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TEMPORAL VARIATION IN DIVERGENT SELECTION ON SPINE NUMBER IN THREESPINE STICKLEBACK

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Abstract.—Short-term temporal cycles in ecological pressures, such as shifts in predation regime, are widespread in nature yet estimates of temporal variation in the direction and intensity of natural selection are few. Previous work on threespine stickleback (*Gasterosteus aculeatus*) has revealed that dorsal and pelvic spines are a defense against gape-limited predators but may be detrimental against grappling insect predators. In this study, we examined a 15-year database from an endemic population of threespine stickleback to look for evidence of temporal shifts in exposure to these divergent predation regimes and correlated shifts in selection on spine number. For juveniles, we detected selection for increased spine number during winter when gape-limited avian piscivores were most common but selection for decreased spine number during summer when odonate predation was more common. For subadults and adults, which are taken primarily by avian piscivores, we predicted selection should generally be for increased spine number in all seasons. Among 59 comparisons, four selection differentials were significant (Bonferroni corrected) and in the predicted direction. However, there was also substantial variability in remaining differentials, including two examples with strong selection for spine reduction. These reversals were associated with increased tendency of the fish to shift to a benthic niche, as determined from examination of stomach contents. These dietary data suggest that increased encounter rates with odonate predation select for spine reduction. Strong selection on spine number was followed by changes in mean spine number during subsequent years and a standard quantitative genetic formula revealed that spine number has a heritable component. Our results provide evidence of rapid morphological responses to selection from predators and suggest that temporal variation in selection may help maintain variation within populations. Furthermore, our findings indicate that variable selection can be predicted if the agents of selection are known.

Key words.—Defense, natural selection, predation, selective heterogeneity, stickleback, survival.

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Although natural selection is often predicted to deplete variation within populations (Fisher 1930; Lewontin 1964), intrapopulation variability can be maintained by temporal or spatial heterogeneity in selection pressures (Felsenstein 1976; for review see Hedrick et al. 1976; Hedrick 1986). Studies directly demonstrating selection in natural populations have flourished over the last few decades (Endler 1986; for review see Kingsolver et al. 2001), yet the mechanisms of selection are often unknown and heterogeneity in selective pressure not evaluated. Detection of spatial variability in selection (e.g., Reimchen 1979, 1980; Bell et al. 1985; Arnqvist 1992; Losos et al. 1998; Blanckenhorn et al. 1999; Butler et al. 2000) is comparatively easy relative to detecting temporal heterogeneity in selection, which may require multiple-generation comparisons (Price and Grant 1984; Schluter and Smith 1986; Reimchen 1995). Temporal variation in selection is generally thought to slow the loss of variation rather than maintain variation indefinitely and it can be difficult to predict. Empirical studies of temporal variation in selection where the agents of selection are reasonably well known can help clarify the relative importance of this process in the maintenance of variation and may also allow determination of the causes of variable selection (e.g., Benkman and Miller 1996; Jann et al. 2000).

Antipredator defenses provide useful characters for examining heterogeneity in selection (Reimchen 1979, 1994; Vermeij 1987; Swain 1992; Crespi and Sandoval 2000) as predation regime differs spatially and temporally. The dorsal and pelvic spines of threespine stickleback (*Gasterosteus aculeatus*) comprise a defensive adaptation against gape-limited predators such as predatory fish (Hoogland et al. 1957). Geographical surveys of stickleback populations in North America and Europe show substantive evidence for site-specific selection as populations exposed to elevated levels of vertebrate predators have larger or more robust spines than populations with reduced levels of vertebrate predation (Hagen and Gilbertson 1972; Moodie and Reimchen 1976; Gross 1978; Reimchen 1983). In several localities from throughout the North American and European distribution of stickleback, populations have been identified that exhibit variability in number of dorsal and pelvic spines (e.g., Moodie and Reimchen 1976; Bell 1987). Such intrapopulation variability in the major defensive structure on the fish is atypical and offers opportunities for evaluating strength and mode of selection in natural populations.

One of these spine-variable stickleback populations inhabits Boulton Lake on the Queen Charlotte Islands, western Canada. Reimchen (1980) observed that stickleback of both sexes with greater number of spines were more prevalent in limnetic regions, whereas spine-deficient morphs were most abundant in littoral zones. Predation by diving birds, which are gape-limited piscivores, may produce selection favoring increased complement of spines (Moodie and Reimchen 1976; Gross 1978; Reimchen 1988). Conversely, odonate naiads are grappling predators and may produce selection favoring spine reduction (Reimchen 1980; Reist 1980; Ziuganov and Zotin 1995). Thus intrapopulation variability in spine number appears to be a functional adaptation to spatial

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variability in the two predator groups, diving birds being more prevalent in the limnetic regions and odonates being most common in littoral and benthic regions. Notably, the lake is devoid of piscine predators. In addition to the spatial congruence between spine phenotypes and predators in Boulton Lake, there were also temporal changes in predation regime. Avian piscivores such as loons (*Gavia immer*) are intermittent in spring and summer, whereas mergansers (*Mergus* spp., *Lophodytes*) and grebes (*Podiceps* spp.) are mainly present during autumn and winter (Reimchen 1980). Macroinvertebrate piscivores such as *Aeshna* are resident throughout the year but should be more active during the higher temperatures of spring and summer (Agryopoulou and Stamou 1993; Nakakita and Ikenaga 1997). This may result in temporal variability in divergent selection. As a result, stickleback were sampled in spring and autumn over a 15-year period to test for temporal shifts in selection on spine number.

We first examine yearly variability in mean spine number, predicting that any fluctuations between the sexes and among age classes should be correlated if a common process drives yearly variation. The activity and metabolic demands of invertebrate predators should be greatly increased by elevated temperatures and, consequently, we expected that mean spine number would be inversely related to monthly temperature within a season at least for younger age classes of stickleback ($<$ 45 mm SL), which are most susceptible to odonate predation (Reimchen 1980). Although such a correlation between an environmental variable and morphology suggests selection has occurred (Endler 1986), it does not measure fitness differences among individuals. Consequently, we also directly estimated within-generation phenotypic selection on spine number during episodes of juvenile, subadult, and adult survival for two seasonal bouts of selection (summer survival, winter survival; method VII, Endler 1986) for each year. We predicted that exposure to increased avian predation would select for elevated spine number, whereas greater exposure to odonate predation would select for spine reduction. We assessed variation in exposure to these different predation regimes by: (1) seasonal shifts in the relative abundance of the two predator groups; and (2) temporal shifts in habitat use as assessed by examining variation in stickleback diet, predicting that increased use of littoral or benthic foods will increase exposure to odonate predators and, consequently, result in selection for spine reduction.

Furthermore, we used the intergenerational response to selection and standard quantitative genetic formula to estimate the heritability of spine number. Evidence for temporal heterogeneity in divergent predatory regimes acting on a heritable trait would provide evidence that natural selection is capable of producing rapid morphometric shifts within populations and would suggest that variable selection can be predicted if the agents of natural selection are known. Notably, stickleback serve as a particularly useful model for estimating selection due to previous studies on the functional significance of defense morphology (Hagen and Gilbertson 1972; Moodie and Reimchen 1976; Gross 1978; Reimchen 1983, 1994) and trophic morphology (Schluter 1993, 1994, 1995).

MATERIALS AND METHODS

Temporal Fluctuation in Mean Spine Number

Collections of stickleback were made from 1970 to 1987 using minnow traps, trawls, and beach seines. When possible, juvenile and adult age cohorts were collected during spring and autumn from 1975 through to 1987 to obtain a sequential, replicated series of samples that would allow tests for selection on spine number. The spring samples represented age 0+ cohort that survived the winter as well as subadults and adults, whereas the autumn samples represented young of the year, 1+, and adult cohorts.

A total of 20,368 stickleback were scored for the multiple meristic and metric characters including numbers of dorsal, pelvic, and anal spines (for details see Reimchen 1980, 1997). Stickleback could have from zero to six spines per fish (maximum three dorsal spines, two pelvic spines, and an anal spine). We estimated selection acting on the spines within age cohorts using two methods: (1) comparison of changes in the relative frequency of each of six separate pelvic and dorsal spine phenotypes through time (three dorsals, full pelvis; three dorsals, half pelvis; three dorsals, absent pelvis; two dorsals, full pelvis; two dorsals, half pelvis; two dorsals, absent pelvis) and; (2) comparison of the changes in mean spine number per fish (range = 0–6).

We assessed variability among years in mean spine number for yearlings and subadults ($<$ 45 mm) and adults ($\geq$ = 45 mm) using ANOVA. We examined whether mean yearly spine number was correlated between males and females and tested (ANCOVA) whether this occurred in both subadults and adults (i.e., test for homogeneity of slopes, size class as a factor). We also tested whether mean monthly spine number was correlated with mean monthly temperature in subadults and adults within two separate seasons (winter: January–April and October–December; summer: May–September) using ANCOVA analyses with sex as a factor and mean monthly temperature as a covariate.

Estimates of Selection on Spine Number

Selection can be estimated by comparing trait frequency distributions between age or life-history classes collected at a single point in time (cross-sectional data) or by following cohorts through time (longitudinal data; Endler 1986). We adopted the latter method; selection was estimated by comparing trait distributions before and after seasonal bouts of selection (winter, summer) for three different cohorts (juvenile, subadult, adult). For winter, we compared the stickleback cohort captured from September to October to the same cohort collected from March to April in the subsequent year. For summer, we compared the stickleback cohort captured from March to April to the same cohort collected from September to October of the same year. The juvenile and subadult cohorts can be differentiated with length frequency curves, although there is overlap between the 1+ and 2+ cohorts. Stickleback in this lake grow approximately 2–3 mm/month (Reimchen 1992). We compared fish before and after seasonal bouts of selection that differed by 10 mm SL, thus accounting for seasonal growth and improving the resolution of our cohort analyses. For all comparisons within cohorts between the two time periods (t_{begin}, t_{end}), we assigned absolute fitness values of zero and one, respectively. Comparisons were made for the two size classes within each cohort: juvenile survival (20–35 mm vs. 35–45 mm), subadult survival (35–45 mm vs. 45–55 mm), and adult survival (45–55 mm vs. 55–65 mm). Selection analyses were performed for all samples where there were $\geq$20 fish in each fitness category. The sexes differ in spine number (Reimchen 1980) and, consequently, were treated separately for all analyses.

We tested whether mean spine number differed before and after each separate bout of selection using *t*-tests and report standardized directional selection differentials (equivalent to selection intensities, *i*; Endler 1986). Notably, estimates of selection calculated in this manner (i.e., without direct reference to individuals who survived or perished) will tend to underestimate the strength of selection, rather than detect selection when it has not occurred (Endler 1986). Corrections for multiple comparisons are not standard in estimation of selection differentials, and thus we retain a critical alpha of 0.05. However, for each episode of survival we also present significance levels corrected for multiple comparisons within genders (sequential Bonferroni method; Rice 1989). We tested for trends in the direction of selection using binomial tests and Fisher's exact tests. Variance (nonlinear) selection acting on spine number was also estimated but this was rarely detected and was not related to season in any size class. Consequently, these quadratic selection differentials are not reported.

We also tested whether dietary niche shifts (pelagic vs. benthic) were correlated with the direction of selection on spine number that was detected. Unparasitized stickleback ($N = 9089$) were examined and for each specimen the presence or absence of various food items was recorded (for scoring methodology and general descriptions of the diet, see Reimchen and Nosil 2001a,b,c). As a measure of how benthic the diet of stickleback was within each episode of selection, we calculated the proportion of individuals who had benthic macroinvertebrates (amphipods, zygopterans, or trichopterans) present in their stomachs. We calculated this dietary variable separately for subadult and adult survival (juveniles do not consume macroinvertebrates) and for each episode of selection. Because the diet varies substantially between the sexes (Reimchen and Nosil 2001a), the proportion of benthic fish within a sample was also calculated separately for each of the sexes. We used bivariate correlation and ANCOVA analyses to test whether the proportion of fish with macroinvertebrates in their stomach contents within an episode of selection was correlated with the estimated selection differential from that same sample. In some samples, one or more of these three food items was not scored systematically and these samples were excluded. All statistical analyses were conducted using SPSS (ver. 10, SPSS, Inc., Chicago, IL).

For juvenile survival there was evidence of seasonal heterogeneity in the direction of linear selection. To visualize the form of directional selection acting on the spine number during the two seasonal bouts of selection, we calculated univariate fitness functions using the nonparametric cubic spline approach of Schluter (1988). This technique does not assume any a priori form of selection on a character and may detect local optima not readily detectable with parametric regression techniques. However, because individuals were not individually marked, cubic splines will be approximations of the relationship between phenotype and fitness. Fitness functions and standard errors (based on bootstrap resampling) from 1000 bootstraps were calculated for all samples where changes in mean spine number approached or attained statistical significance ($P < 0.10$) using software developed by D. Schluter (GLMS, ver. 3).

Response to Selection and Estimates of the Heritability of Spine Number

For episodes of selection where selection differentials attained statistical significance, we also estimated the heritability (h^2) of spine number using selection intensities (*i*) and the response the selection (*R*), where $R = h^2 i$ and *R* represents the change in mean phenotype between generations (mean in second generation − mean in first generation). For these analyses, we calculated mean spine number in the generation after selection in the following manner (using 20- to 35-mm fish as the next generation in all cases): (1) juvenile winter and summer survival, fish 20–35 mm in the following year's fall were considered the next generation; (2) subadult winter survival, fish from the current fall were considered the next generation; (3) subadult summer survival, fish from the following year's fall were considered the next generation; (4) adult winter survival, fish from the current fall were considered the next generation; and (5) adult summer survival, fish from the following year's fall were considered the next generation.

RESULTS

Temporal Variability in Mean Spine Number

There were temporal fluctuations in mean spine number for subadult stickleback (year, $F_{14,9979} = 9.32$, $P < 0.001$; sex, $F_{1,9979} = 88.47$, $P < 0.001$; sex × year, $F_{14,9979} = 1.58$, $P = 0.08$, two-way ANOVA) and for adult stickleback (year, $F_{14,10252} = 9.27$, $P < 0.001$; sex, $F_{1,10252} = 115.30$, $P < 0.001$; sex × year, $F_{14,10252} = 1.09$, $P = 0.36$, two-way ANOVA; Fig. 1). Mean yearly spine number in adult fish was correlated with mean spine number for subadult stickleback from the previous year (Fig. 2; $r^2 = 0.67$, $B = 0.39$, SE of $B = 0.17$, $F_{1,28} = 5.48$, $P < 0.05$, ANCOVA), with no differences in slope between the sexes ($F_{1,28} = 0.17$, $P = 0.68$, test of parallelism). We also tested whether changes in spine number were congruent between the sexes and this was confirmed ($r^2 = 0.19$, $F_{1,30} = 6.16$, $P < 0.05$) and was independent of size class (spine number × size class interaction, $F_{1,30} = 0.77$, $P = 0.39$, ANCOVA).

Mean Spine Number and Temperature

We tested whether mean monthly spine number in either subadults or adults was associated with mean monthly temperature in winter or summer seasons. Increased temperature within seasons should lead to increased odonate activity and this could result in stronger selection against spines in juvenile fish. Results show a negative association between spine number in subadults and temperature in summer that approaches statistical significance (slope = -0.03, SE =

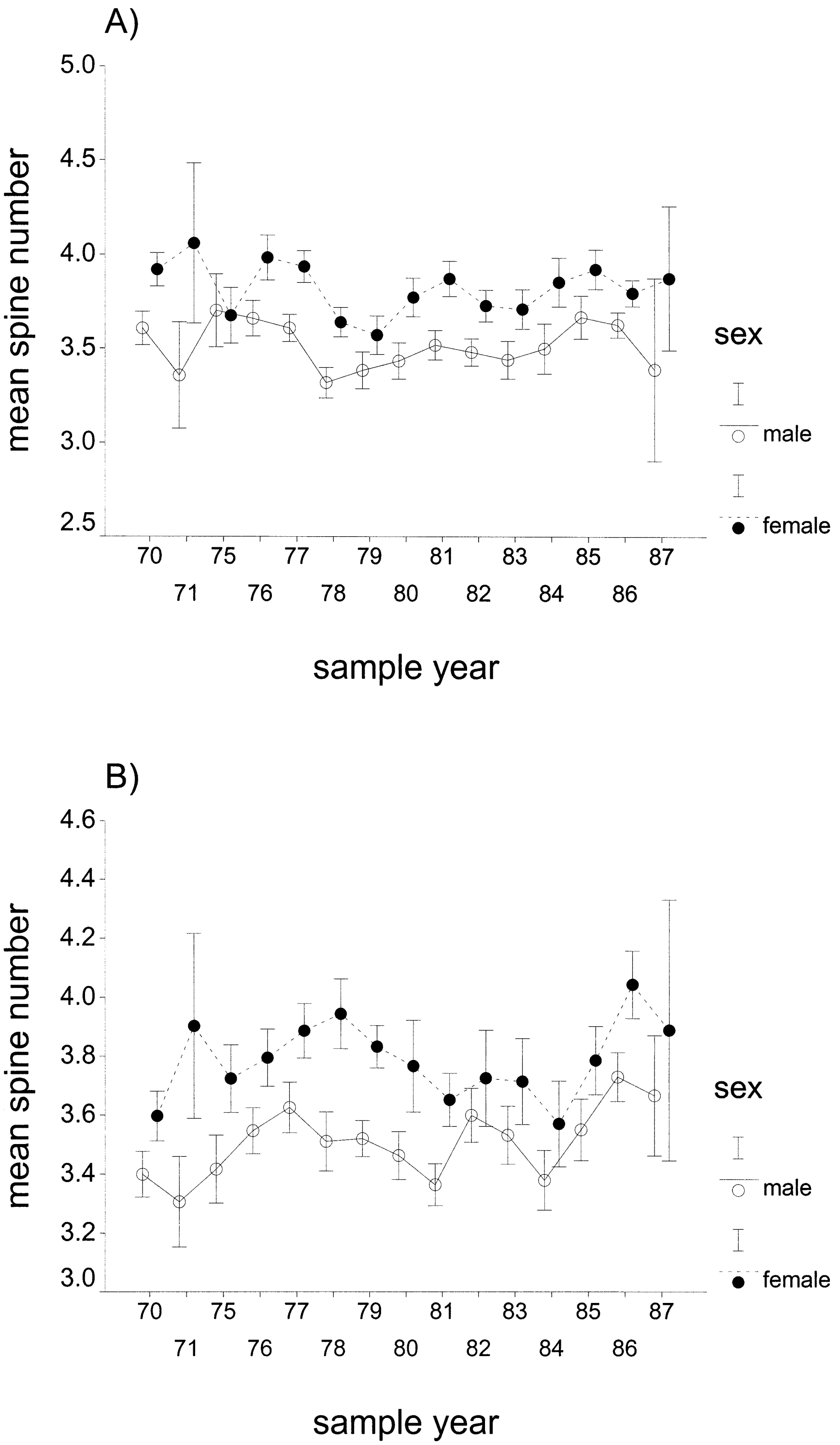

FIG. 1. Temporal variability in mean yearly spine number for male and female stickleback collected from Boulton Lake between 1970 and 1987. Mean yearly spine number was correlated between the sexes in both adults and subadults ($r^2 = 0.19$, $P < 0.05$): (A) subadults (< 45 mm); (B) adults (≥ 45 mm).

0.16; $F_{1,69} = 3.47$, $P = 0.06$, ANCOVA). This association between temperature and spine number did not differ between the sexes (all $P > 0.05$, test for homogeneity of slopes). None of the other associations with temperature approached significance (all $P > 0.25$).

Estimates of Selection on Spine Number

For each sample year, we estimated directional (linear) selection acting on spine number for winter and summer survival in juveniles, subadults, and adults (Tables 1, 2, 3, re-

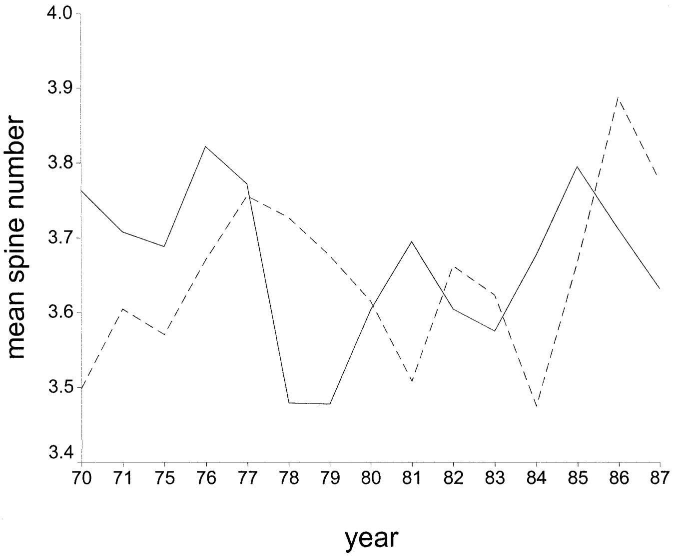

FIG. 2. Mean yearly spine number for subadult (< 45 mm, solid line) and adult (≥ 45 mm, dotted line) stickleback from Boulton Lake between 1970 and 1987. Mean yearly spine number in adult fish was correlated with mean spine number for subadult stickleback from the previous year ($r^2 = 0.67$, $P < 0.05$), with no differences in slope between the sexes ($P = 0.68$, ANCOVA test of parallelism).

spectively). Mean absolute directional selection differential (all size classes) was 0.24 (maximum = 0.88; see Fig. 3). Mean signed directional selection did not differ significantly from zero (mean = 0.01, $t_{82} = 0.27$, $P = 0.79$, one-sample *t*-test), indicating selection for decreased and increased spine number was comparable.

In general, we predicted selection for increased spine number during times of bird activity (winter) but for decreased spine number during times of odonate activity (summer). For juvenile survival, there was no evidence of such directional trends when the entire dataset was considered (15 of 24 tests in the predicted direction, $P = 0.31$, binomial test, Table 1). However, when we restricted our analyses to the differentials that were statistically significant, we detected selection in the predicted directions; strong directional selection on the spines tended to be positive during winter, but negative during summer. Among the five cases where changes in mean spine number attained statistical significance, three occurred during summer survival where spine number decreased, and two occurred during winter where spine number increased ($P = 0.10$, Fisher's exact test). This association was strengthened when we included samples where changes in mean spine number approached statistical significance ($P < 0.10$), with three negative differentials occurring during summer and five positive differentials occurring during winter ($P < 0.05$, Fisher's exact test). Graphical analyses using the cubic spline were largely consistent with statistical analyses, with fitness tending to increase with increasing spine number during winter but decrease with increasing spine number in summer (Fig. 4).

We suspected pooling spine number across the three types of spines (dorsal, pelvic, anal) may confound associations between phenotype and fitness (survival) and, consequently, we performed analyses of the relationship between phenotype

TABLE 1. Juvenile stickleback and estimates of selection on spine number. Results show standardized directional (linear) selection differentials (*i*) for survival through winter (W) and summer (S) seasons. Also shown for each seasonal bout of selection is mean (SD) spine number before ($\bar{X}_B$) and after ($\bar{X}_A$) selection and results from independent-samples *t*-tests for differences in mean spine number before and after selection. Selection tended to favor increased spine number through winter seasons but favor decreased spine number through summer seasons. Results in bold remain significant even after correction for multiple comparisons within genders (sequential Bonferroni method; Rice 1989).

Date	Sex	*i*	$\bar{X}_B$ (SD)	$\bar{X}_A$ (SD)	*t*	*N*
W 1970–71	males	−0.24	3.58 (0.93)	3.36 (0.91)	1.50	408
S 1977	females	0.12	3.95 (0.17)	3.97 (0.20)	−0.05	73
W 1977–78	males	0.11	3.46 (0.95)	3.56 (1.11)	−0.60	159
	females	−0.03	3.87 (0.99)	3.84 (0.95)	0.17	161
S 1978	females	0.09	3.56 (0.76)	3.63 (1.17)	−0.33	103
W 1978–79	males	0.13	3.45 (1.00)	3.58 (0.85)	−0.68	99
	females	−0.26	3.83 (0.85)	3.61 (0.95)	1.16	95
W 1980–81	males	0.34	3.44 (0.79)	3.71 (1.08)	−2.16*	226
	females	**0.49**	3.68 (0.88)	4.17 (1.07)	**−2.88****	198
S 1982	males	−0.28	3.67 (0.79)	3.45 (0.85)	1.23	92
	females	0.07	3.93 (1.04)	4.00 (1.08)	−0.25	52
W 1982–83	males	0.25	3.38 (0.69)	3.55 (0.95)	−1.71	324
	females	−0.05	3.63 (0.81)	3.59 (0.91)	0.29	280
S 1983	males	−0.23	3.63 (1.10)	3.38 (0.67)	0.89	45
W 1983–84	males	0.23	3.34 (0.90)	3.55 (0.99)	−1.21	137
	females	0.17	3.68 (0.86)	3.83 (1.09)	−0.78	138
S 1984	males	0.29	3.23 (0.72)	3.44 (1.01)	−1.02	70
	females	−0.52	3.83 (1.01)	3.30 (0.77)	2.44*	73
W 1984–85	males	0.05	3.69 (1.03)	3.74 (0.97)	−0.27	90
	females	−0.16	4.02 (0.96)	3.87 (0.78)	0.80	103
W 1985–86	males	0.48	3.51 (0.73)	3.86 (1.06)	−1.94	318
	females	0.39	3.76 (0.94)	4.13 (1.10)	−1.79	155
S 1986	males	**−0.43**	3.70 (0.88)	3.32 (0.72)	**4.48*****	396
	females	**−0.37**	3.94 (0.99)	3.57 (0.97)	**4.55*****	529

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 2. Subadult stickleback and estimates of selection on spine number. Results show standardized directional (linear) selection differentials (i) for survival through (W) and summer (S) seasons. Also shown for each seasonal bout of selection is mean (SD) spine number before (X_B) and after (X_A) selection and results from independent-samples t-tests for differences in mean spine number before and after selection. Results in bold remain significant even after correction for multiple comparisons within genders (sequential Bonferroni method; Rice 1989).

Date	Sex	i	$\bar{X}_B$ (SD)	$\bar{X}_A$ (SD)	t	N
W 1970–71	males	−0.50	3.86 (1.03)	3.35 (0.79)	2.61*	87
	females	−0.20	4.13 (1.02)	3.93 (1.11)	0.77	68
W 1976–77	males	**−0.87**	4.19 (0.94)	3.37 (0.84)	**4.61*****	107
	females	−0.13	4.00 (1.06)	3.86 (1.02)	0.75	158
S 1977	males	**−0.58**	4.16 (1.11)	3.52 (0.98)	**3.33****	208
	females	0.06	3.71 (0.90)	3.76 (1.00)	−0.21	224
W 1977–78	females	−0.06	3.97 (1.10)	3.90 (1.00)	0.32	140
S 1978	males	−0.05	3.56 (1.11)	3.51 (1.12)	0.28	141
	females	−0.04	3.84 (0.95)	3.80 (1.04)	0.25	170
W 1978–79	females	−0.09	3.63 (1.17)	3.52 (0.96)	0.48	138
S 1979	males	−0.18	3.58 (0.85)	3.43 (0.88)	1.12	265
	females	0.25	3.61 (0.95)	3.85 (1.01)	−1.59	241
W 1979–80	males	**0.75**	3.20 (0.63)	3.67 (0.85)	**−3.02****	133
	females	0.35	3.53 (0.99)	3.88 (0.91)	−1.89	129
W 1980–81	males	−0.51	3.66 (0.86)	3.22 (0.70)	2.70**	111
S 1981	males	−0.07	3.71 (1.08)	3.63 (0.92)	0.34	113
S 1982	males	0.02	3.58 (0.91)	3.60 (0.79)	−0.19	201
	females	−0.13	3.76 (0.94)	3.64 (0.90)	0.58	148
W 1982–83	males	0.18	3.45 (0.85)	3.60 (0.92)	−0.80	126
	females	−0.35	4.00 (1.08)	3.62 (0.89)	1.58	70
S 1983	males	−0.12	3.55 (0.95)	3.44 (0.90)	0.67	126
	females	0.26	3.59 (0.91)	3.83 (0.79)	−1.17	69
W 1983–84	males	0.24	3.38 (0.67)	3.54 (0.89)	−0.75	67
S 1984	males	−0.33	3.55 (0.99)	3.22 (0.80)	2.02*	127
	females	−0.39	3.83 (1.09)	3.41 (0.84)	1.95	94
W 1984–85	males	−0.27	3.44 (1.01)	3.17 (0.82)	1.42	114
	females	0.39	3.30 (0.77)	3.60 (0.81)	−1.78	117
S 1985	males	−0.14	3.74 (0.97)	3.60 (0.99)	0.54	59
	females	0.12	3.87 (0.78)	3.96 (1.02)	−0.43	65
S 1986	males	0.15	3.86 (1.06)	3.70 (1.00)	1.72	510
	females	−0.06	4.13 (1.10)	4.06 (1.07)	0.63	234
W 1986–87	males	**0.49**	3.32 (0.74)	3.68 (0.91)	**−3.25****	323
	females	−0.45	3.57 (0.87)	3.96 (1.08)	−2.15*	320

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

and fitness using each of six dorsal and pelvic spine phenotypes (see Materials and Methods) for all bouts of juvenile survival where changes in spine number approached significance ($P < 0.10$). This analysis gave results congruent with previous analyses. The most common phenotype in Boulton Lake is low spined (two dorsals, absent pelvis). For the samples in which directional selection on spine number was detected, the relative frequency of this phenotype decreased over winter and increased in summer (Table 4). Conversely, the high-spined phenotype (three dorsals, full pelvis) increased in relative frequency during winter but decreased in frequency during summer.

Directional selection on spine number was also detected in subadults and adults. Significant changes in mean spine number were detected for eight cases of subadult survival (differentials: three positive, five negative) and for five cases of adult survival (differentials: three positive, two negative). However, there were no significant associations with season for either age class ($P = 0.46$, $P = 1.00$, for subadult and adult, respectively, Fisher's exact test).

We then tested whether shifts from a relatively pelagic to a more benthic dietary niche were correlated with the direction and strength of selection on spine number, predicting that a highly benthic diet would increase exposure to odonate predators and thus would be associated with selection for spine reduction. Consistent with this prediction, benthic macroinvertebrate consumption during summer episodes of selection was inversely correlated with the value of selection differentials, and this occurred for both subadult and adult survival (Fig. 5; subadults: seven samples from males, four samples from females, $B = -0.01$, $r = -0.74$, $P < 0.01$, $n = 11$; adults: all samples from males as no females consumed macrobenthos, $B = -0.02$, $r = -0.85$, $P < 0.05$, $n = 7$). Pooling the results for between the sexes in subadults was justified as both sexes exhibited a negative correlation between macrobenthos consumption and selection differentials, and this association did not differ between the sexes in slope or elevation (tests for homogeneity; slopes: $F_{1,11} = 0.50$, $P = 0.51$; y-intercepts: $F_{1,11} = 0.30$, $P = 0.60$, ANCOVA). No significant associations between diet and selection differentials were detected during episodes of winter survival in any case (all $P > 0.20$).

Response to Selection and Estimates of the Heritability of Spine Number

Strong directional selection in our data was often associated with predictable changes in mean population spine num-

TABLE 3. Adult stickleback and estimates of selection on spine number. Results show standardized directional (linear) selection differentials (i) for survival through winter (W) and summer (S) seasons. Also shown for each seasonal bout of selection is mean (SD) spine number before ($\bar{X}_B$) and after ($\bar{X}_A$) selection and results from independent-samples t-tests for differences in mean spine number before and after selection. Results in bold remain significant even after correction for multiple comparisons within genders (sequential Bonferroni method; Rice 1989).

Date	Sex	i	$\bar{X}_B$ (SD)	$\bar{X}_A$ (SD)	t	N
W 1970–71	males	−0.48	3.74 (1.00)	3.26 (0.74)	2.60*	89
W 1976–77	males	−0.06	3.65 (0.97)	3.59 (1.04)	0.38	253
	females	0.02	3.91 (1.03)	3.93 (1.07)	−0.13	230
S 1977	males	0.51	3.37 (0.84)	3.80 (1.03)	−2.76**	146
W 1977–78	males	0.13	3.52 (0.98)	3.65 (1.00)	−0.94	247
	females	**0.60**	3.76 (1.00)	4.36 (1.04)	**−2.84****	221
S 1978	males	0.13	3.46 (0.91)	3.58 (1.06)	−0.65	136
W 1978–79	males	0.10	3.51 (1.12)	3.62 (1.03)	−0.81	262
	females	0.25	3.80 (1.04)	4.06 (1.19)	−1.78	220
S 1979	males	0.04	3.33 (0.90)	3.37 (0.86)	−0.28	184
W 1979–80	males	0.11	3.43 (0.88)	3.53 (0.91)	−0.78	274
S 1980	males	−0.31	3.67 (0.85)	3.41 (0.80)	2.26*	196
	females	−0.19	3.88 (0.91)	3.71 (0.81)	0.75	68
W 1980–81	males	0.19	3.28 (0.81)	3.43 (0.95)	−1.15	195
	females	−0.10	3.77 (0.90)	3.68 (1.03)	0.44	95
W 1981–82	males	0.27	3.63 (0.92)	3.88 (1.01)	−0.99	58
S 1982	males	0.18	3.52 (0.92)	3.69 (0.63)	−1.14	223
W 1982–83	males	−0.13	3.60 (0.79)	3.50 (0.95)	0.58	122
S 1983	males	−0.22	3.60 (0.92)	3.40 (0.76)	1.33	145
W 1983–84	males	−0.07	3.44 (0.90)	3.38 (0.77)	0.41	156
	females	−0.03	3.83 (0.79)	3.81 (0.92)	0.08	57
S 1984	males	0.18	3.54 (0.89)	3.70 (1.03)	−0.63	66
W 1984–85	males	0.05	3.22 (0.80)	3.26 (0.82)	−0.24	122
S 1985	males	**0.88**	3.17 (0.82)	3.89 (1.18)	**−3.93*****	125
W 1985–86	males	0.22	3.60 (0.99)	3.82 (0.96)	−0.97	249
	females	0.11	3.96 (1.02)	4.07 (1.05)	−0.47	144

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

ber in the larger fish during the following year. For example, selection for increased spine number was detected in juveniles over the winter of 1980–1981, and this was followed by an increase in mean spine number in adults between 1981 and 1982 (see Fig. 1). As well, the strongest positive selection detected in adult fish occurred among females in winter 1977–1978 and among males in summer 1985. Among the 15 years of sampling, the second highest mean spine number in females was detected in 1978 and the highest mean spine number in males was detected in 1986.

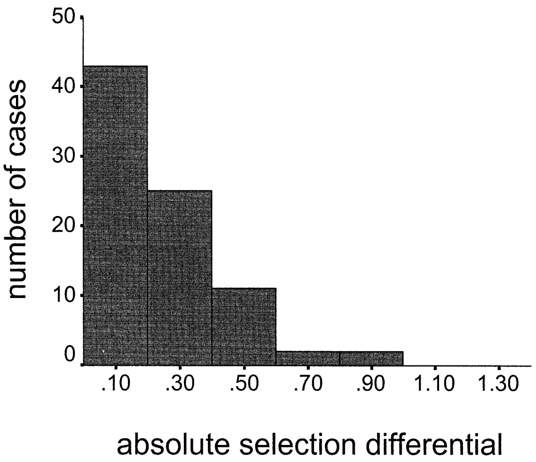

FIG. 3. Frequency distribution of absolute directional selection differentials for selection on spine number of Boulton Lake stickleback.

For the episodes of selection where we detected strong directional selection and where samples from the next generation were available ($n = 10$), we estimated the heritability of spine number (using the response to selection and the calculated selection differentials; see Materials and Methods for details). Of the eight episodes of selection on juvenile survival where selection differentials approached significance ($P < 0.10$), there were four cases where we had samples from which to calculate the response to selection and thus the heritability of spine number. For subadult and adult survival, there were six cases where we detected significant selection differentials and had samples from the next generation. For two of these 10 samples (both for juvenile winter survival), the response to selection was actually in the opposite direction to that expected from the selection differential, yielding negative heritabilities. The other samples yielded heritability estimates ranging from 0.12 to 1.24. The mean estimated heritability was significantly greater than zero both when the samples with negative heritabilities were excluded ($h^2 = 0.52$, SD = 0.50, $t_7 = 2.95$, $P < 0.05$; one-sample t-test) and when they were included ($h^2 = 0.38$, SD = 0.52, $t_9 = 2.35$, $P < 0.05$; one-sample t-test).

DISCUSSION

Examination of temporal trends revealed that mean spine number in Boulton Lake stickleback varied greatly among the 15 years of sampling. Yearly spine number was correlated between the sexes for both subadult and adult fish, suggesting that a common process drives temporal fluctuations in spine

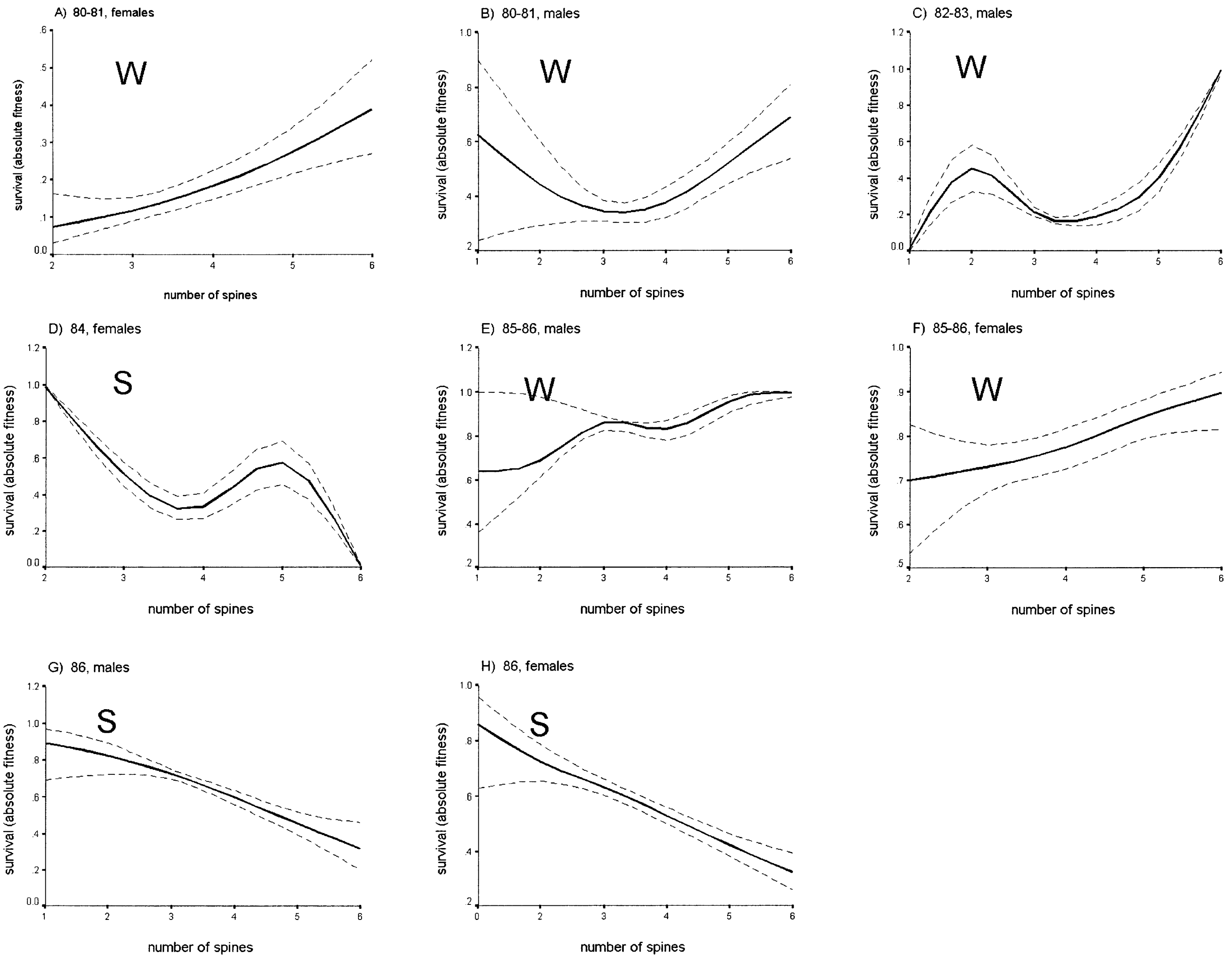

FIG. 4. Fitness functions showing the relationship between spine number and fitness (juvenile survival). Fitness functions (solid lines) and bootstrap SE (dashed lines, 1000 replicates) are estimated from nonparametric regressions using the cubic spline technique (Schluter 1988). Results are shown for seasonal bouts of selection where changes in mean spine number approached or attained statistical significance ($P < 0.10$). Selection on spine number tended to be directional and positive during winter (W) but directional and negative during summer (S). A) W80–81, females. B) W80–81, males. C) W82–83, males. D) S84, females. E) W85–86, males. F) W85–86, females. G) S86, males. H) S86, females.

number. These fluctuations appear to represent population-level effects as changes in spine number among subadults were highly correlated with that of adults when lagged by one year. Predation is one of the important selective processes influencing morphological evolution in stickleback populations (Reimchen 1994), and temporal variability in predation regimes provides a plausible mechanism to account for temporal fluctuations in spine number. Consequently, we estimated selection acting on spine number during contrasting predation regimes by following cohorts between successive seasons. We found average directional selection differentials near 0.24 (range = 0.02–0.88), comparable to those reported in other studies of selection in natural populations (Endler 1986; for review see Kingsolver et al. 2001). Furthermore, 17 of 83 (20%) estimates of selection were significant (nine of 83 following Bonferroni correction), generally congruent with a recent review by Kingsolver et al. (2001), who found that 25% of all selection differentials reported in the literature reached statistical significance. Our estimates of selection are probably conservative because they compare distributions before and after selection, without direct reference to individuals who survived or perished. By using a trait with known functional significance, our estimates of selection are unlikely to be confounded by selection acting on correlated traits.

Spines on stickleback are an advantage against gape-limited piscivores (Hoogland et al. 1957; Hagen and Gilbertson 1972; Moodie and Reimchen 1976; Gross 1978; Reimchen 1988) but potentially a disadvantage against grappling predators such as odonate naiads (Reimchen 1980; Reist 1980; Ziuganov and Zotin 1995). At Boulton Lake, odonate naiads are one of the primary predators on juvenile stickleback (Reimchen 1980) and, consequently, we predicted that the

TABLE 4. Relative frequencies of six spine phenotypes in stickleback before (% B) and after (% A) seasonal bouts of selection. Results are for juvenile survival during winter (W) and summer (S) seasons. Estimates of winter survival compare samples of fish 20–35 mm SL taken in the fall (September–November) to samples of fish 35–45 mm SL taken in the spring (March–April). Estimates of summer survival compare fish 20–35 mm SL sampled in spring to fish 35–45 mm SL sampled in fall. Also shown are results from chi-square analyses comparing frequencies of spine phenotypes before and after selection. N, sample size.

Spine phenotype	W 1980–81		W 1982–83		W 1985–86		S 1984		S 1986	
	% B	% A	% B	% A	% B	% A	% B	% A	% B	% A
Males										
3 dorsals, absent pelvis	11.7	10.0	10.9	8.5	27.0	10.8	—	—	19.3	13.6
3 dorsals, half pelvis	1.5	4.4	1.2	4.9	2.7	7.0	—	—	5.2	2.3
3 dorsals, full pelvis	2.9	10.0	0.0	2.4	2.7	6.3	—	—	3.0	0.4
2 dorsals, absent pelvis	66.4	54.4	65.6	58.5	51.4	41.6	—	—	46.7	66.3
2 dorsals, half pelvis	10.2	10.0	14.2	11.0	13.5	15.7	—	—	11.1	10.2
2 dorsals, full pelvis	7.3	11.1	8.1	14.6	2.7	18.5	—	—	14.8	7.2
Chi-square	8.89	$N = 227$	13.90*	$N = 329$	14.15*	$N = 323$			20.04**	$N = 399$
Females										
3 dorsals, absent pelvis	25.8	20.0	—	—	30.3	14.3	17.5	20.6	19.2	27.2
3 dorsals, half pelvis	4.3	8.6	—	—	6.1	7.1	0.0	5.9	9.6	4.0
3 dorsals, full pelvis	4.9	14.3	—	—	6.1	14.3	12.5	0.0	9.2	4.0
2 dorsals, absent pelvis	52.1	31.4	—	—	42.4	34.1	47.5	67.6	37.5	52.3
2 dorsals, half pelvis	4.9	8.6	—	—	9.1	11.1	17.5	2.9	15.0	7.6
2 dorsals, full pelvis	8.0	17.1	—	—	6.1	19.0	5.0	2.9	9.6	5.0
Chi-square	11.07*	$N = 198$			8.56	$N = 159$	11.81*	$N = 74$	33.06***	$N = 542$

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

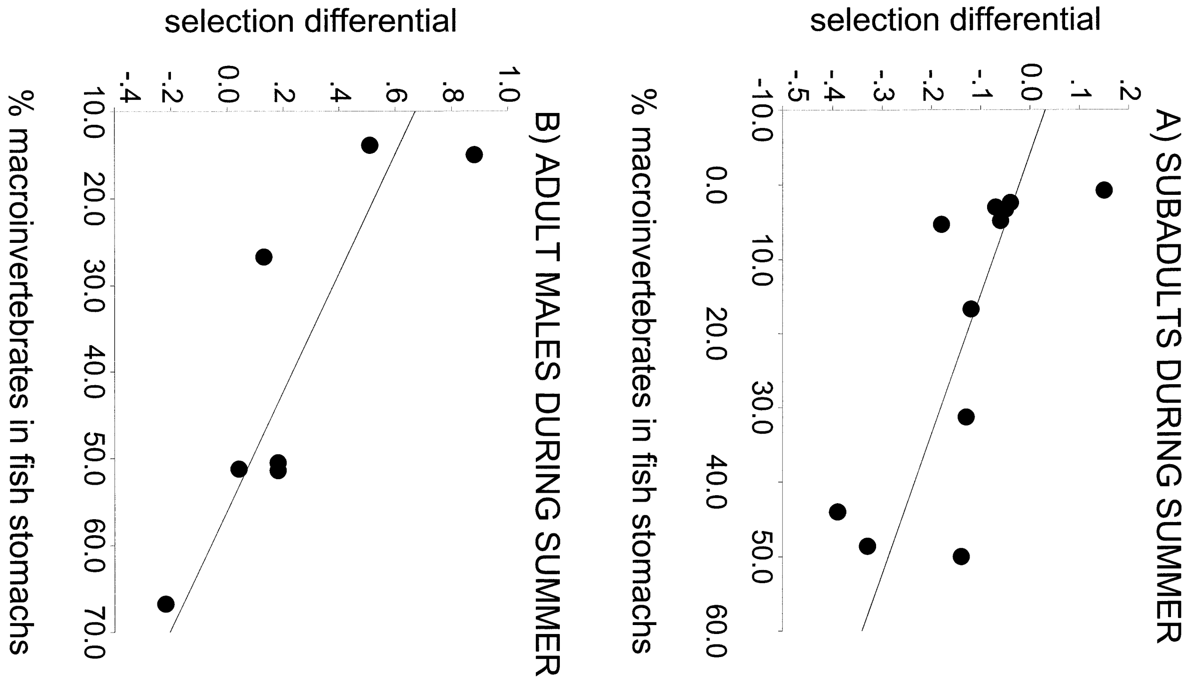

FIG. 5. Correlations between the direction and strength of estimated selection differentials and the proportion of stickleback with benthic macroinvertebrates in their stomach contents during an episode of selection: (A) subadult stickleback; and (B) adult stickleback. In both age classes, benthic macroinvertebrate consumption was negatively and significantly correlated with selection differentials (subadults, $B = -0.01$, $r = -0.74$; adults, $B = -0.02$, $r = -0.85$, both $P < 0.05$). This suggests increased exposure to odonate predation selects for spine reduction.

seasonal variability in predatory regimes (birds in winter, odonates in summer) would result in seasonal variability in selection on spine number. Concordant with this prediction, significant selection on juveniles during winter was always for increased spine number, whereas significant selection on juveniles during summer was always for decreased spine number. This effect was unchanged when each of six dorsal and pelvic spine phenotypes was considered separately. Yet, we also observed substantial variability in both direction and strength among the selection differentials that did not reach formal statistical significance. Such temporal variability may simply reflect sampling effects. However, it also possible that such variation reflects weak but nevertheless biologically meaningful instances of differential predation, as recently demonstrated for weak temporal selection on lateral plates (Reimchen 1995). As well, mean juvenile spine number during summer was inversely related to mean monthly temperature. Such an association could arise if elevated temperature results in high odonate activity (i.e., predation) and thus increased selection against spines.

Subadult and adult stickleback in Boulton Lake are taken primarily, but not exclusively, by avian piscivores and selection should generally favor increased spine number (Reimchen 1980, 1988). We detected such positive selection on spine number in several cases. However, we also detected significant selection for reduced spine number in two years. Our measurements of selection in subadult and adult fish, although frequently statistically insignificant, nevertheless exhibit a predictable association with temporal shifts in the spatial niche occupied by the fish, such that increased occurrence of macrobenthos was associated with the negative differentials. Odonates are prevalent in the nearshore regions of the lake but virtually absent in the central parts of the lakes (Reimchen 1980), and both predatory odonate larvae and the macroinvertebrates that stickleback prey upon would clearly not reside in the water column. Thus, it is likely that exposure to odonate predation would be greater during periods of time where a high frequency of stickleback had macroinvertebrates in their stomachs than during periods where such benthic prey were consumed at a lower frequency. Conceivably, selection for spine reduction in larger stickleback only occurs during such times of high odonate exposure. Conversely, while foraging on pelagic food items stickleback are exposed to increased avian predation, which selects for greater spine number. We stress that although juveniles are most susceptible to odonate predation, adult stickleback are regularly consumed by odonates in minnow traps and in controlled predation experiments odonate larvae captured both subadult and adult stickleback from Boulton Lake (Reimchen 1980, pers. obs.). Furthermore, experiments with adult ninespine stickleback (SL = 51–62 mm) in large ponds revealed a selective advantage of the loss of pelvic spines under insect predation but a selective disadvantage against gape-limited predatory fish (Ziuganov and Zotin 1995).

In fact, the correlation between the direction and magnitude of selection differentials and benthic diet was stronger in adults than in subadults ($r = -0.74, -0.85$, respectively). Although predation by odonates is more common in smaller age classes of stickleback this does not necessarily mean that selective predation is absent in the adults. Strength of selection on prey defenses should be a function of predator failures (Vermeij 1982; Reimchen 1994). Because odonates have greater manipulation difficulty of larger than smaller stickleback (Reimchen 1980), there are a larger proportion of adults that escape after capture and as such, selective predation may be equally prevalent in adults (i.e., heavily spined adults are more commonly ingested than low-spined phenotypes). Further experimental work is required to explicitly test this hypothesis. The results from all age classes are consistent with the hypothesis that increased exposure to insect predation selects for spine reduction, whereas exposure to avian predation selects for increased spine number.

Several alternative explanations for spine reduction exist. First, reduced spine number might be favored if subadult and adult stickleback evade predators by hiding in mud substratum as suggested by Nelson (1969) for spine loss in *Culaea inconstans*. As well, there are mortality factors other than predation that may be nonrandom with respect to spine phenotype, for example, differential parasitism (Reimchen 1997; Reimchen and Nosil 2001a,b,c). Finally, it is possible that spine reduction is linked to chemical limitation (e.g., calcium shortage). However, if spine reduction was simply the result of chemical limitation selecting for reduced spine number, then phenotypes with relatively few spines would be favored more uniformly through time and space and we would not have detected such rapid and common shifts in trait means, we would not have detected so many instances of selection for increased spine number, and most of the fish in the lake would exhibit low spine number. Finally, previous work (Reimchen 1980; Ziuganov and Rosanov 1987) and the results of the current study indicate spine number has a strong heritable component, making it unlikely that variation in spine number is simply the result of phenotypic induction resulting from a shortage of calcium.

We stress that we estimated selection on a trait with known functional significance. Spines are clearly a defense against gape-limited predators and may be detrimental against grappling predators. It is logical to infer that shifts in exposure to these divergent predation regimes account for the correlated shifts in selection on spine number. By using a trait with known functional significance we also reduce the possibility that our estimates of selection on spine number are confounded by selection acting on correlated traits.

Although our results suggest selective elimination driven by differential predation, potential effects of differential dispersal and movement among phenotypes can confound estimates of selection (Endler 1986). Previous work on the Boulton Lake population revealed spine phenotypes in juveniles and subadults were not randomly distributed among regions of the lake during summer (Reimchen 1980). Specifically, there was an excess of fully spined phenotypes in pelagic regions of the lake relative to the littoral region. This could occur if there was dispersal of spined phenotypes from littoral regions, where these may be at a disadvantage against invertebrate predators, into open water regions free of such grappling predators. Therefore, comparisons of phenotype frequencies between seasons in the littoral region would result in a reduction in the frequency of spined phenotypes that was not the result of differential survival. Several lines of evidence suggest that this is not a tenable explanation for our data. First, we detected an increase in higher-spined phenotypes in littoral zones during winter, a trend opposite to that predicted from differential movements. Second, the nonrandom spatial distribution of phenotypes reported in Reimchen (1980) only compared samples from summer. The samples compared in this study were from spring and fall, and in both these seasons mean spine number in juveniles ($\leq$ 35 mm) did not differ significantly between beach and central areas of the lake (spring, males $t_{189} = -0.97$, $P = 0.43$, females $t_{274} = -1.09$, $P = 0.28$; fall, males $t_{1670} =$

-0.50, $P = 0.61$, females $t_{1779} = -1.914$, $P = 0.07$). Third, we detected selection on spine number in adult fish yet adult spine phenotypes are randomly distributed between littoral and pelagic regions of the lake. Finally, the yearly fluctuations in population mean spine number and the congruence between the sexes suggest temporal variation in selection rather than biased-dispersal patterns.

In several of the fitness functions calculated in this study there was evidence of complex patterns of nonlinear selection (i.e., multiple dips and/or modes; e.g., Fig. 4 c, d). The standard errors generated by bootstrapping suggest these dips and modes are real and that the relationship between phenotype and fitness was not strictly linear in some cases. This suggests the different spine phenotypes are maintained via a combination of divergent selection from different predator groups and complex patterns of nonlinear selection, perhaps associated with monthly shifts in predation regime not detectable from seasonal analyses.

Complete descriptions of natural selection include both estimates of within-generation phenotypic selection as well as an analysis of the intergeneration response to selection (i.e., between-generation shifts in the population mean; Endler 1986). Strong directional selection in our data was often associated with predictable changes in mean population spine number in the larger fish during the following year. These trends suggest that our estimates of selection are biologically meaningful rather than sampling or statistical biases. Furthermore, the observed responses to selection suggest spine number has a heritable basis. However, due to wide range of the heritability estimates, we feel that our calculations serve to strengthen the probability that the morphometric shifts observed are the result of selection on a heritable trait, rather than acting as precise estimates of the heritability of this trait.

Although natural selection can reduce variation within populations (Fisher 1930; Lewontin 1964), multiple forces can influence intrapopulation variability (Felsenstein 1976; for reviews, see Hedrick et. al. 1976; Hedrick 1986), including spatial heterogeneity in selection (Giesel 1970; Reimchen 1979, 1980; Arnqvist 1992; Losos et al. 1998; Butler et al. 2000), temporal heterogeneity in selection (Boag and Grant 1981; Price and Grant 1984; Bell et al. 1985; Schluter and Smith 1986; Benkman and Miller 1996; Fairbairn and Preziosi 1996; Reimchen 1997; Blanckenhorn et al. 1999; Jann et al. 2000) and gene flow (for review Slatkin 1987; Sandoval 1994). The Boulton Lake population is small and does not incur any gene flow (Reimchen 1980), so one might expect little variability in morphology (Hedrick et al. 1976; Hedrick 1986; Slatkin 1987). Contrary to this expectation, spine number is highly variable in this population. Predictable yet heterogeneous patterns of selection provide the most likely mechanism to account for the variability observed in this population. The absence of predatory fish and competitors may have facilitated the colonization of multiple niches within the lake. Such ecological release would result in heterogeneous patterns of selection among habitats and over time, given the seasonal variability in selection by predators detected in this study.

Heterogeneity in selection has been studied in other stickleback populations. In one of the classic examples of rapid shifts in phenotype frequencies from the Miocene fossil record of *Gasterosteus*, Bell et al. (1985) showed evidence for bidirectional shifts in mean dorsal spine number among 5000-year sampling intervals over a 120,000-year time series. Although potentially attributable to temporal variation in selection, Bookstein (1988) suggests that the pattern cannot be distinguished from a random walk. Results from the current study on *Gasterosteus* from Boulton Lake suggest that temporal shifts in selection produce shifts in phenotypes frequencies, implicating ecological determinism in morphological evolution. Similar results have been observed in a lake population of giant stickleback from the Queen Charlotte Islands, where cyclical changes in lateral plate number were associated with seasonal changes in the relative proportion of bird versus trout predators, with increased predation by trout resulting in increases in mean lateral plate number (Reimchen 1995). Furthermore, adaptation to different trophic niches via spatial variation in selection has been shown to drive speciation in sympatric stickleback populations (Schluter and McPhail 1992; Schluter 1993, 1994, 1995; Nagel and Schluter 1998; Rundle et al. 2000; Taylor and McPhail 2000). Collectively, these results suggest spatial and temporal selective heterogeneity is an important evolutionary force favoring both the origin and maintenance of biological diversity within populations.

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