisons, we describe the qualitative differences in skull shape between samples in terms of predictions

based on trophic ecology and functional models. We could not formally test such predictions in the present study due to lack of replication. However, by determining whether trophic morphological variation among samples is consistent with what is expected given that our samples are otherwise morphologically comparable to populations from their respective habitats, we can assess the feasibility of extending our comparisons to additional samples which would be necessary to test predictions and examine the correlation between habitat and trophic morphology. Thus, by providing detailed descriptions of trophic morphological variation among samples from a range of habitats, we establish a base line for more comprehensive comparisons to investigate stickleback diversification mechanisms using modern geometric morphometrics. Such studies will yield insight into the selective forces responsible for evolution of diverse trophic morphology in teleosts.

#### MATERIALS AND METHODS

Threespine stickleback were sampled from four populations in the Mat-Su Valley region of Cook Inlet, Alaska: a stream population (Meadow Creek, 61.56°N, 149.81°W), two lacustrine populations (Visnaw Lake, 61.62°N, 149.68°W) and (Mud Lake, 61.56°N, 148.95°W), and an anadromous population (Rabbit Slough, 61.54°N, 149.25°W). The Visnaw Lake and Mud Lake populations were selected because they appeared to represent planktivorous and benthic-feeding ecotypes, respectively (Walker, 1997). Although Visnaw Lake is shallow, it is nearly devoid of rooted vegetation, and the stickleback have low body depth (Walker, 1997) and high gillraker counts ( $\bar{x} = 22.95 \pm 0.245$ ,  $n = 20$ ) characteristic of limnetic threespine stickleback (Schluter and McPhail, 1992). In contrast, Mud Lake is less than 2 m deep, and rooted vegetation occurs across the entire lake. Mud Lake stickleback are deep bodied (J. A. Walker, pers. comm.) and have significantly lower gillraker counts ( $\bar{x} = 17.85 \pm 0.243$ ,  $n = 20$ ,  $F_1 = 217.2$ ,  $P < 0.0001$ ) than those in Visnaw Lake, conforming to expectations for benthic threespine stickleback. Although diet was not measured directly, gillraker counts can be used as an indirect measure. Populations with high gillraker numbers eat smaller food items (Hart and Gill, 1994), and gillraker count differences between our populations are comparable to those between members of benthic-limnetic species pairs, whose diets have been well characterized (McPhail, 1984, 1994; Schluter and McPhail, 1992). The samples were collected either with a

seine (3.05 m long, 1.22 m high, 0.318 cm mesh) or with six to 10 minnow traps (chamber 44.45 cm long, 22.86 cm diameter; openings 2 cm diameter; mesh 0.318 cm). Traps were baited with sharp cheddar cheese and set overnight (about 20 h) near vegetation within a few meters of shore, usually at 1–2 m depth. Specimens were anesthetized in MS-222 (Tricaine Methanesulfonate), washed to remove the MS-222, fixed in 10% formalin, and transferred to 50% isopropyl alcohol for storage.

Specimens were sexed by gonad inspection and, because soft tissue is destroyed during trypsin digestion, the caudal fin of females was notched for future reference. We also counted gillrakers on the first gill arch of the left side of 20 stickleback from each population. Specimens were cleared and stained roughly following Pothoff (1983) to improve the visibility of skeletal elements. Alcian blue cartilage stain was used in preliminary work but proved unnecessary for distinguishing cranial landmarks. Therefore this time-consuming step was eliminated from the present study.

Landmarks for the geometric morphometric analysis were chosen (1) to cover the geometric form of the head, orbit, and nasal capsule; (2) to capture the configuration of the four-bar linkage jaw-abduction mechanism (see Fig. 1); and (3) to encompass linear measurements used in previous studies (e.g., Lavin and McPhail, 1985; Schluter and McPhail, 1992; Schluter, 1993). Landmark positions were plotted on paper using a dissecting microscope and camera lucida, and their coordinates were digitized with an IBM-compatible personal computer, CCD video camera, 70 mm macro lens, and MorphoSys software (C. Meacham and T. Duncan, MorphoSys, vers. 1.29, University of California, 1990, unpubl.). Sample size in a morphometric study should equal at least twice the number of landmarks (for two-dimensional data) to have fewer variables than observations. Thus, we used 23 landmarks per specimen (Figs. 1–3A, Table 1) and 50 males and 50 females per sample.

The 400 specimens were superimposed using the generalized least squares criterion (Rohlf and Slice, 1990) in the GRF-ND software package (D. E. Slice, GRF-ND, Dept. of Ecology and Evolution, State University of New York, Stony Brook, 1994, unpubl.), and the mean coordinates of selected landmarks were connected to form an outline, which aids in visualizing the reference configuration. This reference configuration was used in subsequent analyses.

After removing nonbiological variation, the thin-plate spline function was used to calculate

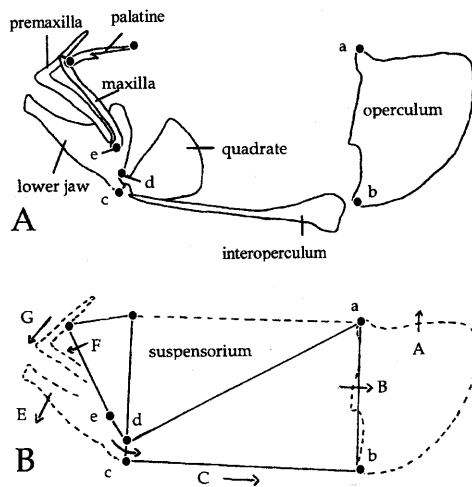


Fig. 1. (A) Landmarks corresponding to four-bar linkage model of *Gasterosteus aculeatus* jaw mechanics. (B) Four-bar linkage mechanics of the skull of *Gasterosteus aculeatus*. The levator operculi muscle exerts a force (A) on the operculum causing it to pivot dorsally about point (a). This rotation exerts a caudad directed force (B) on the interoperculum (b). This transfers a force (C) to point (c) causing it to move caudally relative to (d), which depresses the lower jaw (force E). Depression of the lower jaw pulls point (e) which rotates the maxilla in the direction of force (F). The premaxilla is simply pulled along by the maxilla and is protruded (G).

uniform and nonuniform components of shape for each specimen (Bookstein, 1989, 1991). The thin-plate spline utilizes variation in the parameters of an interpolating function to express variation among specimens. Specifically, the shape of a reference configuration of landmarks is used to generate this interpolating function, which is then decomposed into a series of geometrically orthogonal elements called principal warps (Bookstein, 1989). The principal warps are multidimensional shape axes that represent a decomposition of the possible shape deformations that could occur starting from the reference configuration. By projecting each specimen onto these shape axes, the deviations in shape of that specimen from the reference configuration is expressed as a set of shape variables, or partial-warp scores, which represent the deformation of the reference configuration into that particular specimen (Bookstein, 1989; Rohlf, 1993). Because six degrees of freedom are lost in the analysis of two-dimensional data (two for translation, one for rotation, one for scale, and two for the uniform components of shape), there were 40 ( $2p-6$ ) partial-warp scores

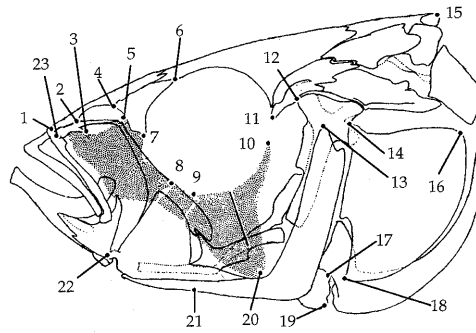


Fig. 2. Positions of landmarks used in morphometric analyses of threespine stickleback skull morphology. Figure reproduced with modification from Bowne (1994). (See Table 1 for descriptions.)

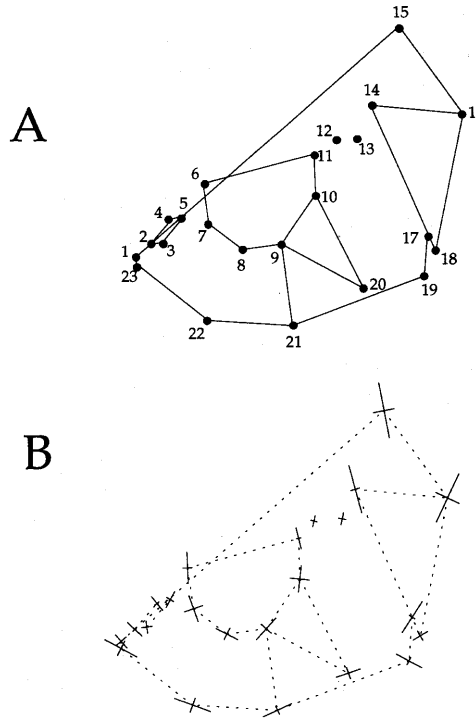


Fig. 3. (A) Outline representation of stickleback skull obtained by connecting the 23 landmarks to selected other landmarks used in this study. (B) Generalized least squares (Procrustes) superimposition of 400 specimens after translation, rotation, and scaling each specimen. Variation present at each landmark is represented by principal axes, and the outline formed by connecting the mean coordinates of each landmark represents the reference configuration.

TABLE 1. MORPHOLOGICAL LANDMARKS USED IN THIS STUDY. Landmark types are discussed in the Materials and Methods.

Landmark	Description	Type
1	Anterior extent of Nasal	2
2	Nasal—palatine at nasal capsule	1
3	Palatine—lachrymal at nasal capsule	1
4	Nasal—lachrymal at nasal capsule	1
5	Lateral ethmoid—lachrymal at nasal capsule	1
6	Lateral ethmoid—frontal suture at orbit	1
7	Lachrymal—lateral ethmoid suture at orbit	1
8	Lachrymal—second suborbital suture at orbit	1
9	Second suborbital—third suborbital suture at orbit	1
10	Dorsal extent of third suborbital	2
11	Anterioventral extent of Sphenotic at orbit	2
12	Anterior extent of hyomandibula—sphenotic	2
13	Dorsal extent of preopercular	2
14	Operculum—hyomandibula	1
15	Posterior extent of supraoccipital	2
16	Posteriordorsal extent of operculum	2
17	Posteriordorsal extent of interoperculum	2
18	Anterioventral extent of operculum	2
19	Caudoventral extent of interoperculum	2
20	Ventral extent of third suborbital	2
21	Ventral border of preoperculum perpendicularly ventral to (9)	3
22	Quadrate—articular	1
23	Anterior extent of palatine	2

for each specimen (in which  $p$  is the number of landmarks). These partial-warp scores were then used in multivariate statistical analyses to compare the samples.

After superimposition of the specimens, and calculation of the two uniform and 40 nonuniform shape components using the TPSWTS program in NTSYS-PC (F. J. Rohlf, vers. 1.80, Exeter Software, Setauket, NY, 1993, unpubl.), a principal components analysis was performed on the partial-warp scores (i.e., a relative warps analysis with  $\alpha = 0$ ). Thus, the major trends in nonuniform shape variation were identified (Rohlf, 1993). The first principal components axis, or first relative warp (Bookstein, 1991; Rohlf, 1993), represents the direction of maximal nonuniform shape variation among all specimens. Shape deformations along this axis were described and evaluated in relation to functional morphology, habitat type, and presumptive prey type.

In addition to the principal components analysis, a two-way MANOVA was performed to test for differences among populations and between the sexes in the uniform and nonuniform components of shape (e.g., Rohlf et al., 1996; Adams and Funk, 1997). A canonical variates analysis (CVA) was also performed in NTSYS-PC (F. J. Rohlf, vers. 1.80, Exeter Software, Setauket,

NY, 1993, unpubl.) using males and females within each sample as separate groups. This method allows one to identify patterns of shape similarity among populations by providing an ordination of the populations in a morphological space. Following these analyses, all possible pairwise comparisons were performed on the generalized Mahalanobis distances between populations using their corresponding  $F$ -values (Krzanowski, 1988; Marcus, 1993; F. J. Rohlf, NTSYS-pc, vers. 1.80, Exeter Software, Setauket, NY, 1993, unpubl.). An experiment-wise error rate of  $\alpha = 0.05$  was maintained using the Bonferroni method, yielding a critical  $\alpha$  of 0.00178. Finally, predicted mean shapes for each population were generated using the program TPSREGR (F. J. Rohlf, Dept. of Ecology and Evolution, State University of New York, Stony Brook, 1993, unpubl.) to regress the partial-warp scores for the mean landmark configuration of each population onto a dummy variable with a separate state for each population. (The mean landmark configuration of each sample was found by performing a generalized least-squares superimposition of the specimens within that population.) Representations of the mean shape for each population were qualitatively compared to the reference configuration

to describe patterns of shape difference between populations.

#### RESULTS

*General description of shape variation.*—The first relative warp (RW1) explained 59.3% of the variation in nonuniform shape (Fig. 4), and the first two relative warp axes explained 91.7%. Because so much of the nonuniform variation among specimens was explained by RW1, we briefly describe the trends in shape variation along the first relative warp. The positive extreme of RW1 is characterized by a relatively short (landmarks 1 and 16) and deep skull (landmarks 15 and 19) and a smaller orbit (landmarks 6–11). In addition, the snout is directed more ventrally and is shorter and deeper, and the area ventral to the orbit (i.e., suspensorium) is greater. The negative extreme of RW1 is characterized by the converse arrangement.

*Comparisons of samples.*—Gillraker number varies significantly among the four populations ( $F_s = 107.95$ ,  $P < 0.0001$ ), with the Rabbit Slough ( $\bar{x} = 22.35 \pm 0.326$ ) and Visnaw Lake ( $\bar{x} = 22.95 \pm 0.245$ ) samples having higher counts than the Meadow Creek ( $\bar{x} = 18.35 \pm 0.182$ ) and Mud Lake ( $\bar{x} = 17.85 \pm 0.243$ ) samples.

Two-way MANOVA revealed significant differences among samples and between sexes, for both uniform and nonuniform components of shape (Table 2). The design could not be collapsed into a one-way canonical variates analysis (CVA) because the interaction between population and sex was also significant. Thus, we performed the canonical variates analysis and ordinated the mean for each sex in each sample along the first three canonical axes (Fig. 5). This ordination reveals separation of the four samples and of males and females within each population. The Meadow Creek and Mud Lake samples are most similar, whereas the Rabbit Slough and Visnaw Lake populations are somewhat different from the other two and diverge in different directions along the first two canonical axes. A minimum length spanning tree connects each group to its nearest neighbor in morphospace.

Using the  $F$ -value corresponding to each generalized Mahalanobis distance, we performed all pairwise comparisons between treatments (i.e., each sex in each sample). All but five of 27 comparisons were significant, indicating significant differentiation among the populations (Table 3). Three of the five nonsignificant comparisons involved males and females from the

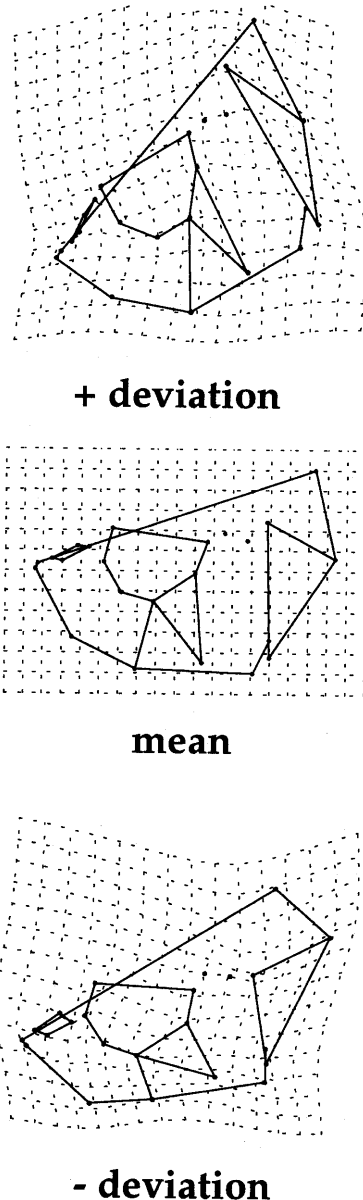


Fig. 4. Overall deformations in shape along the first relative warp for all 400 specimens. The first relative warp is the direction of maximal variation found by a principal components analysis of the partial-warp scores for all specimens. Positive deformations along this axis represent more benthivore-like trophic forms, whereas negative deformations along this axis are more planktivorous in morphology. (Landmarks specifically discussed in the text have been labeled on the mean configuration.)

TABLE 2. TWO-WAY MANOVA FOR (A) UNIFORM AND (B) NONUNIFORM COMPONENTS OF SHAPE. Analyses include all 400 specimens, using two variables for uniform components and 2 $\times$ 6 (40) variables for nonuniform components.

Source	df	Wilk's L	Fs	df1	df2	P
(A) Uniform						
Pop	3	0.04894	458.7536	6	782	<0.0001
Sex	1	0.62443	117.5832	2	391	<0.0001
Sex $\times$ Pop	3	0.45428	63.0386	6	782	<0.0001
(B) Nonuniform						
Pop	3	0.00141	70.0685	120	1058	<0.0001
Sex	1	0.42515	11.9323	40	353	<0.0001
Sex $\times$ Pop	3	0.12847	8.6763	120	1058	<0.0001

same population (Mud Lake, Rabbit Slough, and Visnaw Lake). Thus, it seems that the significant differences between sexes found in the MANOVAs (Table 2) were primarily due to the Meadow Creek population. The remaining non-significant comparisons were between Meadow Creek females and Mud Lake males and females.

To evaluate the canonical vectors for classification purposes, we calculated the generalized distance from each specimen to the mean of each group and then assigned each specimen to the group to which it had the smallest generalized distance. Using all eight groups (i.e., males and females treated separately), we found that 345 of 400 specimens (86%) were assigned to the correct group. However, because only one population (Meadow Creek) was signifi-

cantly sexually dimorphic (Table 3), we collapsed the model by disregarding sex and reassigning the specimens to the original four samples. This analysis showed that 382 of 400 specimens (95.5%) were correctly classified, indicating that 37 of the previous misclassifications were assigned to the correct sample but the incorrect sex. Thus the canonical functions performed reasonably well in discriminating between samples from the four test populations.

We generated graphical representations of landmark configurations of the mean specimen for each of the four samples to obtain a more general understanding of shape variation (Fig. 6). Compared to the Mud Lake sample, fish from Visnaw Lake had long and shallow skulls and snouts with a low supraoccipital crest, a large orbit, a short opercular link relative to the interopercular link (see Fig. 1), and a small area beneath the orbit (i.e., suspensorium). The similarity between the Mud Lake and Meadow Creek samples was conspicuous, except that Meadow Creek stickleback had more ventrally directed snouts and deeper skulls. The anadromous fish (Rabbit Slough) appeared similar to the Visnaw Lake phenotype in most respects but with a larger orbit, shallower suspensorium, and higher supraoccipital crest (Fig. 6).

#### DISCUSSION

We used geometric morphometrics to compare skull shape among four samples from the range of threespine stickleback habitats. We used MANOVA and unplanned pairwise comparisons to detect differences in skull shape among populations and between sexes. We found statistically significant differences among the four populations, but sexes were significantly different only in the Meadow Creek sample (Tables 2–3). We establish the ability of geometric morphometrics to distinguish among

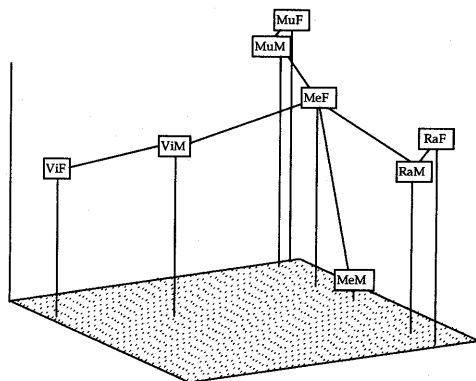


Fig. 5. Canonical variates ordination of the samples used. The first three canonical variates axes represent 45%, 30%, and 10% of the variation, respectively. The populations are connected by a minimum spanning tree. Abbreviations: Me = Meadow Creek; Mu = Mud Lake; Ra = Rabbit Slough; Vi = Visnaw Lake. M and F suffixes on lake acronyms refer to male and female specimens.

TABLE 3. MULTIPLE COMPARISONS OF NONUNIFORM COMPONENTS OF SHAPE BETWEEN ALL POSSIBLE PAIRS OF TEST SAMPLES (SEXES TREATED AS SEPARATE SAMPLES). Significant unplanned comparisons based on generalized Mahalanobis distances are denoted with an asterisk (\*), whereas those comparisons that are not significant are denoted by (ns). Abbreviations described in the legend of Figure 5.

Group	MeM	MeF	MuM	MuF	RaM	RaF	ViM	ViF
Mem	0							
MeF	6.55	0						
	*							
MuM	7.58	4.11	0					
	*	ns						
MuF	8.08	4.67	1.70	0				
	*	ns	ns					
RaM	9.43	7.79	10.19	10.65	0			
	*	*	*	*				
RaF	10.19	8.60	10.61	10.94	2.62	0		
	*	*	*	*	ns			
ViM	8.60	6.38	7.09	7.98	8.28	9.12	0	
	*	*	*	*	*	*		
ViF	11.20	9.42	9.97	10.65	10.99	11.64	4.76	0
	*	*	*	*	*	*	ns	

and describe the four samples included in this study.

We used relative warps analysis and thin-plate spline deformations to visualize qualitative differences in skull shape among samples from different habitats. The major trend in shape variation (first relative warp), ranges from short, deep skulls at one extreme to shallow, elongate skulls at the other (Fig. 4). The thin-plate spline deformations representing mean landmark configurations for samples from the two lacustrine habitats, Visnaw and Mud Lakes (Fig. 6), are almost identical to the extremes along the first relative warp (Fig. 4), with the positive extreme of RW1 resembling Mud Lake specimens and the negative extreme resembling Visnaw Lake fish. Thus, although both of these samples are from lakes, they exhibit substantial differences in skull shape. The skull morphology of the resident stream sample (Meadow Creek) closely resembles that of the Mud Lake sample, although the stream fish have deeper skulls and more ventrally directed snouts than the Mud Lake fish. The anadromous sample (Rabbit Slough) resembles the Visnaw Lake sample in most respects.

We selected anadromous, resident stream, lacustrine benthic, and lacustrine limnetic populations to span the range of habitats of threespine stickleback. Visnaw Lake fish and Mud Lake fish have limnetic and benthic morphologies, respectively (Walker, 1997), and are expected to be, based on lake characteristics, behaviorally limnetic and benthic (Walker, 1995).

Mud Lake is consistently shallow (< 2 m), and the water column is dominated by macrophytes (M. A. Bell, pers. comm.; Walker, 1997), providing conditions that favor benthic foraging (Hart and Gill, 1994). Visnaw Lake is also relatively shallow but virtually free of benthic macrophytes (M. A. Bell, pers. comm.), suggesting that it is a plankton-dominated system (Scheffer et al., 1993). Previous morphometric studies of trophic morphology have shown that, relative to benthics, limnetic threespine stickleback have long snouts (Lavin and McPhail, 1985; McPhail, 1994), shallow bodies (McPhail, 1994; Walker, 1995), and small eyes (McPhail, 1994). Gillraker number and ecotype are also correlated, with limnetics having a higher count than benthics (Gross and Anderson, 1984; Reimchen et al., 1985; McPhail, 1994). The gillraker counts and morphological differences between Mud and Visnaw Lake fish are consistent with the results of these previous studies. Similarly, gillraker counts of anadromous stickleback tend to be higher (17–25; McPhail, 1994) than those of typical freshwater populations (12–19; Hagen and Gilbertson, 1972; McPhail, 1994). The average number of gillrakers for our Rabbit Slough sample ( $22.35 \pm 0.326$ ) was within the range for anadromous fish, providing evidence that the Rabbit Slough population is not atypical in trophic morphology for populations from similar habitats. Finally, the resident stream sample (Meadow Creek) had a mean gillraker number not significantly different from the benthic sample (Mud Lake) and within the



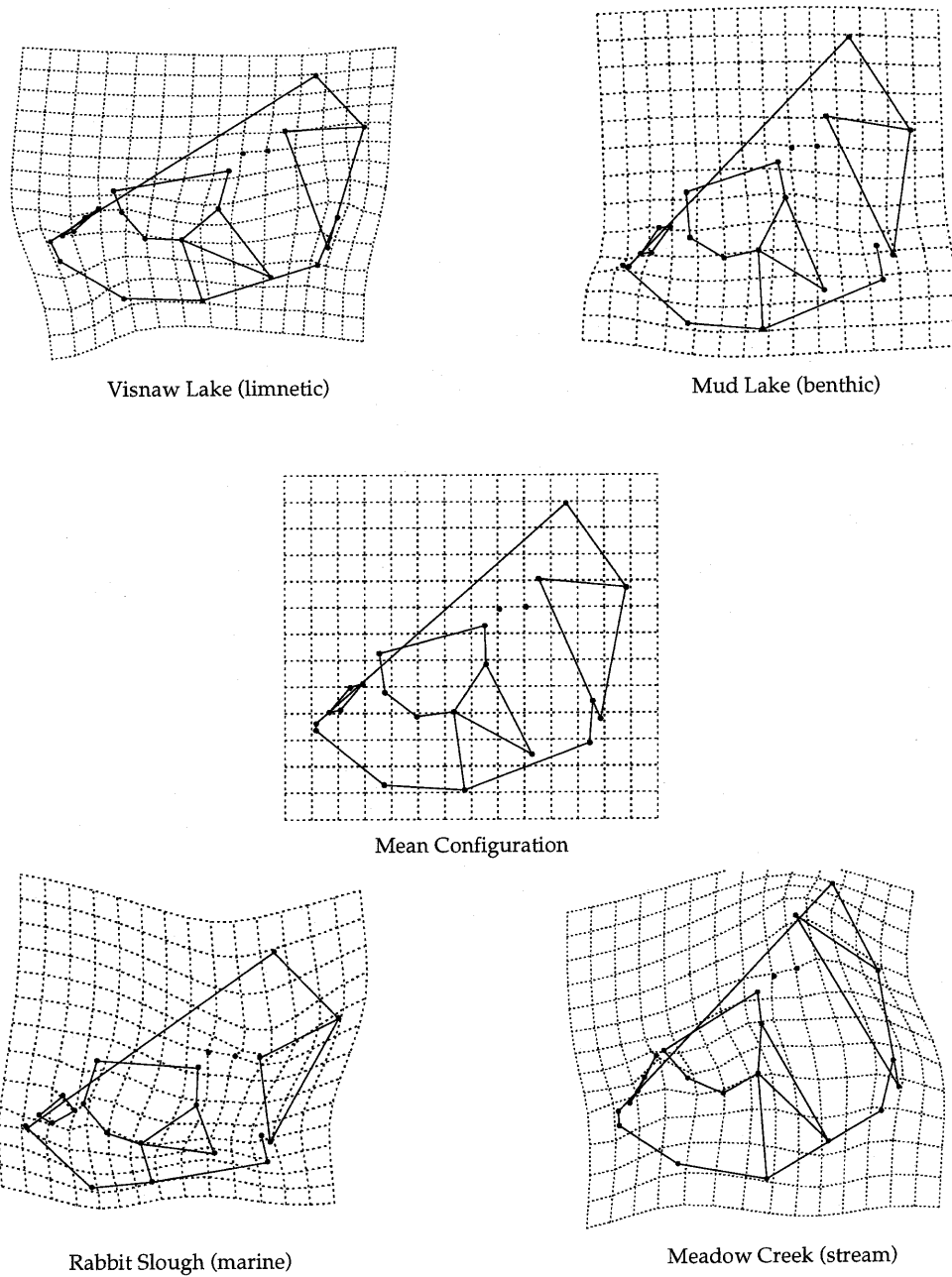


Fig. 6. Reconstructions of typical specimens from each of the four samples. Shape differences are slightly exaggerated to emphasize the differences between populations.

range of gillraker counts for previously studied populations (16.11–18.3; Reimchen et al., 1985; Schluter and McPhail, 1992; McPhail, 1994). Morphological similarities between our study

populations and those studied previously, combined with consideration of functional morphology suggests the possibility that previously undescribed variation in skull shape detected

with geometric morphometrics may prove to be characteristic of ecotypic differentiation of threespine stickleback.

Because our four samples apparently represent populations adapted to different prey types and habitats, we may explain phenotypic differences among them in terms of trophic ecology. In our samples, benthic specimens had short, deep skulls with high supraoccipital crests, deep suspensoria, short and deep snouts, and long opercular links relative to interopercular links. The limnetic specimens had long, shallow skulls with low supraoccipital crests, shallow suspensoria, long shallow snouts, and short opercular links relative to interopercular links. Liem (1993) described the differences in skull morphology typically found between suction-feeding and ram-feeding teleosts. Suction-feeders tend to have a cone-shaped buccopharyngeal cavity for maximizing buccal volume and suction velocity, and a high supraoccipital crest for insertion of epaxial musculature used in skull-elevation for hyoid depression and jaw abduction. The suspensory apparatus is deep for insertion of the levator arcus palatini muscle used in expansion of the buccal cavity. The converse is true for ram feeders, which have a cone-shaped buccopharyngeal cavity and little specialization for generating suction. Our analysis shows that Visnaw and Mud Lake samples conform to all of these expectations for ram- and suction-feeding morphologies, respectively. Orbit size, which was larger in the limnetic population, increases resolving power to detect small planktonic prey (Meer and Anker, 1984; Hart and Gill, 1994; Walton et al., 1994). Finally, with reference to the four-bar linkage model (Westneat, 1990), which describes teleost jaw abduction, the planktivorous sample had a short opercular link relative to the interopercular link, which should maximize jaw abduction speed relative to the contrasting arrangement in the benthic sample. Although this result seems confusing because jaw abduction speed should be more important in a suction feeder, Anker (1978) found that the opercular four-bar mechanism may be of primary importance only for respiratory jaw movements, with a second linkage mechanism involving the sternohyoideus muscle being responsible for feeding movements. Thus, a more detailed analysis including additional landmarks from both linkages seems warranted.

The stream sample (Meadow Creek) is morphologically similar to the lacustrine benthic sample (Mud Lake), with Meadow Creek females statistically indistinguishable from Mud Lake specimens. This counterintuitive (see Ben-

zen and McPhail, 1984) similarity of females from a sexually dimorphic sample to specimens from benthic rather than limnetic samples is consistent with findings from previous work on sexual dimorphism in stickleback dentition (WJC, unpubl. data) and poses the question of whether this dimorphism is driven by ecological or sexual selection. The similarity between the lacustrine benthic and stream samples is expected because they both feed on relatively large prey items associated with the substratum (Hart and Gill, 1994). Mean gillraker counts for Meadow Creek and Mud Lake populations were lower than counts for the two planktivorous populations (Rabbit Slough and Visnaw Lake). Because gillraker number is highly correlated with trophic ecotype (Gross and Anderson, 1984; Reimchen et al., 1985; McPhail, 1994), the similarity in gillraker number between these two sets of populations implies similar feeding habits. The Meadow Creek specimens have shorter, deeper, more ventrally directed snouts than those of Mud Lake specimens, which may be a general feature of stream-resident *G. aculeatus* to permit feeding with the body at a low angle to the substratum (Hart and Gill, 1994).

The skull morphology of the anadromous sample (Rabbit Slough) was similar to Visnaw Lake morphology, tending to be exaggerated in limnetic characteristics (e.g., shallower suspensorium) but with a higher supraoccipital crest. This deep posterior skull is generally a benthic suction-feeding characteristic but may be a non-trophic adaptation in anadromous stickleback which also tend to have deep bodies (Walker, 1995). Body form is correlated with locomotory and foraging characteristics, which would lead to the prediction that anadromous fish, which are primarily planktivorous, should more closely resemble lacustrine planktivores, putting anadromous fish and lacustrine benthics at opposite morphological extremes. Walker (1995) addressed this paradox by postulating that differences in selection on body form by predatory fishes may have caused anadromous fish to evolve deep bodies for fast acceleration. Thus, the unexpected similarity in body form between anadromous fish and lacustrine benthics could be the result of factors not directly related to foraging or feeding. Our analysis supports this conclusion, the skulls of anadromous fish being relatively deep posteriorly, which is, along with body depth, a factor in the lateral profile of the fish.

Geometric morphometric methods revealed qualitative differences in skull shape between planktivorous (anadromous and lacustrine limnetic) and benthic-feeding (stream and lacus-

trine benthic) *G. aculeatus* populations, which have not previously been described or not described in detail. The observed variation was consistent with predictions based on trophic ecology and functional morphology. Assuming that the four sampled populations are representative of their respective ecotypes, this study demonstrates the potential for geometric morphometric study of correlation between habitat and trophic morphology. Additional comparisons of skull shape among populations with known trophic ecology will determine whether our tentative conclusions are generally applicable to threespine stickleback from different habitats. This would allow a more powerful and sensitive examination of the relationship between trophic morphology and habitat than possible with linear measurements or meristic counts. Further study of sexual trophic dimorphism may also prove useful in reducing confounding factors common in comparisons among populations. Such analyses should help to elucidate the mechanisms for evolution of trophic morphology in lacustrine planktivores and benthic feeders from anadromous ancestors and, more generally, the diversification of trophic morphology in teleost species.

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