

Published in final edited form as:

*Biol J Linn Soc Lond.* 2010 November 1; 101(3): 595–608. doi:10.1111/j.1095-8312.2010.01531.x.

## Classification of threespine stickleback along the benthic-limnetic axis

James J. Willacker<sup>1,2</sup>, Frank A. von Hippel<sup>1</sup>, Peter R. Wilton<sup>3</sup>, and Kelly M. Walton<sup>4</sup>

<sup>1</sup>Department of Biological Sciences, University of Alaska Anchorage, 3211 Providence Dr., Anchorage AK 99508, USA

<sup>3</sup>Department of Biology, Carleton College, 300 N. College St., Northfield, MN 55057, USA

<sup>4</sup>Alaska Natural Heritage Program, University of Alaska Anchorage, 707 A St., Anchorage, AK 99501, USA

### Abstract

Many species of fish display morphological divergence between individuals feeding on macroinvertebrates associated with littoral habitats (benthic morphotypes) and individuals feeding on zooplankton in the limnetic zone (limnetic morphotypes). Threespine stickleback (*Gasterosteus aculeatus* L.) have diverged along the benthic-limnetic axis into allopatric morphotypes in thousands of populations and into sympatric species pairs in several lakes. However, only a few well known populations have been studied because identifying additional populations as either benthic or limnetic requires detailed dietary or observational studies. Here we develop a Fisher's linear discriminant function based on the skull morphology of known benthic and limnetic stickleback populations from the Cook Inlet Basin of Alaska and test the feasibility of using this function to identify other morphologically divergent populations. Benthic and limnetic morphotypes were separable using this technique and of 45 populations classified, three were identified as morphologically extreme (two benthic and one limnetic), nine as moderately divergent (three benthic and six limnetic) and the remaining 33 populations as morphologically intermediate. Classification scores were found to correlate with eye size, the depth profile of lakes, and the presence of invasive northern pike (*Esox lucius*). This type of classification function provides a means of integrating the complex morphological differences between morphotypes into a single score that reflects the position of a population along the benthic-limnetic axis and can be used to relate that position to other aspects of stickleback biology.

### Keywords

benthic morphotypes; Cook Inlet Basin; discriminant analysis; *Esox lucius*; *Gasterosteus aculeatus*; geometric morphometrics; limnetic morphotypes; northern pike; trophic morphotypes

## INTRODUCTION

Many species display local adaptations that allow for the efficient use of available food resources. In some groups, these adaptations result in two or more specialized morphotypes with distinct differences in body morphology, cranial-facial morphology, feeding behavior, or niche use that are recognizable across many independent populations. In freshwater fish, trophic polymorphisms are exceptionally common; they have been described for 97 species comprising 52 genera and 17 families (Robinson & Wilson, 1994). Of the accounts that deal

<sup>2</sup>Corresponding author, jjwillac@gmail.com, (907) 786-4012 (phone), (907) 786-1314 (fax).

with lacustrine species, the majority (37 out of 48) include divergence between morphotypes feeding predominantly on macroinvertebrates associated with the substrates of shallow littoral habitats (benthic morphotypes) and morphotypes feeding primarily on zooplankton suspended in the limnetic zones of lakes (limnetic morphotypes; McPhail, 1984; Robinson & Wilson, 1994; Uchii *et al.*, 2007). Differentiation along this benthic-limnetic axis is generally continuous but sometimes results in populations that feed almost exclusively on one prey type or another (McPhail, 1984).

The differences between these two morphotypes consist of numerous traits that influence the foraging efficiency of fish feeding in different habitats and on different types of prey (Schluter & McPhail, 1992; McPhail, 1994; Robinson & Wilson, 1994; Wimberger, 1994). Benthic fish typically have deeper heads and bodies, fewer gill rakers, more robust pharyngeal teeth, and smaller eyes. Benthic fish generally live in shallow areas of lakes where structurally complex habitats dominate and macroinvertebrates associated with the substrate or submerged vegetation are the most abundant prey type (Scheffer, 1998). In this environment, the deep body of the benthic morphotype provides a mechanical advantage that minimizes turning radius and increases maneuverability (Walker, 1997; Gerstner, 1999; Blake, 2004), thus increasing foraging efficiency (Werner, 1977; Ehlinger & Wilson, 1988; Walker, 1997; Svanbäck & Eklöv, 2003). The deep head of the benthic morphotype confers increased suction feeding performance, which is advantageous when feeding on large prey that are often embedded in or clinging to the substrate (Webb, 1984; Norton & Brainerd, 1993; Ferry-Graham, Bolnick & Wainwright, 2002; Carroll *et al.*, 2004). Fewer and more widely spaced gill rakers may improve benthic foraging efficiency by allowing debris taken in during suction to pass out of the buccal cavity through the gills (Werner, 1977; Gross & Anderson, 1984; Amundsen, Bohn & Vaga, 2004). In many taxa, benthic morphotypes display robust pharyngeal plates and molarform teeth which increase foraging efficiency when feeding on hard bodied prey such as snails and Sphaeriid clams that require crushing (Vermeij & Covich, 1978; Meyer, 1989; Wainwright, 2006). Smaller eyes in benthic morphotypes have not been directly linked to increased foraging performance in littoral habitats; however, Hulsey, Mims, & Streelman (2007) provide some evidence that reduced eye size may allow for more developed facial musculature.

Limnetic morphotypes have traits that are well suited for feeding on planktonic prey. Zooplankton often display patchy abundance and distributions within lakes (Omori & Hamner, 1982; del Giorgio & Gasol, 1995) and planktivorous fish often travel relatively large distances to find productive patches (Webb, 1984; Walker, 1997); this selects for the more fusiform body shape of limnetic morphotypes. The numerous, fine gill rakers of limnetic morphotypes likely increase the efficiency of feeding on small planktonic organisms by acting as a sieve to filter them out as water is forced from the buccal cavity (Magnuson & Heitz, 1971; Gibson, 1988; Gerking, 1994; Langeland & Nøst, 1995; but see Wright, O'Brien & Luecke, 1983; Drenner *et al.*, 1987; Gerking, 1994). The relatively large eye size associated with limnetic morphotypes is assumed to be an adaptation to feeding on small prey since visual acuity increases with eye size in fishes (Protasov, 1970; Land & Nilsson, 2002).

Many structures associated with feeding in teleosts are located on the skull (Liem, 1993; Gerking, 1994; Caldecutt & Adams, 1998). Jaw structure and arrangement, shape and location of the opercular bones, orbit morphology, arrangement of the suspensorial bones, and many other osteological features influence the effectiveness of feeding (Norton, 1991; Wainwright, 1996; Huckins, 1997) and vary depending on the feeding mechanism being employed (Gosline, 1973; Liem, 1993; Westneat, 2004). Since fish feeding on benthic prey typically utilize a suction feeding mechanism while fish feeding on planktonic prey typically rely more on ram feeding (Gerking, 1994), many of these features should differ between

benthic and limnetic morphotypes. These features are widespread over the skull (Liem, 1993), and thus it is difficult to measure differences in their morphology as a whole. Geometric morphometrics, a landmark based technique of measuring and analyzing shape, allows for the examination of the integrated effects of many features on the skull morphology of fish (Zelditch *et al.*, 2004).

The morphological differences found between benthic and limnetic morphotypes represent local adaptation to ecologically contrasting habitats. Trophic traits are genetically controlled (McKaye *et al.*, 1984; Lavin & McPhail, 1985; Hindar, Ryman & Stahl, 1986; McPhail, 1994; Bernatchez *et al.*, 1996; Robinson & Wilson, 1996; Bernatchez, Chouinard & Lu, 1999; Gíslason *et al.*, 1999; Lu & Bernatchez, 1999; Peichel *et al.*, 2001; Proulx & Magnan, 2004), and often evolve in parallel in populations that are independently derived (Bell & Foster, 1994b; Robinson & Wilson, 1994). Additionally, similar variation is found across many unrelated taxa (Robinson & Wilson, 1994; Taylor, 1999; Robinson & Schluter, 2000). However, some plasticity in these traits exists (Day & McPhail, 1996; Robinson & Wilson, 1996; Peres-Neto & Magnan, 2004; Proulx & Magnan, 2004).

Differentiation along the benthic-limnetic axis is evident both within and between populations and between species (Bell & Andrews, 1997). Because differentiation along the benthic-limnetic axis occurs at levels on both sides of the species “boundary”, it has been implicated as a potential means of speciation in several groups of freshwater fishes such as Arctic char (Malmquist *et al.*, 1992; Savvaitova, 1995; Adams *et al.*, 1998; Jonsson & Skúlason, 2000; Jonsson & Jonsson, 2001; Guiguer *et al.*, 2002; McCarthy *et al.*, 2004), sunfish (Mittelbach, Osenberg & Wainwright, 1992; Robinson & Wilson, 1996; Jastrebski & Robinson, 2004), and numerous other groups (Schluter & McPhail, 1992; Robinson & Wilson, 1994; Bernatchez *et al.*, 1996; Schluter, 1996; Logan *et al.*, 2000; Grey, 2001; Ostbye *et al.*, 2006).

The threespine stickleback (*Gasterosteus aculeatus* L.; hereafter referred to as ‘stickleback’) is a small fish, widely distributed throughout the boreal and temperate zones of the northern hemisphere. The species inhabits coastal marine, brackish, and an astounding array of freshwater habitats ranging from tiny ephemeral streams in arid desert regions to large Arctic lakes. Among these habitats, populations can be wholly marine, anadromous, or strict residents of fresh water (Bell & Foster, 1994b). A broad geographical and ecological distribution, together with the fragmentation of the gene pool into many thousands of isolated populations in freshwater habitats, has generated immense phenotypic diversity within the species complex. This diversity, coupled with the high incidence of repeated parallel evolution between populations, have made the species complex a model system for the study of ecological speciation (Bell & Foster, 1994b; Hendry *et al.*, 2009). Benthic and limnetic morphotypes are common in threespine stickleback and have evolved repeatedly in parallel both allopatrically in thousands of lakes around the northern hemisphere, and sympatrically in several lakes in coastal British Columbia (McPhail, 1984; Schluter & McPhail, 1992; Baker *et al.*, 2005).

Many aspects of stickleback behavior, morphology, and life-history are thought to be linked to foraging (Bell & Foster, 1994b), and have been intensively studied in the sympatric species pairs of British Columbia (Bentzen & McPhail, 1984; Nagel & Schluter, 1998; Hatfield & Schluter, 1999; Odling-Smee, Boughman & Braithwaite, 2008) and in a few well known allopatric populations (Walker, 1997; Caldecutt & Adams, 1998; Baker *et al.*, 2005; Purnell *et al.*, 2006; Travis, 2007; Snowberg & Bolnick, 2008). A relatively small number of populations have been used extensively in studies of stickleback trophic morphology because identifying additional populations as either benthic or limnetic requires detailed dietary or observational studies for all populations being considered. In this study we

develop a classification function based on the skull morphology of previously confirmed benthic and limnetic stickleback populations from the Cook Inlet Basin of Alaska and test the feasibility of using this function to identify other morphologically divergent populations. This type of classification function provides a means of integrating the many complex morphological differences between morphotypes into a single score that reflects the relative position of a population along the benthic-limnetic axis. We also demonstrate how the “benthic-limnetic” scores produced by such a classification function can be used to test hypotheses regarding the relationship between trophic morphotype and lake habitat.

## METHODS

### Fish Collection and Preservation

Threespine stickleback were collected from 45 freshwater lakes throughout the Cook Inlet Basin (Fig. 1; Table 1) between 2002 and 2009 using unbaited 1/8" or 1/4" wire mesh minnow traps set from shore. Trapped stickleback were sacrificed with an overdose of MS-222 anesthetic, rinsed in lake water, and fixed in 10% formalin buffered with dolomite. After 2 to 8 weeks fish were removed from formalin, rinsed in a running water bath, and preserved in 70% ethanol. All fish were then bleached in a 0.05% hydrogen peroxide solution to remove pigmentation and stained with 1% alizarin red S to facilitate the analysis of bony structures.

### Morphological Analysis

Morphological analyses were completed using approximately 40 (minimum of 36) fish from each population. Only fish measuring > 32 mm standard length (SL; anterior tip of premaxilla to posterior border of hyperal plate) were included because most stickleback have developed adult body shape by this size (Walker, 1993). Photographs of the left lateral aspect of the head of each fish were taken using a digital camera mounted on a fluorescent dissecting scope (Leica MZFLIII microscope with a Leica DFC340FX camera). Photographs were adjusted for brightness and contrast. In order to cover all features of the head, up to four photographs were taken; multiple photographs of a specimen were merged into a single composite image using a reposition only function in Adobe Photoshop (version 10.0.1).

Twenty biologically homologous anatomical landmarks that cover the form of the stickleback skull (Fig. 2; Cresko & Lum, unpublished) were digitized using the tpsDIG2 program (version 2.12; Rohlf, 2008a). One landmark was not included in analyses due to inconsistent visibility and repeatability. The remaining 19 landmarks were aligned using the generalized Procrustes procedure and the resulting configurations used to obtain partial warps in the tpsRelw software package (version 1.46; Rohlf, 2008b). In addition to geometric morphometric data, vertical orbit diameter was also measured from the digital images using the measuring tool in tpsDig2.

### Development of Classification

Partial warp scores were used in the R statistical package (version 2.9.1; www.r-project.org) to develop a Fisher's linear discriminant function (FLDA) to classify specimens along the axis that best separates benthic and limnetic stickleback. A FLDA was used rather than a standard linear discriminant analysis (LDA) because it does not assume normally distributed classes or equal class covariances as LDA does. When the assumptions of LDA are met the two methods are equivalent (Hair et al., 2009). When both FLDA and LDA were compared with these data the results were qualitatively the same (Willacker, 2009).

The FLDA was developed using the variance-covariance matrix of partial warp scores from three known “extreme” benthic populations (Corcoran, Mud, and Tern Lakes) and three known “extreme” limnetic populations (Long, Lynne, and South Rolly Lakes) as a training set for establishing the separation of benthic and limnetic morphotypes. The populations used to develop this discriminant function were chosen based on studies of stickleback diet and feeding behavior that have shown them to be the most extreme of each morphotype known in the Cook Inlet Basin (Walker, 1997; Caldecutt & Adams, 1998; Foster, Scott & Cresko, 1998; Purnell *et al.*, 2006; Purnell *et al.*, 2007; Travis, 2007; Karve, von Hippel & Bell, 2008; von Hippel, 2008; M. Travis, unpublished data; P. Park, unpublished data). Following FLDA, a Hotelling’s  $T^2$  test was performed in R to confirm that the differentiation between the groups was significant. Cross validation using a leave-one-out procedure that drew repeated (10 000 iterations) random samples ( $n = 100$ ) from each training group was used to predict the group to which each individual in the training set belonged and assess the accuracy of classifications within the training set. A leave-one-out approach was used to cross-validate the training set because the division of the dataset into separate development and validation samples would have resulted in a loss of statistical power. This method does not compromise the validity of the cross-validation (Hair *et al.*, 2009). Following cross-validation, the FLDA classification function was used to calculate discriminant scores for each individual from the populations of uncertain morphotype. These individual discriminant scores were then used to calculate population mean discriminant scores (PMDS). The distributions of individual discriminant scores across populations were normally distributed, as were the PMDS (Willacker, 2009). The PMDS were adjusted so that the inter-population mean was zero. The divergences between PMDS and the inter-population mean were then used to classify populations as extreme benthic/limnetic adapted (PMDS  $>2$  SD from inter-population mean), moderately benthic/limnetic adapted (PMDS 1-2 SD from inter-population mean), or intermediate (PMDS  $<1$  SD from inter-population mean).

### Statistical Analysis

Data on 13 lake habitat variables (Table 2), stocking history and the presence of invasive northern pike (*Esox lucius* L.; Table 1) were included in habitat analyses. Statistical analyses were carried out using the R statistical package (version 2.9.1). The PMDS was used in all statistical tests utilizing discriminant scores.

Linearly measured morphometric traits are impacted by the size of the fish. Therefore, the mean orbit diameter of each population was regressed against its mean centroid size and the residuals were used in subsequent analyses. The association between PMDS and the residuals of orbit diameter was tested using Pearson’s product-moment correlation.

Principle components analysis was used to reduce the dimensionality of the lake habitat variables and identify groups of variables that best characterized lake habitat differences. The significance of loadings on each principle component (PC) was assessed using the power-level method outlined in Hair *et al.* (2009). Using this method, only loadings of  $\pm 0.80$  or greater were considered significant. The relationships between principle components with significant loadings and PMDS were assessed using Pearson’s product-moment correlation. Complete habitat data were only available for 17 of the 45 lakes and therefore the sample size in these correlations was small. Because of this, the correlations were also performed using the non-parametric Spearman rank correlation. Results were qualitatively the same and only the parametric correlations are reported.

Two *t*-tests were conducted to test for differences in PMDS due to the stocking of salmonids and the invasion of northern pike.

## RESULTS

### Classification Function

The discriminant scores for individuals in the training set showed a bimodal distribution (Fig. 3). Individual scores for fish from the benthic training group ( $n = 120$  fish from three populations) ranged from  $-10.83$  to  $-0.09$  with a mode of  $-5.98$ , while individual scores for the limnetic training group ( $n = 120$  fish from three populations) ranged from  $-0.69$  to  $10.28$  with a mode of  $4.23$ . Less than 2% of individuals fell into the range of overlap.

Morphological differences between the two training groups were readily apparent in images of the fish (Fig. 4 A and B). Relative warps analysis and thin-plate spline deformations illustrate that fish with lower discriminant scores (more benthic) typically have shorter, deeper skulls and a shorter snout while fish with higher discriminant scores have shallow, elongate skulls with longer snouts (Fig. 4 C and D). The results of a Hotelling's  $T^2$  test confirmed that the apparent differences in the two training groups were significant ( $T^2 = 32.30$ ;  $F_{34, 203} = 1.49$ ;  $p < 0.0001$ ). Cross-validation of the training set showed that 97.1% of all individuals were classified into the correct morphotype when repeated random samples were drawn.

When the classification function was applied to individuals from populations of uncertain morphotypes, individual scores ranged from  $-12.28$  to  $11.35$  (mode =  $0.62$ ). Extreme populations had mean discriminant scores greater than  $\pm 5.45$  ( $2+ SD$  from the inter-population mean), moderately adapted populations had scores between  $\pm 2.73$  and  $\pm 5.45$  ( $1-2 SD$ ), and intermediate populations were less than  $\pm 2.73$  ( $<1 SD$ ; Willacker, 2009). Of the 45 populations examined, three were identified as morphologically extreme (Fig. 5): Mud and Tern Lakes were classified as extreme benthic populations (PMDS =  $-6.95$  and  $-6.87$  respectively), while South Rolly Lake was classified as an extreme limnetic (PMDS =  $5.48$ ). Additionally, Corcoran, Walby and Watson lakes were also classified as moderately adapted to benthic habitat (PMDS =  $-5.11$ ,  $-4.33$ , and  $-4.59$  respectively), while Boot, Caswell, Grouse, Long, Lynne, and Milo lakes were classified as moderately adapted to limnetic conditions (PMDS =  $2.89$ ,  $2.73$ ,  $2.84$ ,  $4.08$ ,  $3.71$ , and  $3.88$  respectively). The remaining 33 populations were classified as morphologically intermediate. All six populations used in the training set (Mud, Tern, Corcoran, Long, Lynne, South Rolly) had PMDS  $>1SD$  from the inter-population mean (Fig. 5).

### Orbit Diameter

Population mean discriminant score was significantly correlated with residual orbit diameter; fish from populations with a more benthic PMDS had a significantly larger orbit diameter than fish from populations with a more limnetic score ( $r = -0.47$ ,  $df = 43$ ,  $P = 0.001$ ).

### Habitat Covariates

Of the 13 habitat variables measured, five yielded significant loadings on a principle component. The percent of the lake less than 1.52 m deep, the percent of the lake less than 3.04 m deep, and the mean lake depth all had significant loadings on PC1 (loadings =  $0.93$ ,  $0.91$ , and  $-0.83$  respectively), while alkalinity and calcium concentration had a significant loading on PC2 (loadings =  $0.86$ , and  $0.80$  respectively). Principal component 1 was found to be significantly correlated with PMDS ( $r = -0.70$ ,  $P = 0.007$ ); however, PC2 was not ( $r = -0.50$ ,  $P = 0.084$ ).

## Predatory Fish

In lakes where northern pike had been recorded, stickleback had a significantly higher discriminant score (PMDS = 1.16,  $n = 15$ ) than in lakes where pike were not recorded (PMDS = -0.57,  $n = 30$ ,  $t = -2.08$ ,  $df = 43$ ,  $P = 0.022$ ). No significant difference in PMDS was found between lakes stocked with salmonids (PMDS = 0.54,  $n = 19$ ) and unstocked lakes (PMDS = -0.24,  $n = 26$ ,  $t = -1.75$ ,  $df = 43$ ,  $P = 0.087$ ).

## DISCUSSION

Results of discriminant analysis indicate differences in the skull morphologies of known benthic and limnetic stickleback populations, and that these differences accurately separate the two groups. Stickleback with a lower discriminant score typically had shorter, deeper heads as has been reported previously for benthically feeding fish and likewise the skull morphology of fish with higher discriminant scores was characteristic of that expected for a limnetically feeding fish (Bentzen & McPhail, 1984; Bentzen, Ridgway & McPhail, 1984; McPhail, 1984; Bell & Foster, 1994a; Caldecutt & Adams, 1998). Cross-validation demonstrated that the separation of these two groups was independent of the individuals included in the training set. These results show that skull morphology is an accurate and effective means of differentiating between benthic and limnetic morphotypes of threespine stickleback.

We are unable to test the applicability or comparability of this classification function in other contexts (e.g., with stickleback benthic-limnetic species pairs from British Columbia) due to the lack of comparable morphometric data. While the classification function presented here is specific to benthic-limnetic divergence in Cook Inlet populations of threespine stickleback, similar functions could be constructed for other systems and compared between systems. However, it should be noted that the effectiveness of this technique is maximized by using training sets specific to the region and traits of interest.

Comparisons of eye size between populations show that fish from populations with a lower (more benthic) PMDS have significantly larger eyes than populations with a higher (more limnetic) score. These results are contrary to what has been reported for threespine stickleback (e.g., McPhail, 1984; Schluter, 1993; Walker, 1997), and many other species of fish (e.g., Robinson & Wilson, 1994; Snorrason et al., 1994; Taylor, 1999) that show morphological variation along the benthic-limnetic axis. However, larger eyes have been observed in benthic morphotypes of some fish species (e.g., cichlids: Kassam *et al.*, 2003a; Kassam *et al.*, 2003b) and in a polymorphic population of threespine stickleback in Benka Lake, Alaska (W. Cresko, personal communication).

Larger eye size is associated with the need for increased visual sensitivity due to reduced light availability and/or the need for high spatial resolution (Walls, 1963; Archer, 1999; Motani *et al.*, 1999; Land & Nilsson, 2002; Thomas *et al.*, 2006). Larger eye size is generally associated with limnetic morphotypes because the prey are small. In contrast, large eyes are assumed to be unnecessary for benthic feeding because benthic prey are relatively large. However, not all benthic prey are large; in some lakes the benthic macroinvertebrate community is dominated by small water mites, Chironomidae, or oligochaets that are the size of many zooplankton (Hanson, Prepas & Mackay, 1989; Rasmussen, 1993). Additionally, the littoral environment is typically more complex and benthic prey are better concealed (Ivlev, Magill & Scott, 1964; Diggins, Summerfelt & Mnich, 1979; Crowder & Cooper, 1982). Thus, high visual acuity may be required to identify and target prey amongst the structural components (Kassam et al., 2003b). Finally, littoral habitats often have reduced light availability due to increased turbidity, dissolved compounds (staining), and/or shading (Utne, 1997; Steedman, Kushneriuk & France, 2001; Wetzel, 2001), necessitating

larger eyes to retain adequate visual sensitivity. For these reasons larger eyes could be advantageous for benthic fish under some conditions.

Of the 13 habitat lake variables examined, five displayed significant loadings onto a PC. Principle component 1 included three significant variables relating to the depth profile and accounted for 34% of the variation between lakes. Discriminant score was positively correlated with PC1, indicating that shallow lakes (higher percentage of lake area less than 1.52 and 3.05 m deep, and a shallower mean depth) had lower discriminant scores and thus more benthic morphologies. This result was expected since shallow lakes have a greater relative littoral area and thus more benthic habitat than deeper lakes. In shallow lakes, benthic prey would almost certainly dominate, and zooplankton would most likely comprise only a small portion of the available prey.

Principle component 2 included two significant variables (alkalinity and calcium concentration), both representing the prevalence of dissolved cations in the water, and accounted for an additional 20% of the variation between lakes. Population mean discriminant score was not significantly correlated with PC2, suggesting that while these variables represent important habitat differences between lakes, they are not important determinants of the observed morphological variation.

While salmonids did not have a significant impact on stickleback skull morphology, the presence of invasive northern pike was correlated with higher (more limnetic) PMDS. The difference in the impact of these two predators likely results from their different foraging behaviors. Salmonids typically forage in both littoral and limnetic habitats (Sandlund *et al.*, 1987; L'Abée-Lund, Langeland & Saegrov, 1992; Vander Zanden & Vadeboncoeur, 2002) while pike are specialized to forage in littoral environments (Chapman & Mackay, 1984; Vollestad, Skurdal & Qvenild, 1986). When littoral predators, such as pike, dominate a lake system, prey fish may be forced out of the littoral zone and into pelagic refugia (Grimm & Backx, 1990; Jacobsen & Perrow, 1998; Burks *et al.*, 2002). Under these conditions, benthic feeding in the littoral zone carries the cost of increased predation risk and thus there may be increased selection for effective limnetic foraging. Therefore, the increased prevalence of stickleback with limnetic skull morphology in lakes known to contain invasive pike is likely the result of a predator driven habitat shift. It is noteworthy that pike in Cook Inlet Basin lakes tend to drive stickleback populations to extinction except in those lakes with limnetic refugia (Haught, 2009).

This study demonstrates the ability to distinguish benthic and limnetic morphotypes of threespine stickleback using a classification function based on skull morphology. Such a classification function can be used to identify populations that are divergent along the benthic-limnetic axis for studies of the influence of trophic morphology on genetics, life history, reproductive behavior, physiology, or other traits. This classification could be improved upon by the inclusion of other variables such as gill-raker and dental morphology, diet, and stable isotope ratios of tissues. Classification functions such as the one presented here allow for the reduction of the many, often complex, differences between morphotypes into a single score that reflects the relative position of a population along the benthic-limnetic axis, and are thus a powerful tool for examining the role trophic specialization plays in the divergence of species. As illustrated in this paper, these classification functions can also be used to gain insights into the ways in which populations evolve in response to ecological conditions, such as lake morphometry and exotic predators.

## Acknowledgments

The authors would like to thank W. Cresko and K. Lum for providing the skull morphology landmark set and three anonymous reviewers for their comments on the manuscript. D. Adams, W. Aguirre and J. Rohlf provided technical



assistance with geometric morphometrics. M.A. Bell provided specimens from Walby and Beaver House Lakes. All other stickleback were collected under Alaska Department of Fish and Game permit numbers SF-2001-062, SF-2002-002, SF-2003-019, SF-2004-012, SF-2005-020, SF-2006-017, SF-2007-026, SF-2008-059 and SF-2009-016. All work was approved by the UAA IACUC. Funding was provided by National Science Foundation grant DEB 0320076 and University of Alaska Anchorage Faculty Development Grants to FAvH, and a National Science Foundation Research Experiences for Undergraduates site award to UAA. Additional support came from the Alaska INBRE program, grant number 5P20RR016466 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NCRR or NIH.

## LITERATURE CITED

- Adams CE, Fraser D, Huntingford FA, Greer RB, Askew CM, Walker AF. Trophic polymorphism amongst Arctic charr from Loch Rannoch, Scotland. *Journal of Fish Biology*. 1998; 52:1259–1271.
- Amundsen PA, Bohn T, Vaga GH. Gill raker morphology and feeding ecology of two sympatric morphs of European whitefish (*Coregonus lavaretus*). *Annales Zoologici Fennici*. 2004; 41:291–300.
- Archer, SN. Adaptive Mechanisms in the Ecology of Vision. Kluwer Academic Publishers; Boston, MA: 1999.
- Baker JA, Cresko WA, Foster SA, Heins DC. Life-history differentiation of benthic and limnetic ecotypes in a polytypic population of threespine stickleback (*Gasterosteus aculeatus*). *Evolutionary Ecology Research*. 2005; 7:121–131.
- Bell, MA.; Andrews, CA. Evolutionary consequences of postglacial colonization of freshwater by primitively anadromous fishes. In: Streit, B.; Stadler, T.; Lively, CM., editors. *Evolutionary Ecology of Freshwater Animals*. Birkhauser Verlag; Boston, MA: 1997. p. 323-363.
- Bell, MA.; Foster, SA. *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press; New York, NY: 1994a.
- Bell, MA.; Foster, SA. Introduction to the evolutionary biology of the threespine stickleback. In: Bell, MAaF, S. A., editor. *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press; Oxford: 1994b. p. 1-27.
- Bell MA, Ortí G. Pelvic reduction in thespine stickleback from Cook Inlet lakes: geographical distribution and intrapopulation variation. *Copeia*. 1994; 2:314–325.
- Bell MA, Ortí G, Walker JA, Koenings JP. Evolution of pelvic reduction in threespine stickleback fish: a test of competing hypotheses. *Evolution*. 1993; 47:906–914.
- Bentzen P, McPhail JD. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): specialiation for alternative trophic niches in the Enos Lake species pair. *Canadian Journal of Zoology*. 1984; 62:2280–2286.
- Bentzen P, Ridgway MS, McPhail JD. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): spatial segregation and seasonal habitat shifts in the Enos Lake species pair. *Canadian Journal of Zoology*. 1984; 62:2436–2439.
- Bernatchez L, Chouinard A, Lu G. Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, *Coregonus* sp., as a case study. *Biological Journal of the Linnean Society*. 1999; 68:173–194.
- Bernatchez L, Vuorinen JA, Bodaly RA, Dodson JJ. Genetic evidence for reproductive isolation and multiple origins of sympatric trophic ecotypes of whitefish (*Coregonus*). *Evolution*. 1996; 50:624–635.
- Blake RW. Fish functional design and swimming performance. *The Journal of Fish Biology*. 2004; 65:1193–1222.
- Burks RL, Lodge DM, Jeppesen E, Lauridsen TL. Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. *Freshwater Biology*. 2002; 47:343–365.
- Caldecutt WJ, Adams DC. Morphometrics of trophic osteology in the threespine stickleback, *Gasterosteus aculeatus*. *Copeia*. 1998:827–838.
- Carroll AM, Wainwright PC, Huskey SH, Collar DC, Turingan RG. Morphology predicts suction feeding performance in Centrarchid fishes. *Journal of Experimental Biology*. 2004; 207:3873–3881. [PubMed: 15472018]

- Chapman CA, Mackay WC. Direct observation of habitat utilization by northern pike. *Copeia*. 1984;255–258. 1984.
- Crowder LB, Cooper WE. Habitat structural complexity and the interaction between bluegills and their prey. *Ecology*. 1982; 63:1802–1813.
- Day T, McPhail JD. The effect of behavioural and morphological plasticity on foraging efficiency in the threespine stickleback (*Gasterosteus* sp). *Oecologia*. 1996; 108:380–388.
- del Giorgio PA, Gasol JM. Biomass distribution in freshwater plankton communities. *The American Naturalist*. 1995; 146:135–152.
- Diggins MR, Summerfelt RC, Mních MA. Altered feeding electivity of the bluegill from increased prey accessibility following macrophyte removal. *Proceedings of the Oklahoma Academy of Science*. 1979; 59:4–11.
- Drenner R, Hambright KD, Vinyard GL, Gophen M. Particle ingestion by *Tilapia galilaea* is not affected by removal of gill rakers and microbranchiospines. *Transactions of the American Fisheries Society*. 1987; 116:272–276.
- Ehlinger TJ, Wilson DS. Complex foraging polymorphism in bluegill sunfish. *Proceedings of the National Academy of Sciences*. 1988; 85:1878–1882.
- Ferry-Graham LA, Bolnick DI, Wainwright PC. Using functional morphology to examine the ecology and evolution of specialization. *Integrative and Comparative Biology*. 2002; 42:265–277.
- Foster SA, Scott RJ, Cresko WA. Nested biological variation and speciation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*. 1998; 353:207–218.
- Gerking, SD. *Feeding Ecology of Fish*. Academic Press; San Diego, CA: 1994.
- Gerstner CL. Maneuverability of four species of coral-reef fish that differ in body and pectoral-fin morphology. *Canadian Journal of Zoology*. 1999; 77:1102–1110.
- Gibson RN. Development, morphometry and particle retention capability of the gill rakers in the herring, *Clupea harengus* L. *Journal of Fish Biology*. 1988; 32:949–962.
- Gíslason D, Ferguson MM, Skúlason S, Snorrason SS. Rapid and coupled phenotypic and genetic divergence in Icelandic Arctic char (*Salvinus alpinus*). *Canadian Journal of Fisheries and Aquatic Sciences*. 1999; 56:2229–2234.
- Gosline, WA. *Functional Morphology and Classification of Teleostean Fishes*. University of Hawaii Press; Honolulu, HI: 1973.
- Grey J. Ontogeny and dietary specialisation in brown trout (*Salmo trutta* L.) from Loch Ness, Scotland, examined using stable isotopes of carbon and nitrogen. *Ecology of Freshwater Fish*. 2001; 10:168–176.
- Grimm MP, Backx J. The restoration of shallow eutrophic lakes, and the role of northern pike, aquatic vegetation and nutrient concentration. *Hydrobiologia*. 1990; 200:557–566.
- Gross HP, Anderson JM. Geographic variation in the gillrakers and diet of european threespine sticklebacks, *Gasterosteus aculeatus*. *Copeia*. 1984; 1984:87–97.
- Guiguer K, Reist JD, Power M, Babaluk JA. Using stable isotopes to confirm the trophic ecology of Arctic charr morphotypes from Lake Hazen, Nunavut, Canada. *Journal of Fish Biology*. 2002; 60:348–362.
- Hair, JF.; Black, WC.; Anderson, RE.; Babin, BJ. *Multivariate Data Analysis*. Prentice Hall; Upper Saddle River, NJ: 2009.
- Hanson JM, Prepas EE, Mackay WC. Size distribution of the macroinvertebrate community in a freshwater lake. *Canadian Journal of Fisheries and Aquatic Sciences*. 1989; 46:1510–1519.
- Hatfield T, Schluter D. Ecological speciation in sticklebacks: environment-dependent hybrid fitness. *Evolution*. 1999; 53:866–873.
- Haight, SB. Unpublished Master of Science. University of Alaska Anchorage; 2009. Threespine stickleback extirpation and evolution in the face of northern pike invasion.
- Hendry AP, Bolnick DI, Berner D, Peichel CL. Along the speciation continuum in stickleback. *Journal of Fish Biology*. 2009; 75:2000–2036. [PubMed: 20738669]
- Hindar K, Ryman N, Stahl G. Genetic differentiation among local populations and morphotypes of Arctic charr, *Salvelinus alpinus*. *Biological Journal of the Linnean Society*. 1986; 27:269–285.

- Huckins CJF. Functional linkages among morphology, feeding performance, diet, and competitive ability in molluscivorous sunfish. *Ecology*. 1997; 78:2401–2414.
- Hulseley CD, Mims MC, Streebman JT. Do constructional constraints influence cichlid craniofacial diversification? *Proceedings of the Royal Society B*. 2007; 274:1867–1875. [PubMed: 17519189]
- Ivlev, VS.; Magill, DW.; Scott, D. *Experimental Ecology of the Feeding of Fishes*. Yale University Press; New Haven, CT: 1964.
- Jacobsen L, Perrow MR. Predation risk from piscivorous fish influencing the diel use of macrophytes by planktivorous fish in experimental ponds. *Ecology of Freshwater Fish*. 1998; 7:78–86.
- Jastrebski CJ, Robinson BW. Natural selection and the evolution of replicated trophic polymorphisms in pumpkinseed sunfish (*Lepomis gibbosus*). *Evolutionary Ecology Research*. 2004; 6:285–305.
- Jones JR, Bell MA, Baker JA, Koenings JP. General limnology of lakes near Cook Inlet, southcentral Alaska. *Lake and Reservoir Management*. 2003; 19:141–149.
- Jonsson B, Jonsson N. Polymorphism and speciation in Arctic charr. *Journal of Fish Biology*. 2001; 58:605–638.
- Jonsson B, Skúlason S. Polymorphic segregation in Arctic charr *Salvelinus alpinus* (L.) from Vatnshlidarvatn, a shallow Icelandic lake. *Biological Journal of the Linnean Society*. 2000; 69:55–74.
- Karve AD, von Hippel FA, Bell MA. Isolation between sympatric anadromous and resident threespine stickleback species in Mud Lake, Alaska. *Environmental Biology of Fishes*. 2008; 81:287–296.
- Kassam DD, Adams DC, Ambali AJD, Yamaoka K. Body shape variation in relation to resource partitioning within cichlid trophic guilds coexisting along the rocky shore of Lake Malawi. *Animal Biology*. 2003a; 53:59–70.
- Kassam DD, Adams DC, Hori M, Yamaoka K. Morphometric analysis on ecomorphologically equivalent cichlid species from Lakes Malawi and Tanganyika. *Journal of Zoology*. 2003b; 260:153–157.
- Koenings, JP.; Edmundson, JA.; Kyle, GB.; Edmundson, JM. *Limnology field and laboratory manual: methods for assessing aquatic production*. Alaska Department of Fish and Game: FRED Division Report; 1987. p. 212Series 71
- L'Abée-Lund JH, Langeland A, Saegrov H. Piscivory by brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.) in Norwegian lakes. *Journal of Fish Biology*. 1992; 41:91–101.
- Land, MF.; Nilsson, D-E. *Animal Eyes*. Oxford University Press; New York, NY: 2002.
- Langeland A, Nøst T. Gill raker structure and selective predation on zooplankton by particulate feeding fish. *Journal of Fish Biology*. 1995; 47:719–732.
- Lavin PA, McPhail JD. The evolution of freshwater diversity in the threespine stickleback (*Gasterosteus aculeatus*): site-specific differentiation of trophic morphology. *Canadian Journal of Zoology*. 1985; 63:2632–2638.
- Liem, KF. Ecomorphology of the teleostean skull. In: Hanken, J.; Hall, BK., editors. *The Skull: Functional and Evolutionary Mechanisms*. University of Chicago Press; Chicago, IL: 1993. p. 422–452.
- Logan MS, Iverson SJ, Ruzzante DE, Walde SJ, Macchi PJ, Alonso MF, Cussac VE. Long term diet differences between morphs in trophically polymorphic *Percichthys trucha* (Pisces: Percichthyidae) populations from the southern Andes. *Biological Journal of the Linnean Society*. 2000; 69:599–616.
- Lu G, Bernatchez L. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. *Evolution*. 1999; 53:1491–1505.
- Magnuson JJ, Heitz JG. Gill raker apparatus and food selectivity among mackerels, tunas, and dolphins. *Fish. Bull.* 1971; 69:361–370.
- Malmquist HJ, Snorrason SS, Skúlason S, Jonsson B, Sandlund OT, Jónasson PM. Diet Differentiation in Polymorphic Arctic Charr in Thingvallavatn, Iceland. *The Journal of Animal Ecology*. 1992; 61:21–35.
- McCarthy ID, Fraser D, Waldron S, Adams CE. A stable isotope analysis of trophic polymorphism among Arctic charr from Loch Ericht, Scotland. *Journal of Fish Biology*. 2004; 65:1435–1440.

- McKaye KR, Kocher T, Reinthal P, Harrison R, Kornfield I. Genetic evidence for allopatric and sympatric differentiation among color morphs of a Lake Malawi Cichlid fish. *Evolution*. 1984; 38:215–219.
- McPhail JD. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. *Canadian Journal of Zoology*. 1984; 62:1402–1408.
- McPhail, JD. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. In: Bell, MA.; Foster, SA., editors. *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press; Oxford, UK: 1994. p. 399-437.
- Meyer A. Cost of morphological specialization: feeding performance of the two morphs in the trophically polymorphic cichlid fish, *Cichlasoma citrinellum*. *Oecologia*. 1989; 80:431–436.
- Mittelbach GG, Osenberg CW, Wainwright PC. Variation in resource abundance affects diet and feeding morphology in the pumpkinseed sunfish (*Lepomis gibbosus*). *Oecologia*. 1992; 90:8–13.
- Motani R, Rothschild BM, Wahl W Jr. Large eyeballs in diving ichthyosaurs. *Nature*. 1999; 402:747.
- Nagel L, Schluter D. Body size, natural selection, and speciation in sticklebacks. *Evolution*. 1998; 52:209–218.
- Norton SF. Capture success and diet of cottid fishes: the role of predator morphology and attack kinematics. *Ecology*. 1991; 72:1807–1819.
- Norton SF, Brainerd EL. Convergence in the feeding mechanics of ecomorphologically similar species in the Centrarchidae and Cichlidae. *Journal of Experimental Biology*. 1993; 176:11–29.
- Odling-Smee LC, Boughman JW, Braithwaite VA. Sympatric species of threespine stickleback differ in their performance in a spatial learning task. *Behavioral Ecology and Sociobiology*. 2008; 62:1935–1945.
- Omori M, Hamner WM. Patchy distribution of zooplankton: behavior, population assessment and sampling problems. *Marine Biology*. 1982; 72:193–200.
- Ostbye K, Amundsen PA, Bernatchez L, Klemetsen A, Knudsen R, Kristoffersen R, Naesje TF, Hindar K. Parallel evolution of ecomorphological traits in the European whitefish *Coregonus lavaretus* (L.) species complex during postglacial times. *Molecular Ecology*. 2006; 15:3983–4001. [PubMed: 17054498]
- Peichel CL, Nereng KS, Ohgi KA, Cole BLE, Colosimo PF, Buerkle CA, Schluter D, Kingsley DM. The genetic architecture of divergence between threespine stickleback species. *Nature*. 2001; 414:901–905. [PubMed: 11780061]
- Peres-Neto PR, Magnan P. The influence of swimming demand on phenotypic plasticity and morphological integration: a comparison of two polymorphic charr species. *Oecologia*. 2004; 140:36–45. [PubMed: 15127286]
- Protasov, VR. *Vision and Near Orientation in Fish*. Israel Program for Scientific Translations; Jerusalem, Israel: 1970.
- Proulx R, Magnan P. Contribution of phenotypic plasticity and heredity to the trophic polymorphism of lacustrine brook charr (*Salvelinus fontinalis* M.). *Evolutionary Ecology Research*. 2004; 6:503–522.
- Purnell MA, Hart PJB, Baines DC, Bell MA. Quantitative analysis of dental microwear in threespine stickleback: a new approach to analysis of trophic ecology in aquatic vertebrates. *Journal of Animal Ecology*. 2006; 75:967–977. [PubMed: 17009760]
- Purnell MA, Hart PJB, Baines DC, Bell MA. Correlated evolution and dietary change in fossil stickleback. *Science*. 2007; 317:1887. [PubMed: 17901325]
- Rasmussen JB. Patterns in the size structure of littoral zone macroinvertebrate communities. *Canadian Journal of Fisheries and Aquatic Sciences*. 1993; 50:2192–2207.
- Robinson, BW.; Schluter, D. Natural selection and the evolution of adaptive genetic variation in northern freshwater fishes. In: Mousseau, TA.; Sinervo, B.; Endler, JA., editors. *Genetic Variation in the Wild*. Vol. 65. Oxford University Press; New York, NY: 2000. p. 94
- Robinson BW, Wilson DS. Character release and displacement in fishes: a neglected literature. *The American Naturalist*. 1994; 144:596–627.
- Robinson BW, Wilson DS. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). *Evolutionary Ecology*. 1996; 10:631–652.

- Rohlf, FJ. tpsDIG2. version 2.12. Department of Ecology and Evolution, State University of New York at Stony Brook; 2008a.
- Rohlf, FJ. tpsRelw. version 1.46. Department of Ecology and Evolution, State University of New York at Stony Brook; 2008b.
- Sandlund OT, Jonsson B, Malmquist HJ, Gydemo R, Lindem T, Skúlason S, Snorrason SS, Jónasson PM. Habitat use of arctic charr *Salvelinus alpinus* in Thingvallavatn, Iceland. *Environmental Biology of Fishes*. 1987; 20:263–274.
- Savvaitova, KA. Patterns of diversity and processes of speciation in Arctic char. In: Klemetsen, A.; Jonsson, B.; Elliott, JM., editors. *Proceedings of the Third international charr symposium June 13-18 1994; Trondheim, Norway*. Drottningholm, Sweden: Soetvattenslaboratoriet; 1995. p. 81-91.
- Scheffer, M. *Ecology of Shallow Lakes*. Chapman & Hall; New York, NY: 1998.
- Schluter D. Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. *Ecology*. 1993; 74:699–709.
- Schluter D. Ecological speciation in postglacial fishes. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*. 1996; 351:807–814.
- Schluter D, McPhail JD. Ecological character displacement and speciation in sticklebacks. *The American Naturalist*. 1992; 140:85–108.
- Snorrason SS, Skúlason S, Jonsson B, Malmquist HJ, Jónasson PM, Sandlund O, Lindem T. Trophic specialization in Arctic charr *Salvelinus alpinus* (Pisces; Salmonidae): morphological divergence and ontogenetic niche shifts. *Biological Journal of the Linnean Society*. 1994; 52:1–18.
- Snowberg LK, Bolnick DI. Assortative mating by diet in a phenotypically unimodal but ecologically variable population of stickleback. *The American Naturalist*. 2008; 172:733–739.
- Steedman RJ, Kushneriuk RS, France RL. Littoral water temperature response to experimental shoreline logging around small boreal forest lakes. *Canadian Journal of Fisheries and Aquatic Sciences*. 2001; 58:1638–1647.
- Svanbäck R, Eklöv P. Morphology dependent foraging efficiency in perch: a trade-off for ecological specialization? *Oikos*. 2003; 102:273–284.
- Taylor EB. Species pairs of north temperate freshwater fishes: evolution, taxonomy, and conservation. *Reviews in Fish Biology and Fisheries*. 1999; 9:299–324.
- Thomas RJ, Szekely T, Powell RF, Cuthill IC. Eye size, foraging methods and the timing of foraging in shorebirds. *Functional Ecology*. 2006; 20:157–165.
- Travis, MP. Unpublished Doctor of Philosophy. Stony Brook University; 2007. The functional morphology and evolution of feeding modes in threespine stickleback.
- Uchii K, Okuda N, Yonekura R, Karube Z, Matsui K, Kawabata Z. Trophic polymorphism in bluegill sunfish (*Lepomis macrochirus*) introduced into Lake Biwa: evidence from stable isotope analysis. *Limnology*. 2007; 8:59–63.
- USEPA. Survey of the Nation's Lakes. Field Operations Manual. U.S. Environmental Protection Agency; Washington, DC: 2007. EPA 841-B-07-004
- Utne ACW. The effect of turbidity and illumination on the reaction distance and search time of the marine planktivore *Gobiusculus flavescens*. *Journal of Fish Biology*. 1997; 50:926–938.
- Vander Zanden MJ, Vadeboncoeur Y. Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology*. 2002; 83:2152–2161.
- Vermeij GJ, Covich AP. Coevolution of freshwater gastropods and their predators. *The American Naturalist*. 1978; 112:833–843.
- Vollestad LA, Skurdal J, Qvenild T. Habitat use, growth, and feeding of pike (*Esox lucius* L.) in four Norwegian lakes. *Archiv für Hydrobiologie*. 1986; 108:107–117.
- Von Hippel FA. Conservation of threespine and ninespine stickleback radiations in the Cook Inlet Basin, Alaska. *Behaviour*. 2008; 145:693–724.
- Wainwright PC. Ecological explanation through functional morphology: the feeding biology of sunfishes. *Ecology*. 1996; 77:1336–1343.
- Wainwright, PC. Functional morphology of the pharyngeal jaw apparatus. In: Shadwick, RE.; Lauder, GV., editors. *Fish Biomechanics*. Elsevier Academic Press; San Diego, CA: 2006. p. 77-102.

- Walker JA. Ecological morphology of lacustrine threespine stickleback, *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biological Journal of the Linnean Society*. 1997; 61:3–50.
- Walls, GL. *The Vertebrate Eye and its Adaptive Radiation*. Hafner Publishing Co.; New York, NY: 1963.
- Webb PW. Body form, locomotion and foraging in aquatic vertebrates. *American Zoologist*. 1984; 24:107–120.
- Werner EE. Species packing and niche complementarity in three sunfishes. *The American Naturalist*. 1977; 111:553–578.
- Westneat MW. Evolution of levers and linkages in the feeding mechanisms of fishes. *Integrative and Comparative Biology*. 2004; 44:378–389.
- Willacker, JJ. Unpublished Master of Science. University of Alaska Anchorage; 2009. Geometric morphometrics of threespine stickleback in the Cook Inlet Basin, Alaska.
- Wimberger, PH. Trophic polymorphisms, plasticity, and speciation in vertebrates. In: Stouder, DJ.; Fresh, KL.; Feller, RJ., editors. *Theory and Application in Fish Feeding Ecology*. University of South Carolina Press; Columbia, SC: 1994. p. 19-44.
- Wright DI, O'Brien WJ, Luecke C. A new estimate of zooplankton retention by gill rakers and its ecological significance. *Transactions of the American Fisheries Society*. 1983; 112:638–646.
- Zelditch, ML.; Swiderski, DL.; Sheets, HD.; Fink, WL. *Geometric Morphometrics for Biologists*. Elsevier Academic Press; San Francisco: 2004.



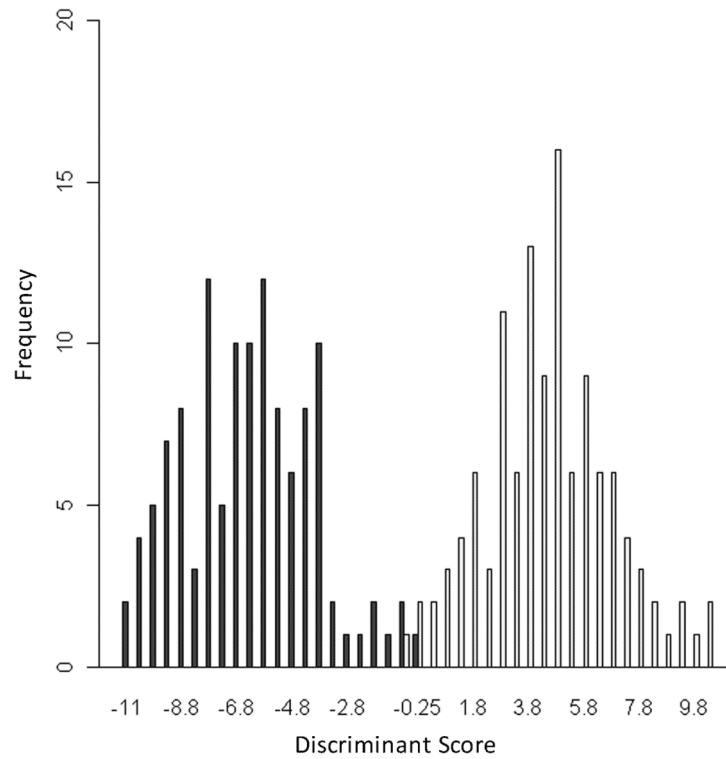
**Figure 1.**

Map of the Cook Inlet Basin showing major geographic regions (separated by dashed borders) and the locations of 45 stickleback populations. Black rimmed squares and arrows indicate populations used in the training sets with 1 signifying populations used in the benthic training group and 2 signifying populations used in the limnetic training group. White circles are populations of uncertain morphotype. North arrow indicates true north.

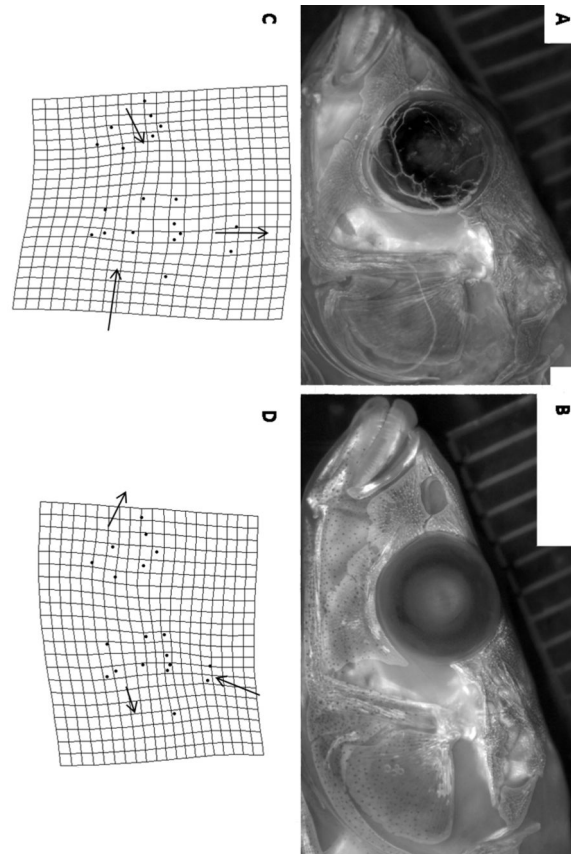


**Figure 2.** Locations of anatomical landmarks used to study skull morphology in threespine stickleback. Note landmark 17 has been excluded. Double sided arrow indicates vertical orbit diameter measurement.

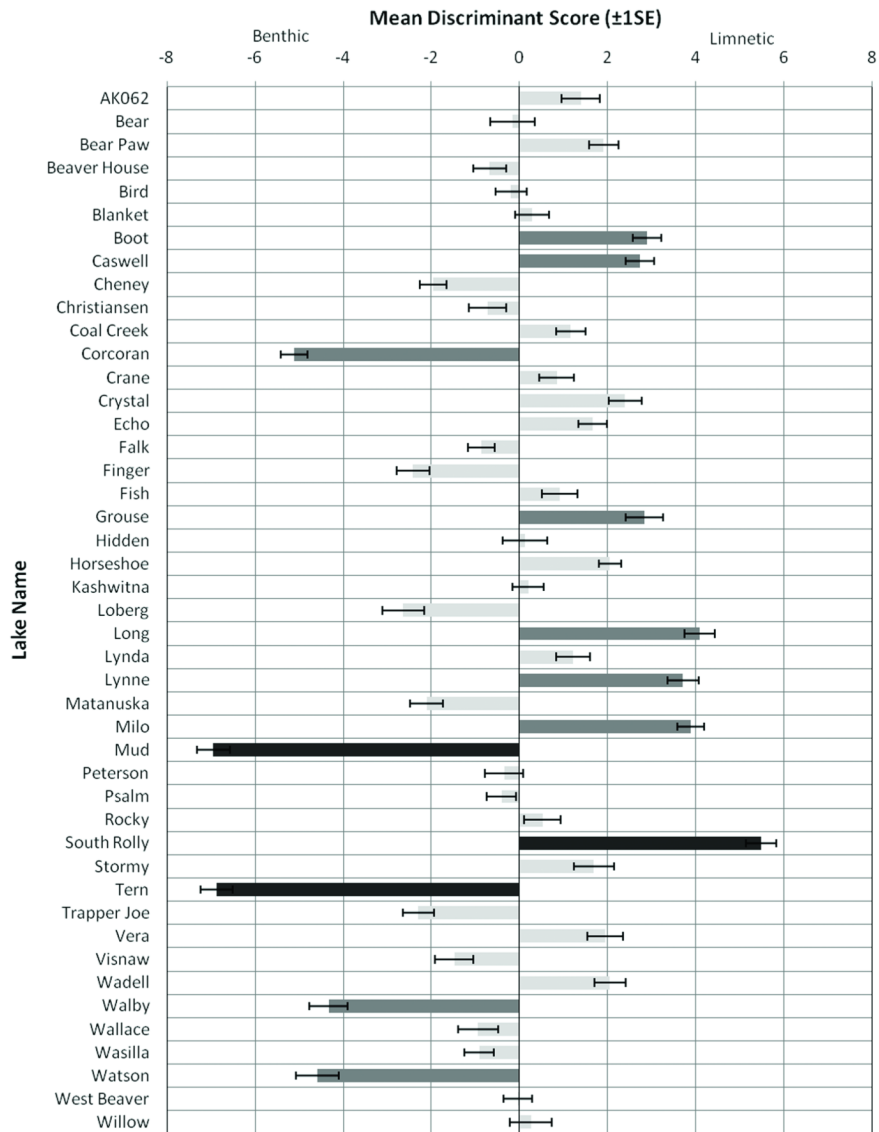




**Figure 3.** Histogram of discriminant scores for benthic (dark bars) and limnetic (light bars) training groups of threespine stickleback from the Cook Inlet Basin, Alaska.



**Figure 4.** Images of fish that most closely correspond to the A) benthic and B) limnetic mean skull morphologies and the distortion of the thin-plate spline that accompanies each configuration (C and D). Note scale bars (millimeters) embedded in images.



**Figure 5.** Population mean discriminant scores for 45 threespine stickleback populations from the Cook Inlet Basin, Alaska. Error bars indicate standard error within each population. The color of the bar indicates the divergence of each population mean discriminant score from the mean discriminant score across all populations; dark grey = >2SD, medium grey = 1SD-2SD, and light grey = <1SD.

Table 1

Locations, putative morphotypes based on previous dietary analysis, and pike/stocking statuses of sampled lakes. Blank morphotype values indicate populations with no published accounts of diet and a question mark following morphotype indicates conflicting or limited evidence. P indicates pike presence and S indicates stocked lakes (data obtained from Alaska Department of Fish and Game lake files).

Lake Name	Region	Latitude	Longitude	Morphotype	Pike/Stocked
AK062 (unnamed)	Kenai Peninsula	60.794	-150.330		
Bear	West-side Cook Inlet	60.420	-152.378		
Bear Paw	Mat-Su Valley	61.614	-149.756	S	S
Beaver House	Mat-Su Valley	61.574	-149.863	Limnetic?, e, h, i, j	S
Bird	Mat-Su Valley	60.972	-150.409		
Blanket	Mat-Su Valley	61.591	-149.874		
Boot	Mat-Su Valley	61.717	-150.117		S
Caswell	Mat-Su Valley	62.017	-149.967		P, S
Cheney	Anchorage	61.203	-149.758		P, S
Christiansen	Mat-Su Valley	62.315	-150.063		S
Coal Creek	Mat-Su Valley	61.489	-151.568		
Corcoran	Mat-Su Valley	61.574	-149.688	Benthic?, f, g, h, i, j	
Crane	Kenai Peninsula	60.792	-150.955		
Crystal	Mat-Su Valley	61.710	-150.100		P, S
Echo	Kenai Peninsula	60.438	-151.161		S
Falk	Mat-Su Valley	61.566	-149.049		
Finger	Mat-Su Valley	61.606	-149.279		P, S
Fish	Mat-Su Valley	62.251	-150.065		P
Grouse	Kenai Peninsula	60.776	-150.287		
Hidden	Kenai Peninsula	60.486	-150.263		
Horseshoe	Mat-Su Valley	61.572	-149.928		P
Kashwitna	Mat-Su Valley	61.833	-150.076	Limnetic?, e, f, g, h, j	P
Loberg	Mat-Su Valley	61.560	-149.258	Limnetic?, h	S
Long	Mat-Su Valley	61.578	-149.764	Limnetic e, f, g, h, i, j	P, S

Lake Name	Region	Latitude	Longitude	Morphotype	Pike/Stocked
Lynda	Mat-Su Valley	61.571	-149.836	Limnetic? <i>e, f, g, h, i, j</i>	
Lynne	Mat-Su Valley	61.712	-150.039	Limnetic <i>b, i</i>	S
Matanuska	Mat-Su Valley	61.556	-149.229		S
Milo	Mat-Su Valley	61.670	-150.092		P
Mud	Mat-Su Valley	61.563	-148.949	Benthic <i>a, c, e, f, g, h, i</i>	
Peterson	Kenai Peninsula	60.525	-150.396		P
Psalm	Anchorage	61.383	-149.563		
Rocky	Mat-Su Valley	61.556	-149.825		P, S
South Rolly	Mat-Su Valley	61.669	-150.126	Limnetic <i>d, e</i>	P, S
Stormy	Kenai Peninsula	60.771	-151.047		P
Tem	Kenai Peninsula	60.533	-149.550	Benthic <i>c, d, h</i>	
Trapper Joe	Kenai Peninsula	60.760	-150.079		
Vera	Mat-Su Valley	61.713	-150.138		S
Visnaw	Mat-Su Valley	61.619	-149.679	Limnetic? <i>a, h, j</i>	S
Wadell	West-side Cook Inlet	60.408	-152.356		
Walby	Mat-Su Valley	61.619	-149.211		S
Wallace	Mat-Su Valley	61.574	-149.572		P
Wasilla	Mat-Su Valley	61.586	-149.396		P
Watson	Kenai Peninsula	60.539	-150.465		
West Beaver	Mat-Su Valley	61.586	-149.844		S
Willow	Mat-Su Valley	61.744	-150.057	Benthic? <i>h, i, j</i>	S

<sup>a</sup> Caldecutt & Adams, 1998,

<sup>b</sup> Foster et al., 1998,

<sup>c</sup> Karve et al., 2008,

<sup>d</sup> M. Travis, unpublished data,

<sup>e</sup> P. Park, unpublished data,

<sup>f</sup>Purnell *et al.*, 2006,

<sup>g</sup>Purnell *et al.*, 2007,

<sup>h</sup>Travis, 2007,

<sup>i</sup>von Hippel, 2008,

<sup>j</sup>Walker, 1997

**Table 2**

Habitat variables tested and their associated sample size, mean, and standard deviation. Water chemistry data were obtained from a dataset compiled by stickleback researchers beginning in 1989 (Bell & Ortí, 1994; Bell et al., 1993; Jones et al., 2003; Walker, 1997) and from the Alaska Lakes Assessment conducted by the U.S. Environmental Protection Agency in 2008. Physical habitat data were obtained from Alaska Department of Fish and Game lake files. See Willacker (2009) for details on the compilation of the dataset.

Parameter	n	$\bar{X}$	SD
Alkalinity	32	43.61	33.96
Ammonia (NH <sub>3</sub> )	28	7.46	12.64
Calcium	34	11.77	9.20
Chlorophyll A	33	1.93	4.42
Color	34	14.53	8.75
Lake area	45	154.35	255.29
Maximum depth	31	34.83	29.15
Mean depth	23	13.12	8.28
Percent of lake <1.52m	17	38.53	17.61
Percent of lake <3.05m	23	55.50	23.41
Total nitrogen (TN)	30	271.97	91.99
Total phosphorus (TP)	34	8.34	3.64
Turbidity	35	0.97	0.45