

A SURVEY OF PHOTOPERIODIC RESPONSE AND MORPHOLOGICAL
VARIATION ACROSS A LATITUDINAL GRADIENT IN THREESPINE
STICKLEBACK

by

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Natural biological variation exists at different geographic scales. We compared phenotype distribution across latitude, region and habitat type in threespine stickleback (*Gasterosteus aculeatus*) to determine local adaptation. To quantify variation in photoperiodic response, the day length cue was used to time sexual maturation and morphological characters across these various scales. Using lab-reared lines, we developed an index of sexual maturation and experimentally determined critical photoperiod for Alaskan and Oregon populations. Results showed that photoperiodic response existed in Alaskan but not Oregon populations. We also collected morphological data and made comparisons between wild Alaskan and Oregon populations and found similarities within habitat type across latitude but differences across region and habitat type. These data support the hypothesis that local adaptation

results in variation across geography and habitat and, in stickleback, parallel evolution of morphological phenotypes within similar but geographically distant habitats.

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CHAPTER I

INTRODUCTION

Natural variation can be examined at different geographic scales, which can be conceived of as a series of nested distributions of organismal range. The largest scale encompasses an organism's entire range with the greatest differences in abiotic factors at the northern and southernmost points of the distribution. Within that total range are smaller, more localized distributions in which there are no differences in seasonal occurrence, but observable differences in local climatic conditions or other abiotic factors. Within a region populations may inhabit different habitat types, which may vary in local abiotic factors and community interactions. In this thesis, differences in phenotype will be described and compared across large-scale geographic distance (latitude), across local geographic area (region within latitude) and among habitat types.

Across large-scale geography, differences among populations might be due to differentiation by isolation. However, the role of local selective forces must also be considered. An observed phenotype may be the result of the selective force exerted by an environmental parameter such as geographic variation or habitat.

We used threespine stickleback (*Gasterosteus aculeatus*) to investigate the relative importance of geographic distance between populations compared to selection for differences in phenotype among habitats. Threespine stickleback are small, holarctic fish found in both marine and freshwater habitats. Ancestral marine populations colonized freshwater habitats and gave rise to derived forms, which underwent extensive phenotypic diversification. Characters such as morphology, courtship behavior, nuptial coloration and the timing of breeding vary among populations. These characters are readily observable and address both physiological and morphological phenotypes. Previous work has focused on either physiological or morphological differences in phenotype, but rarely both. This thesis presents salient data on both types of characters.

Stickleback breed in early spring and the timing of this behavior is cued by length of day (photoperiod). Chapter II is a comparison of photoperiodic response between lab-

reared Oregon and Alaskan lines of stickleback. The resulting data showed that reproduction is highly associated with photoperiod in the Alaskan population, but not in the Oregon population. This suggests a heavier reliance on photoperiod at more northerly latitudes. We propose this may be due the shorter length of breeding and growing time at higher latitudes.

Chapter III compares variation in morphological characters for different populations among habitat types and across regions in Oregon. The data showed differences in body shape and armor phenotypes, suggesting that stickleback are affected by local selective pressures. Additional comparisons of morphology between Oregon and Alaskan populations showed similar patterns. These results support the hypothesis that local selection influences phenotype across geography and habitat type and provide evidence for parallel evolution across geography.

Chapter IV is a summary of the conclusions drawn from the experimental results from chapters II and III.

CHAPTER II
LATITUDINAL VARIATION IN PHOTOPERIODIC RESPONSE OF THE
THREESPINE STICKLEBACK (*GASTEROSTEUS ACULEATUS*) IN WESTERN
NORTH AMERICA

Introduction

At temperate and polar latitudes with distinct summer and winter seasons, the ability to exploit the favorable season, to avoid or mitigate the unfavorable season, and to make a timely transition between the two lifestyles are all components of fitness. Animals use the length of day, or photoperiod, to time their seasonal development, reproduction, migration and dormancy (Bradshaw and Holzapfel 2007). Generally, the influence of day length on the seasonal activities of vertebrates increases with latitude but, compared to arthropods, there are very few studies involving vertebrates and none, to our knowledge, involves latitudinal variation among populations of a single species of fish. Herein, we consider intraspecific variation in response to day length of northern and southern populations of the threespine stickleback, *Gasterosteus aculeatus*.

In fish with long gonadal cycles, reproduction and migration are generally cued by a combination of a circannual clock whose period of oscillation is approximately annual and by increasing and decreasing day lengths that serve to “set” the circannual clock; fish with short gonadal cycles are less dependent on a circannual clock and more dependent on a direct response to a single constant day length (Bromage et al. 2001). The threespine stickleback is a small fish found in marine, estuarine and freshwater habitats (Bell and Foster 1994) that has long been a model for studies of ecology and behavior (Wooten 1976), and has recently been used for studies of the microevolution of developmental processes (Cresko et al. 2007). It has a weak circannual rhythm, has a strong response to constant day lengths and is distributed in the Holarctic from about 35-70°N (Baggerman

1985; Bornestaf and Borg 2000).

The most thorough studies of photoperiodism in stickleback use populations collected from nature, mainly the Baltic Sea (ca. 56-59°N). At this latitude, long days promote reproduction in both males and females during the late spring and early summer (Borg 1982; Borg et al. 2004). In males, long days promote a sexual syndrome of color change from drab to bright, territoriality, kidney hypertrophy and spiggin (glue for nest building) production, nest building, and courtship (Borg et al. 2004). In early fall, fish go through a brief refractory period (Borg 1982). Later in the fall, fish become reproductively responsive to long days and critical photoperiod (the median day length required to trigger breeding phenotype) declines, likely due to decreasing autumnal day lengths and temperature (Baggerman 1972, 1985). Increasing day lengths in the spring then promote gonadal maturation in females and spiggin production in males so that, in the southern Baltic, the breeding season of marine stickleback peaks from early May to early July (Borg 1982).

In the Rhône delta at ca. 43°N the breeding season of anadromous stickleback peaks earlier in February and March (Crivelli and Briton 1987). While Crivelli and Briton (1987) did not identify the environmental cues determining the migration and spawning in the Rhône delta, the difference in phenology between the Baltic and Rhône delta populations suggests that there may be a geographic difference in response to day length between northern and southern European populations. To pursue the question of geographic variation in photoperiodic response of threespine stickleback, we determined photoperiodic response of *G. aculeatus* from western North America over a range of 18°N latitude. Previous studies in Europe have considered wild-caught fish whose photoperiodic response may be altered by their environmental history. To determine genetic difference among geographic populations, we used the first or third (Alaskan, 61 generation of laboratory maintained fish, reared them on short days, and upon attaining adult size, exposed them to different constant day lengths at 20°C.

Methods

Threespine Stickleback Collecting and Lines

The Alaskan stock was collected from Rabbit Slough (61°34' N, 149°15' W). In nature, this population has an anadromous life history with spring runs beginning in mid-May and ending in mid-June. The animals used for these experiments were G₃ outbred descendants from wild-caught individuals. The Oregon stickleback were collected from Eel Creek (43°35' N, 124°11' W), which is a small perennial creek contiguous with the Pacific Ocean via a larger, connecting waterway. This population is likely resident within the stream system as all fish trapped in Eel Creek show a consistent low-armor phenotype (indicative of resident freshwater fish) and a unique body morphology that is a subset of that found in the larger connecting creek (Yeates-Burghart and Bolle, unpublished). Furthermore, fish in breeding condition are found in Eel Creek only during a short time from mid-March to late April. The animals used for these experiments were G₁ outbred descendants from wild-caught individuals.

The experimental fish were produced and hatched using standard crossing protocol (Cresko et al. 2004). To prevent transmission of disease or parasites, stickleback embryos were bleached in a 6% solution of sodium hypochlorite on days 2 and 5 post fertilization. After hatching, the fry were put in a 75.7L aquarium at a density of 1.25 fish per liter under a 10:14 light:dark (L:D) cycle in water of 7 ppt salinity (Instant Ocean Brand) at 20°C. Juvenile stickleback were fed dry food (Zeigler Larval AP100, 250-450 microns) and freshly hatched brine shrimp. Adults were fed dry food (Nelson's silver cup fish feed, trout fry, 0.59-1.38 mm).

Photoperiod Cabinets and Husbandry

Experiments were run in light-tight, air-cooled cabinets located in climate-controlled rooms held at 20°C. Photoperiod cabinets consisted of six 29×40×53 cm (H×W×D) chambers with five shelves each. Each shelf accommodated three, 4 L, continuously aerated tanks and was illuminated by a "twilight" lamp (a single Lumex SLX-LX5093UWC/G "water cool" white LED in a 3.5 cm translucent spherical reflector) and a "daytime" fixture (Sylvania white, nine-LED strip light,

LED/UC/W/9/W). Twilight was simulated by turning on the twilight bulb for 15 min before and for 15 min after the daylight fixture. Twilight and daylight were programmed with Chronotrol XT electronic timers. Fish were fed daily and the water was changed with 7 ppt Instant Ocean saline solution every two days in each tank. For experiments, each tank contained one male and one female adult fish, at least 8 months old and at least 50 mm in length. Any fish that died was not replaced. At the end of the experiments all surviving fish were included in the data. Experiments with Alaskan fish were run in two blocks. The first block used a broader span of day lengths to identify the region of the critical photoperiod. The second block repeated day lengths at which there had been incidental death in the first block (to increase sample size) and also used a narrow span of day lengths to refine the estimate of the critical photoperiod. The data from both blocks were combined. Experiments with Oregon fish were run as a single block. Experiments with both Alaskan and Oregonian fish were run for six weeks.

Assays of Sexual Maturation

In stickleback, ovarian growth and kidney hypertrophy are reliable indicators of sexual maturation (Mayer et al. 2004). Ovaries enlarge during the maturation of eggs; male kidneys enlarge during the production of spiggin. To quantify sexual development across light treatments, we calculated the OSI and KSI, the ovary-somatic index and the kidney-somatic index. Kidneys or ovaries were excised and dried to constant mass with their respective owners at 37°C using a Mettler AT261 DeltaRange electronic balance. The OSI was calculated as the ratio of ovary to total body mass and the KSI as the ratio of kidney to total body mass.

Results

As shown in Figure 1, sexual maturation in Alaskan sticklebacks exhibited a sigmoid dose-response curve that increased with day length for both OSI (ANOVA: $F_{7,63} = 6.51$; $P < 0.001$) and KSI (ANOVA: $F_{7,51} = 3.52$; $P = 0.004$). Sexual maturation in Oregon sticklebacks did not vary with day length for either OSI ($F_{5,55} = 1.80$; $P = 0.127$) or KSI ($F_{5,62} = 1.49$; $P = 0.128$).

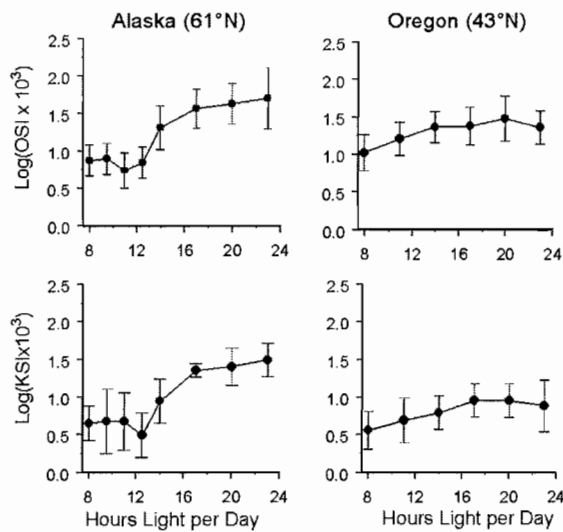


Figure II.1 Photoperiodic response of Alaskan and Oregonian threespine stickleback (*Gasterosteus aculeatus*) from western North America. OSI and KSI, ratio of ovary and kidney to total body mass in females and males, respectively. Error bars show $\pm 2SE$. OSI and KSI are multiplied by 103 to provide positive values on a log10 scale.

Discussion

While photoperiod affects the timing of seasonal activities in many organisms, intraspecific, geographic variation in photoperiodic response has been widely considered only in arthropods (Danilvevkii 1965; Tauber et al. 1986; Danks 1987; Bradshaw and Holzapfel 2007). Even among farmed finfish where photoperiodic control of migration, maturation, and reproduction is widespread (Bromage et al. 2001), there are, to our knowledge, no explicit comparisons of photoperiodic response among populations within a species. In threespine stickleback, all previous studies of photoperiodic response have used wild-caught fish where the environmental influence on reproduction is unknown; none of the studies have considered photoperiodic response over a wide climatic range.

Herein, we have shown that the more northern, Alaskan population of the threespine stickleback, *Gasterosteus aculeatus*, is strongly photoperiodic while the more southern, Oregon population shows no significant response to photoperiod (Fig. 1). Since all of our experiments were run with laboratory-reared fish in the G₁ or G₃ generation to minimize field effects, we conclude that the differences in photoperiodic

response between Alaskan and Oregon populations represent genetic differences between them.

The focal Alaskan population used in our study is anadromous (Cresko 2000, Cresko et al. 2004, Karve et al. 2008), but we do not know whether the migratory behavior of the Oregon population is entirely within fresh water as in upstream populations in the Navarro River of California, USA (Snyder and Dingle 1990), or involves migration from estuarine or marine habitats. A stronger photoperiodic response would be expected in long-distance migrating populations (Clarke et al. 1994, Quinn and Adams 1996). The lack of a discernible photoperiodic response in the Eel Creek, Oregon, population could reflect a strictly freshwater habitat where proximal cues such as food and temperature could be sufficient for timing reproduction.

Nonetheless, the increase in photoperiodic response of northern threespine stickleback is consistent with other vertebrates. In more northern populations of Scandinavian frogs, where there is a strictly limited growing season, day length provides a firm, regulating cue for seasonal reproduction; in more southern populations, frogs use day length to modulate temperature-dependent processes (Laurila et al. 2001). In lizards, with increasing latitude, day length has an increasing effect on metabolic rate (Angilleta 2001), on growth rate (Uller and Olsson 2003), and on ability to maintain a constant body temperature during the spring (Lashbrook and Livezey 1970). In mammals, with increasing latitude, short days have an increasing tendency to induce gonadal regression in mice (Heidemann et al. 1999; Lowrey et al. 2000; Lynch et al. 1981; Sullivan and Lynch 1986) or embryonic dormancy in mustelids (Thom et al. 2004). Hence, the general vertebrate pattern, including that of *Gasterosteus aculeatus*, is a pattern of an increasing influence of day length in the timing of important seasonal life-history events with increasing latitude among intraspecific populations. We propose that increasing reliance on day length by vertebrates at higher latitudes is due to an increasing use of a highly reliable, anticipatory cue to prepare in advance for an increasingly narrow window of opportunity for reproduction and development.

Bridge

We have established clear genetic differences in the critical day length and formal properties of the physiological phenotype of photoperiodic response between an Alaskan and an Oregon population of stickleback. Based on the results of this assay across latitude, we can conclude that phenotypic heterogeneity correlates with geographic location on a large scale. In chapter two, we addressed this question with greater resolution by examining a suite of morphological characters across region and among different habitats in populations of Oregon stickleback.

CHAPTER III
MORPHOLOGICAL VARIATION IN THREESPINE STICKLEBACK ACROSS
DIFFERENT REGIONS AND HABITATS IN OREGON

Introduction

Observations of phenotypic variation among natural populations catalyzed investigations into the processes underlying evolution (Bradshaw and Holzapfel 2007). Examining variation across a geographic scale to identify whether there is a relationship between habitat and character can help resolve questions about the relative importance of isolation by distance as compared to local selective forces on phenotypic diversity (Laurila, Pakkasmaa and Merila 2001; Lynch, Heath and Johnston 1981; O'Malley and Banks 2008). Cataloging and quantifying natural variation is the first step in determining whether there is a causal relationship between selection and phenotype leading to local adaptation (Cresko et al. 2007; Kimmel et al. 2005).

Threespine stickleback (*Gasterosteus aculeatus*) are small fish that exist holarctically from approximately 30 to 70°N (Bell and Foster 1994). Stickleback have long been a model for the study of ecology and behavior (Wootton 1976), and have recently been applied to studies focusing on microevolution of developmental processes (Cresko et al. 2007). Subsequently, a number of laboratory genetic and genomic tools have been developed for stickleback, making them amenable for studies of the genetic basis of evolving traits (Kingsley et al. 2004).

Stickleback can be found in most every type of marine, estuarine and freshwater habitats of the Northern hemisphere, and this naturally abundant species complex possesses a large amount of phenotypic variation (Cresko et al. 2007; Crivelli and Britton, 1987; Mayer, Borg and Pall 2004; Pressley, 1980; Reimchen, 1989; Walker 1997). Threespine stickleback have undergone repeated adaptive radiations since the

deglaciation of northern temperate latitudes approximately 10,500- 12,000 B.P (Bell and Foster 1994; McPhail 1994; Walker 1997; Booth, Troost, Clague and Waitt 2004). The glacial maxima extended south to approximately the 47th parallel. With glacial retreat, invasion of previously ice-locked or unavailable habitat by ancestral anadromous fish allowed for rapid opportunistic colonization into empty niches (Reusch, Wegner and Kalbe 2001). At latitudes falling below the glacial maxima, ice-locked habitat was not a barrier to colonization and invasion of water bodies likely occurred earlier (Booth et al. 2004). Range expansion of threespine stickleback in freshwater habitats produced two outcomes. First, colonization of formerly empty northern waterways led to rapid diversification in behavioral and morphological variation that is the hallmark of threespine stickleback today (Bell and Foster 1994; Baker and Foster, 2002; McKinnon and Rundle 2002). Anadromous stickleback are representative of a more ancestral body shape and armor phenotype (Walker and Bell 2000, McKinnon et al. 2004). The remarkable diversity of deviations from the ancestral marine state in stickleback morphology suggests that novel phenotypes have evolved in response to colonization of previously unavailable habitat by anadromous populations (Walker 1997). Common environmental pressures such as predator regime and prey community composition exist within and between ecotypes and may have substantial impact on the diversity of morphology in stickleback across geography (Walker 1997). Second, the range expansion fell along a north-south axis, creating a geographic distribution with a large latitudinal component (Baumgartner and Bell 1984; Bell 1981). These outcomes provide the elements necessary to investigate the effects of selective pressure on phenotype across habitat type and geography at a variety of scales.

Repeated evolution of similar body morphology between geographically separated populations sharing a common habitat type suggests that widespread parallel selection for phenotype is common in threespine stickleback (McPhail 1994; Walker 1997; McKinnon et al. 2004). Convergence of character states independently derived from ancestral marine populations, such as body size, morphology and, robustness of armoring frequently co-vary across distant, but similar ecotypes (McKinnon and Rundle

2002). Parallel evolution of similar ecomorphs has evolved in geographically separated replicate habitats (Walker 1997; Baumgartner and Bell 1984). However, it is unclear over what magnitude of geographic variation this parallel evolution is likely to occur in threespine stickleback. This paper is a survey of variation among habitat types and geographically separated populations at two different scales, across regions in Oregon, and between Oregon and Alaska. Our goal was to determine the relative importance of geographic distance between populations compared to selection for differences in phenotype among habitats.

Methods

Study Sites

We chose to examine phenotypic variation in populations of stickleback from three regions and compared variation in morphological phenotype among regions and between different habitat types found within each region. These regions differ in local abiotic conditions such as watershed connectivity and salinity and are isolated by distance and geographic barriers that restrict gene flow between adjacent ranges. The coastal region extends from the Pacific Ocean to the foothills of the Cascade Mountains approximately 60 miles east and inland. The valley region encompasses the extent of the Willamette valley floor and the western base of the Cascade Mountains range. The central region begins on the east side of the Cascade Mountains and extends to the foothills of the Ochoco Mountain range (Table III.1).

Table III.1 A description of the regions and habitat types in Oregon

Region:	Description:
Coast	Characterized by typical temperate seasonality, significant connectivity between water bodies and tidal fluxuation at the confluence of river and ocean. Additionally, salinity gradients extend inland and water appears either turbid or clear/tannic. Lakes of all sizes are most common in this region and often have perennial outlet streams.
Valley	The valley floor is characterized by temperate seasonality has reduced perennial connectivity between water bodies; no salinity gradient and water clarity ranges from clear to moderately turbid. Lakes are more isolated with fewer outlet streams.
Central	This region is over 170 miles inland from the coast. It experiences an extreme temperate seasonality in which water body connectivity is significantly reduced for the majority of the year. Water temperatures fluctuate greatly across seasons. There is no salinity gradient and water clarity ranges from clear/tannic to turbid water. Lakes are very isolated in this region and small lakes occur infrequently.
Habitat Type:	Description:
Estuary	Rivers and bays that are directly contiguous with the Pacific Ocean and which undergo cyclical tidal fluctuation. Salinity measurements > 0ppt during tidal events.
River	Rivers and streams that may or may not be contiguous with the Pacific Ocean, but do not undergo tidal fluctuation or have a salinity measurement above 0 ppt at the point of sampling.
Lake	Water bodies that may be minimally connected to other water bodies, but are completely isolated from the ocean by physical barriers or lack of outflow streams.

Habitat types were chosen based on commonality across regions with the exception of the estuarine habitat, which is restricted to the coast range (Table III.1). Each habitat type was defined by the characteristics of the water body in which samples were collected to standardize comparisons between categories. Estuarine habitats were contiguous with the Pacific Ocean and experienced tidal and salinity fluctuation in water levels. Riverine habitats were either contiguous or not contiguous with the ocean, not tidally influenced at the point where samples were collected and had a salinity reading of 0 ppt. Lacustrine habitats had little connectivity to other water bodies and were completely isolated from the ocean via physical barriers or lack of outflow streams.

For coastal locations, fish were categorized as estuarine, riverine and lacustrine if individuals found at the collection site were found in breeding condition. This helped increase the certainty that populations were breeding in the habitats in which they were found.

Collection and Preparation of Stickleback Specimens

Excepting two populations, samples of 30 fish were collected at each of various time points from September 2006 to February 2009 to gain a representative subset of the

morphological variation present in each group across time (Table III.2). This was particularly important for coastal populations which were not as geographically isolated as populations from other regions. Fish were trapped using 1/8" mesh minnow traps baited with salmon eggs set for 24h durations. All individuals were euthanized in the field according to University of Oregon approved IACUC protocols with a buffered MS222 solution, preserved in 100% ethanol. A subset of the fish was haphazardly chosen from each collection and placed in 100% formalin in order to fix tissue. Subsequently fish were stained using Alizarin Red to visualize bones, a technique described previously in Cresko et al. (2004).

Table III.2 17 Oregonian and 7 Alaskan populations from 3 different habitat types were surveyed for morphological variation.

State	Region	Habitat Type	Population	Samples per population	Population Acronym	GPS Latitude	GPS Longitude
Oregon:	Coast	Estuary	Smith River	30	SMES	43.808 °N	124.239 °W
		Estuary	Cushman Slough	30	CSES	43.046 °N	124.178 °W
		Estuary	Dean Creek	30	DCES	43.855 °N	124.006 °W
		Estuary	Winchester Creek S.	30	WCES	43.366 °N	124.409 °W
		Estuary	South Jetty	30	SJES	44.066 °N	124.157 °W
		Estuary	Miner Creek	30	MCES	43.503 °N	124.584 °W
		River	Tenmile Creek	30	TMRV	43.728 °N	124.340 °W
		River	Eel Creek	16	ECRV	43.594 °N	124.218 °W
		Lake	Upper Pony Creek Reservoir	30	UPLA	42.428 °N	124.385 °W
		Lake	Lower Pony Creek Reservoir	30	LPLA	43.422 °N	124.264 °W
	Willamette Valley	River	Riverbend	30	RVRV	44.079 °N	123.029 °W
	Central	River	Deschutes River	30	DERV	43.944 °N	121.551 °W
		River	Crooked River	30	CRRV	44.293 °N	120.839 °W
		Lake	Paulina Lake	30	PALA	43.713 °N	121.358 °W
		Lake	South Twin Lake	30	STLA	43.883 °N	121.798 °W
		Lake	Wickiup Reservoir	30	WILA	43.848 °N	121.848 °W
		Lake	Crane Prairie Reservoir	30	CPLA	43.801 °N	121.079 °W
Alaska:	Coast	Estuary	Mud Bay	30	MBES	59.636 °N	151.500 °W
		Estuary	Resurrection Bay	30	RBES	60.130°N	149.374°W
		Estuary	Salmon Creek	22	SCES	60.130°N	149.374°W
		Lake	Mud Lake	30	MDLA	61.563 °N	148.949 °W

Collection of Environmental Data

To ensure consistency in classing habitat types and consistent collection from the same place we took the GPS coordinates for each site and measured a series of environmental variables including but not limited to contiguity with the ocean, whether or not the area was tidally influenced, salinity (ppt), turbidity and water temperature (Table III.2).

Data Collection

All specimens were photographed under standardized conditions using a Nikon D70 SLR camera. Landmark and linear measurements were collected for a suite of morphological characters including, but not limited to, standard length (SL), lateral plate counts and pelvic spine length from the left side of each specimen and body shape. Every fish scored had a standard length (SL) measurement $> 24\text{mm}$ to ensure adult body proportions and armor character development. SL was taken from the anterior tip of the premaxilla to the posterior end of the caudal peduncle (Fig. III.1A).

To quantify variation in armor phenotypes, the number of lateral plates were counted from anterior to posterior along the left side of each specimen (Fig III.1B) and the length of each left pelvic spine (mm) was measured from base to tip. (Fig. III.1C)

Landmark data to quantify body shape (Fig. III.1D) were collected using the morphometrics program Morpho J (version 1.00b) and linear measurements were digitized using tpsDIG2 (version 2.12). For body shape, we collected data from 26 standardized landmarks from the left side of each specimen. The positioning of landmarks and basic technique was adapted from previous work done using geometric morphometrics on stickleback (Walker 1997; Walker 2000; Adams, Rohlf and Slice 2004).

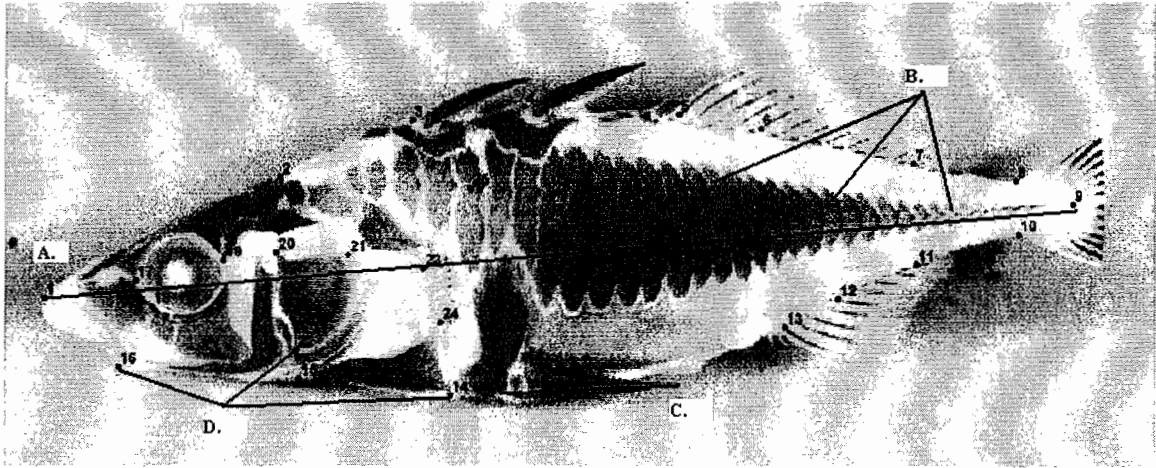


Figure III.1 Linear and morphometric measurements taken for stickleback. A) The standard length of each specimen was taken from the anterior tip of the premaxilla to the posterior end of the caudal peduncle. This allowed all linear and morphometric measurements to be standardized for each individual. B) Lateral plates were counted from the most anterior to posterior along the left side of each specimen. C) The length of each left pelvic spine (mm) was measured from the base of the connecting socket to the tip of the structure. D) We took morphological data based on 26 predetermined physical landmarks from the left side of each specimen. This technique was adapted from previous work that addressed stickleback body shape using geometric morphometrics.

Data Analyses

Data was collected for body shape using geometric morphometrics. This allowed for comparison of shape variation to be addressed at two different levels of resolution. First, this method removed the confounds of natural heterogeneity of size among individuals by standardizing each specimen by the group SL. Second, the standardization of individuals allowed population data to be compiled and compared between groups.

We took the raw landmark data for each specimen and compiled it within population. The data was then graphed on a deformation grid to examine the mean shape of a population based on where the grid warped. We could compare similarities and differences in the amount of grid warping among populations to draw conclusions about shape differences among groups. The shape data was compared using canonical variates analyses (CVA) among populations from the same habitat among different regions and different habitats within the same region. This assessment was performed to correlate any differences observed in phenotype with change in the independent variable. By holding habitat type constant, any differences in phenotype observed in populations among

regions could be associated with region (Walker 1997). By holding geographic effects constant, we could isolate and correlate observed differences with habitat type.

Linear measurements increase with the size of the fish. We standardized all linear measurements to the SL of the individual from which they came. To compare left pelvic spine size with respect to fish SL we regressed each size measurement and retained the residuals from this relationship to use as a SL standardized length of the left pelvic spine (Karve, von Hippel and Bell 2008).

The comparisons made among armor phenotypes in Oregon populations comprised two types. First, we compared variation among regions within river habitats. Next, we compared variation among habitat types within the coastal region.

Results

Body Shape: Habitat (River) X Region (Coast, Valley, Central)

We used Canonical Variates Analysis (CVA) for populations of stickleback from riverine habitats across coast, valley and central regions to determine whether region had an effect on body shape (Table III.2). The resulting data showed tight clustering of river populations with no overlap between different regions (Fig. 111.2). Individuals from replicate habitats in the same region looked more similar to one another than they did to riverine fish from other regions.

The coastal populations varied from the other two regions by having the highest CV1 values. However, they showed similar values to central Oregon populations for CV2. The cluster for the valley population fell below the coastal region and mostly above the central region on the CV1 axis. They also had the highest CV2 values. Central populations showed the lowest CV1 values.

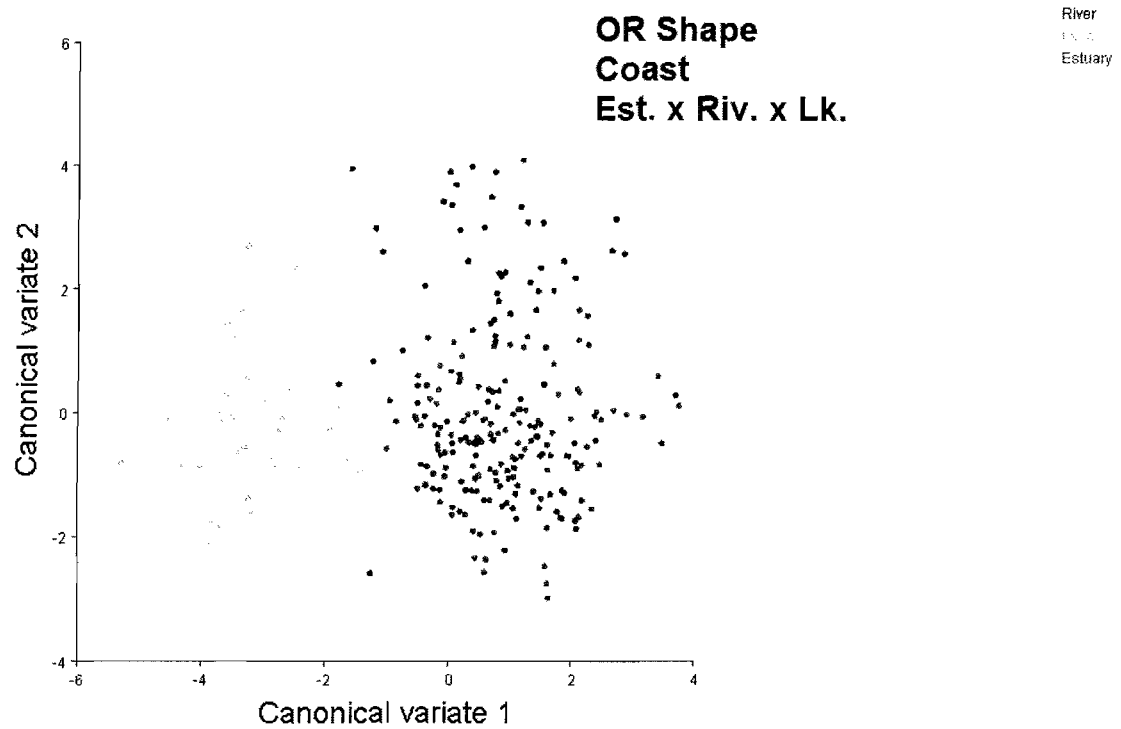


Figure III.2 Results for the comparison of habitat type within coastal region in Oregon. Each dot represents all of the compiled landmark data for each individual within a population, then each population within a habitat type. The data cluster in groups based on habitat type, indicating differences in morphology among groups. There is variation among habitats within region and fish living in different habitats tend to look more similar to one another than to fish from other habitats. Cluster overlap between estuarine and riverine data shows relative interconnectedness of these habitat types within this region. However, there is almost no overlap in the lake data, implying habitat isolation.

Body Shape: Region (Coast) X Habitat Type (Estuary, River, Lake)

We compared CVs for stickleback populations within the coastal region among estuarine, riverine and lacustrine habitat types to determine whether there was an effect on body shape (Table III.2). The resulting data showed clustering of population body shape for each habitat category with moderate overlap among groups.

Populations in estuarine and river habitats showed similar CV1 and CV2 values with the majority of overlap in CV1 (Fig. III.3). The values for CV1 fell slightly higher in estuarine populations than they did in riverine populations. The values for CV2 fell slightly lower in estuarine populations than they did in riverine or lacustrine populations.

There was no overlap in CV1 or CV2 values between estuarine and lacustrine populations. The values for CV1 were different for estuarine and lacustrine populations. Estuarine populations showed a higher CV1 value. Coast and lake habitats had similar values for CV2, with coast data falling slightly lower on the axis.

Riverine populations showed data overlap with both other habitat types. They also had the highest CV2 values for any habitat type. Lacustrine populations had the lowest CV1 values. They also had almost no shape overlap with either estuarine or riverine populations. Estuarine and riverine populations shared more commonalities in body shape with each other than either group did with lacustrine populations.

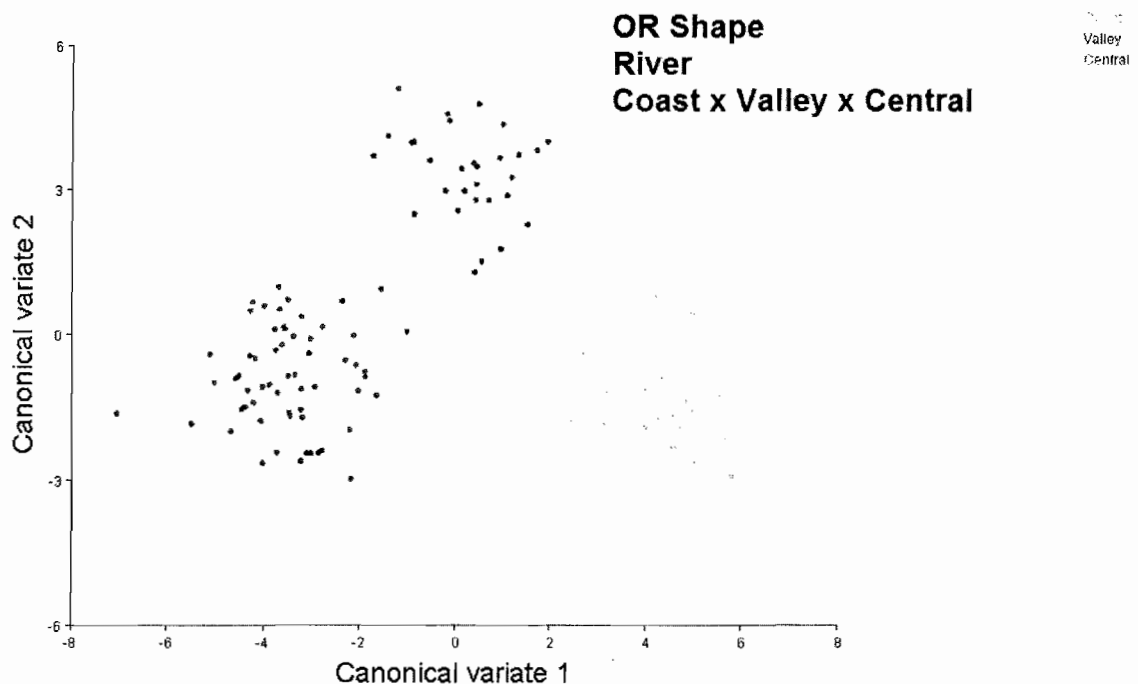


Figure III.3 Results for the comparison of habitat type across region in Oregon. There is variation among regions within habitat type. Distinct clusters show that differentiated ecomorphs exist within rivers across geography. This suggests that habitat isolation curtails gene flow between regions and that selective pressure varies across geography even within the same kinds habitat.

Lateral Plate Count: Habitat (River) X Region (Coast, Valley, Central)

We compared the number of lateral plates for riverine populations across coast, valley and central regions (Table III.2). Coastal riverine populations showed clear differences in

the number of plates present (Fig. III.4). Of the two populations sampled, Tenmile Creek, was high-plated with the average plate number being ~ 27 . The other, Eel Creek, was low-plated with the average being ~ 6 . All individuals within a population were consistent with respect to phenotype. The valley population was polymorphic for plate phenotype. Plate counts ranged from low to high with an average of ~ 13 . Both central populations were low-plated with an average of ~ 6 plates.

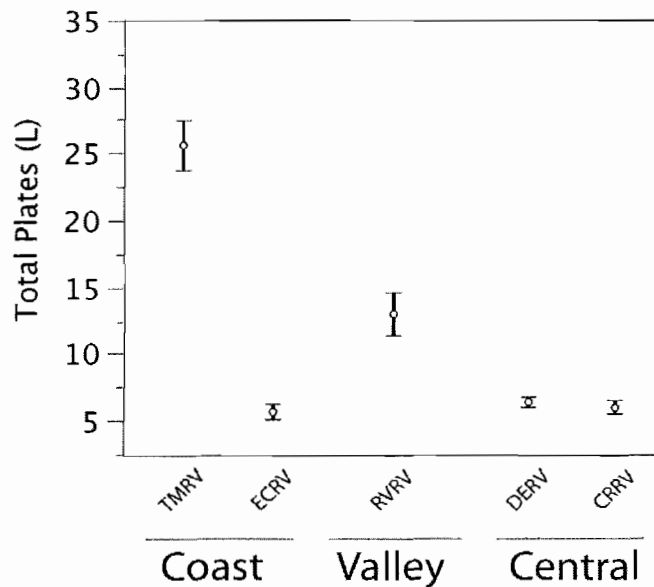


Figure III.4 Lateral plate counts for stickleback in river systems across Oregon regions. Mean counts (95% CIM) of the number of lateral plates on the left side of stickleback in five populations in each of three regions. Coastal populations show distinctly different plating phenotypes that may be the result of differences in the amount of introgression by marine alleles. The valley population has an intermediate phenotype that suggests either some marine gene flow or the initial stages of reduction in this character. Central populations are isolated from other regions and show a consistent low plate phenotype.

Left Pelvic Spine Length: Region (Coast) X Habitat Type (Estuary, River, Lake)

We compared the residuals for length of the left pelvic spine among the different habitat types in the coastal region (Table III.2). Variation in this character was observed across habitat type, however no clear pattern was observed (Fig. III.5) One estuarine population (Cushman Slough) had larger values for spine length than any other group overall. Additionally, two other estuarine populations, Miner Creek and Winchester Creek South,

had lower left pelvic residuals than any other estuarine group, but were not different from one another. There was a significant difference between residuals for the two riverine populations. There were no differences in residuals between the two lake populations. Nor were there differences in residual values between lake populations and fish from any other habitat type.

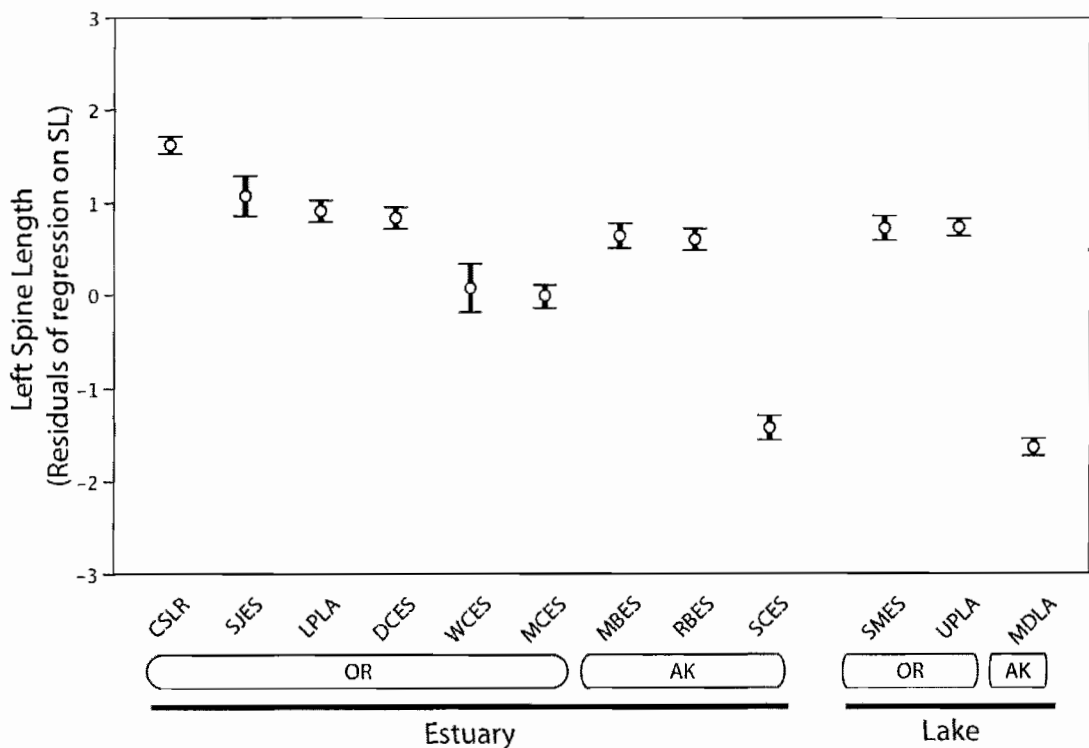


Figure III.5 The resulting residuals for length of the left pelvic spine among the different habitat types in the coastal region of Oregon and Alaska. Mean lengths (95% CIM) of the residuals for the left pelvic spine on the left side of stickleback for nine populations from Oregon and four Alaskan populations from coastal estuaries and lakes. This character was relatively undifferentiated in Oregon estuary and lake populations, with the exception of one putatively anadromous population. Alaskan populations showed distinct differences in spine length between habitat types. These results suggest that there may be differences in local selective pressure across geographic distance.

OR X AK Lateral Plate Count: Region (Coast) X Habitat Type (Estuary, River, Lake)

We compared lateral plate counts for coastal estuarine and lacustrine populations from Oregon and Alaska (Table III.2). The results for both states showed congruent patterns

with the highest plate counts in estuarine populations and the lowest counts in lacustrine populations (Fig. III.6)

The mean number of lateral plates differed very little between Oregon and Alaskan populations within habitat type. Estuarine populations from Oregon had ~26 plates while Alaskan populations had ~29. Lacustrine populations from Oregon had ~5 plates and Alaskan populations had ~7. There was no overlap in the standard errors between Oregon and Alaskan data.

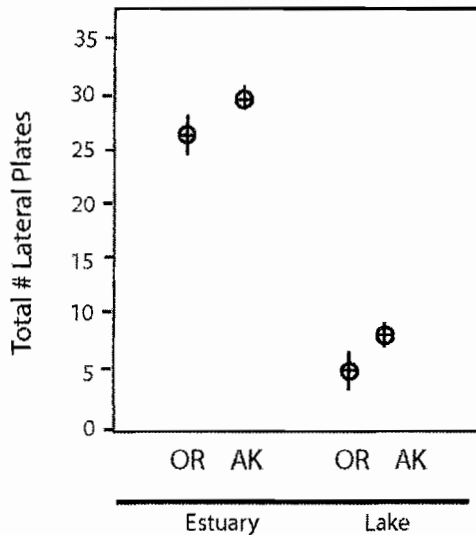


Figure III.6 Comparisons of lateral plate counts for coastal estuary and lake populations from Oregon and Alaska showed parallel phenotypic development across geographic distance. Estuarine populations had the highest lateral plate counts and lake populations had the lowest plate counts. Mean counts (95% CIM) of the number of lateral plates on the left side of stickleback for eight Oregon and four Alaskan estuary and lake populations.

OR X AK Left Pelvic Spine Length: Region (Coast) X Habitat Type (Estuary, River, Lake)

We compared the residuals for length of the left pelvic spine among estuarine and lacustrine habitat types between the coastal regions of Oregon and Alaska (Table III.2) Independent of habitat type, Oregon populations had larger residual values than Alaskan populations in most cases (Fig. III.5) Pelvic spines were longer in Oregon estuarine and lacustrine populations than in comparable Alaskan populations. However, the relative differences across habitat types in Alaska mirrored those in Oregon. Both Alaskan and

Oregon estuarine populations had larger residual values for left pelvic spine length in comparison to lacustrine populations.

Discussion

The Role of Geographic Distance in Partitioning Phenotypic Variation Across Oregon

We examined the effects of geographic separation on phenotype distribution for body shape and lateral plate counts in Oregon populations. Riverine stickleback from different regions across Oregon showed variation in both characters (Fig. III.2, Fig. III. 4).

The body shape data was clustered within habitat type among regions indicating differentiated ecomorphs existing within the same habitat type across geography. Additionally, lateral plate phenotypes clearly varied among regions. The lack of overlap between clusters and the large difference in numbers of lateral plates among regions indicated that there was little or no gene flow between river populations from each region. Populations from river habitats in the same region had more consistent morphology with one another than with populations from other regions, suggesting that local regional factors played a role in body shape. Alternatively, lateral plating phenotypes showed a trend more closely associated with biotic inputs from marine populations. These outcomes suggest that community structure among habitats isolated by distance may have different biotic and abiotic limitations.

A contributing factor to the pronounced shape difference observed in coastal populations compared to valley and central populations is the larger amount of gene flow from marine fish. Permanent connectivity between watersheds is greatest in the coast range, which increases the chances of marine and resident fish meeting and hybridizing. Repeated introgression maintains marine genes in coastal populations such as those found in estuaries, rivers and accessible lakes.

Of the two coastal riverine populations surveyed one had a markedly higher number of lateral plates than the other (Graph III.3). Lateral plating is heritable and fish from Tenmile Creek are more subject to marine genetic input from high-plated fish. The Eel Creek population had very low plate counts that reflected little or no genetic

influence from marine sources. This suggests that the amount of gene flow between resident and migratory fish is an important component in the evolution of this character among populations. The high plated phenotype is more likely to be maintained in the Tenmile Creek population due to repeated exposure to and hybridization with marine fish. The Eel Creek population experiences prezygotic reproductive isolation by breeding in a location in which potential interaction with anadromous fish is absent.

The valley population of stickleback comes from the Mckenzie River, a water body that is ultimately contiguous with the Pacific Ocean. Valley stickleback showed differentiation in body shape and polymorphism in lateral plating, which may be the result of indirect accrual of marine alleles along the river gradient. However, morphological analyses do not reflect substantial similarities between valley and coastal body shape. Alternatively, the differences in shape and variation in lateral plating might be incipient transition to a derived freshwater phenotype. The transition from a high-plated phenotype to a reduced-plate phenotype with concurrent shape change after colonization of freshwater habitats has been documented in other work (Barrett, Rogers and Schluter 2008). The age of this population is undocumented.

There was more similarity in body shape between valley and central populations than the difference expected based on the relative isolation of populations in each region. Comparisons of populations from these regions had more body shape characteristics in common than either group had with coastal populations. A possible explanation for this may be recent anthropogenically-caused introduction of stickleback to central Oregon. The historical presence of these fish is unknown before the 1980's in that area.

The lateral plate counts for central riverine stickleback reflected a uniformly low-plated phenotype. Unlike the coastal populations, riverine fish from the central region were isolated from gene flow with populations east of the Cascade Mountains. Additionally, they came from geographically separated populations within the central region. The biotic and abiotic conditions of both rivers were consistent between populations. The lack of gene flow between populations and the similarity between

environmental factors suggests that habitat type plays a role in shaping morphological outcomes in stickleback.

Central Oregon populations of stickleback exist under abiotic parameters that are markedly different than those observed in the other regions. Connectivity of watersheds is significantly reduced and often seasonal, the climatic disposition of the central region is drier, annual temperature fluctuations are greater among seasons and the geologic makeup of the area is much older than that of the other regions. Physical barriers (dams) prevent all natural gene flow from marine sources and lack of contiguity between watersheds isolates the central region from valley populations.

Phenotypic Variation is Also Distributed Across Different Habitats in the Same Region

We compared body shape and length of pelvic spines among different habitat types within the coastal region. There were distinct differences in body shape associated with habitat type, but pelvic spine length did not show any prevalent trends (Fig. III.5).

One estuarine population, Cushman Slough, had larger residual values than any other population for pelvic spine length. This population is likely anadromous, based on its seasonal abundance and may be reflecting a marine phenotype with respect to pelvic spine length. There is less certainty about the life histories of the other estuarine populations. No other populations from any habitat type showed an independently significant result like Cushman Slough. Possible explanations for the lack of strong correlation outcome include: 1) pelvic spine length may not be a character undergoing evolution in these habitats. In the coastal region, abiotic and biotic factors such as local climate, geology and community composition are similar across habitats. This might reduce potentially divergent selective pressure on the character resulting in a consistent phenotype due to a common environment. 2) Alternatively, pelvic spine length may exhibit a conserved and unvaried phenotype due to heavy selection independent of habitat type.

The data for body shape were loosely clustered among habitats within the coastal region (Fig. III.3). However, the spread of the data suggests that coastal habitats have

greater connectivity and potential for gene flow. A high degree of connectivity among watersheds may act reductively on phenotypic variation among adjacent populations. Abiotic and biotic factors are likely to be more alike in contiguous coastal habitats and the opportunities for gene flow are increased.

Fish from estuarine, riverine and lacustrine habitats within the coast region all clustered phenotypically to varying degrees. Estuarine populations had no clear overlap with lacustrine populations, but showed many similarities to riverine fish. Riverine populations exhibited phenotypic overlap with both lacustrine estuarine populations.

Estuary and river habitat types tend to be highly contiguous and estuarine fish are known to spawn in both brackish and fresh water (Bell and Foster 1994). This strengthens the possibility for gene flow between estuarine and riverine populations and clearly delineates a geographically discernable phenotype gradient that begins at the ocean and moves inland.

The overlap of the estuarine and riverine data can be explained by the distinctly different life histories of the two riverine populations sampled. Interestingly, they both come from the same river system and presumably experience very similar biotic and abiotic pressures. One population showed a shape phenotype more similar to estuarine fish due to hybridization with seasonally co-occurring anadromous stickleback. The second population showed a more derived phenotype unique to the system.

The first population comes from a large slow-moving river that is directly contiguous with the Pacific Ocean called Tenmile Creek in Lane County, Oregon. It has resident populations of stickleback and in early spring, anadromous fish migrate in to spawn. There is overlap in the breeding period between resident and anadromous populations and hybridization is highly likely. This hypothesis is supported by the observation that fish from Tenmile Creek share many morphological similarities with estuarine populations.

The second population comes from Eel Creek, a small perennial stream indirectly contiguous with the Pacific Ocean via the larger, connecting Tenmile Creek. This population is considered resident within the Tenmile-Eel Creek system. Fish migrate

upstream to spawn in Eel Creek during early spring and return to Tenmile Creek by late April. For the remainder of the year, no stickleback reside in Eel Creek. Adult fish are characterized by a unique suite of morphological characteristics that distinguish them from other populations surveyed in the system. This specific morphotype is the only one found in Eel Creek and only during breeding season. Furthermore, anadromous fish have been found above the outlet where Eel Creek flows into Tenmile Creek. However, no anadromous fish have been trapped in Eel Creek despite regular sampling from 2007-2009. Based on these observations, it is unlikely that the Eel Creek population hybridizes with anadromous migrants regardless of overlapping breeding season.

These significantly different populations are representative of the large amount of phenotypic diversity found in Oregon populations and were chosen to illustrate the difficulty of quantifying such variation even at a relatively small scale.

The data showed a small amount of overlap between lacustrine and river populations. This overlap may be due to the relatively recent isolation and early stages of divergence of the lake populations found in Pony Creek Reservoir. The lake sites contain remnant populations of stickleback that historically resided in Pony Creek, a freshwater creek that was transformed into a reservoir approximately 50 years ago. This population does not undergo any natural gene flow with outside populations, but may still retain ancestral riverine characters. Phenotypic differentiation in this case suggests a different set of environmental parameters between lakes and other habitat types.

Parallel Phenotypic Development Between Alaskan and Oregon Populations Supports the Role of Natural Selection in Local Adaptation

Coastal region was held constant between Oregon and Alaska and the number of lateral plates present and pelvic spine length for populations in analogous habitat types was compared across a large geographic scale (Fig. III.5 , III.6). The emergent trend suggests that local habitat may have a greater effect on shaping phenotype than isolation by geographic distance.

Estuarine populations in both Oregon and Alaska had higher lateral plate counts than lacustrine populations from either state. Furthermore, the differences in plate number within habitat type between states were very small. This suggests that differences may be due to variation in local selection.

The standard error for both habitat types was largest for Oregon populations. This indicated a larger amount of phenotypic variation in the plating character that may be explained by the age of Oregon populations relative to those found in Alaska. Older populations have had more time for buried variation to surface and to diverge from the ancestral phenotype. Alternatively, the range of biotic and abiotic factors that influence phenotype may be less specific in Oregon. This may result in more flexibility with respect to phenotypic output.

Additionally, Oregon and Alaskan populations of estuarine and lacustrine stickleback showed differences in the residuals of the left pelvic spine length (Fig III.5). In all except two cases, Oregon estuarine and lacustrine populations had larger residual values than Alaskan populations indicating longer spine length. A possible explanation for the differences observed might be that local selective pressures differ across large geographic distance. In the coastal region, abiotic and biotic factors such as local climate, geology and community composition are similar across habitats within region, but may be different in the same region across latitude. Pelvic spine length may be undergoing divergent evolution in estuarine and lacustrine habitats across latitude resulting in differing phenotypes due to local selective pressure.

Similar environments, independent of geographic proximity, are imbued with groupable categories of biotic and abiotic variables that affect stickleback morphology predictably, resulting in ecomorphic divergence (Walker 1997, Reusch et al. 2001). The widespread examples of parallel evolution of morphology and armor phenotypes that occur across latitude and longitude among populations of threespine stickleback suggest that local selective processes are involved in phenotypic output (McPhail 1994; Walker 1997; McKinnon et al. 2004).

We made comparisons across three geographic scales in Oregon and asked questions about the role of local selective forces on phenotypes and isolation. Body morphology and armoring are local phenotypes that showed two development trends: 1) differentiation within habitat type across region 2) parallel evolution within habitat type and region across larger geographic scales. These results imply that the selective forces associated with habitat type may have a stronger influence on morphological variation than geographic separation.

These data support the hypothesis that local adaptation results in variation across geography and habitat type. Furthermore, there is parallel evolution of morphological phenotype within geographically distant habitat types.

CHAPTER IV CONCLUSIONS

Natural variation occurs at different geographic scales. Species distributions often span such scales and show phenotypic variation that may be the result of selective pressure from the local environment. Understanding the associations between environmental variables and phenotype can illuminate the underpinnings of selection at smaller scales. To address this, we compared physiological (photoperiodic response) and morphological (body shape and armoring) phenotypes from populations of threespine stickleback from different habitat types, regions and across latitude.

We experimentally compared photoperiodic response between an Alaskan and Oregon population and found that the northern population was photoperiodic, but that the southern population was not. Photoperiodic response is a local phenotype that is genetically based and these results suggest local adaptation to photoperiod at different latitudes (Bradshaw and Holzapfel, 2007).

We analyzed data on a suite of morphological characters using geometric morphometrics and linear analyses and made comparisons among different habitat types across coastal, valley and central regions of Oregon. We discovered significant differences in body shape, number of lateral plates and the length of pelvic spines distributed among habitat types and across regions. Additionally, we found similar patterns in these characters across habitat type in comparisons of Oregon and previously well studied Alaskan populations. The parallel association between morphology and habitat type in geographically distinct populations in Oregon and Alaska supports the hypothesis that threespine stickleback are affected similarly by local selection regimes resulting in parallel morphological adaptation.

Future work should build on these findings by addressing the underlying genetic architecture of both photoperiodism and the morphological variation described herein to

determine whether there is a genetic basis for adaptation in these characters. Additionally, it would be worthwhile useful to determine the phylogenetic history between Oregon and Alaskan populations in order to better understand the differences observed between groups.

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