doi:10.1111/j.1558-5646.2008.00520.x

THE ECOLOGY OF ASYMMETRY IN STICKLEBACK DEFENSE STRUCTURES

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Received November 4, 2007
Accepted August 25, 2008

Slight departures from bilateral symmetry are usually associated with reduced fitness. Here we show that an insular freshwater population of *Gasterosteus aculeatus* exhibits spatial and temporal segregation according to the incidence and direction of asymmetry in the number of bony lateral plates, which are important predator-defense structures. We analyzed 11,263 fish collected from 19 full lake transects over three years, and found that signed asymmetries for plate number were slightly left-side biased. Asymmetries occurred in 55% of the stickleback with more asymmetrics found during cold weather, and more left-biased individuals found during windy conditions, possibly due to environmentally driven activity levels that differ among asymmetric forms. Absolute plate asymmetries were randomly distributed in the lake, but there was a strong shift in signed plate asymmetry from a mean of zero in littoral zones to left-side bias in limnetic zones, probably due to microhabitat choices among asymmetric forms. Video data of avian piscivores on the lake show laterality in prey handling, providing a potential mechanism for asymmetric prey morphology. Our results imply a complex relationship between slight asymmetries and niche space that is relevant to the broader literature on the evolutionary implications of developmental instability and intrapopulation variability.

KEY WORDS: **Developmental instability, directional asymmetry, niche segregation, predation.**

Subtle bilateral asymmetries in phenotype are widespread in natural populations and comprise an emerging but controversial direction in evolutionary studies. These subtle asymmetries, often referred to as "fluctuating asymmetry" (FA), generally originate from developmental instability (Waddington 1942); these have received substantial attention due to negative correlations with fitness, including associations with sexual selection (Møller and Hoglund 1991; Watson and Thornhill 1994) and trait function (Mather 1953; Moodie 1977; Palmer and Strobeck 1986; Balmford et al. 1993; Gummer and Brigham 1995; Clarke 1998). In particular, asymmetry has detrimental effects on the biomechanics of predator–prey interactions, by compromising morphological defenses of the prey (Moodie and Reimchen 1976; Reimchen 1983; Bergstrom and Reimchen 2003) as well as the ability of an asymmetric predator to manipulate prey (Swaddle 1997). However, the consequences of asymmetry in these interactions varies among habitats as a function of predation regime (Bergstrom and Reimchen 2003), suggesting a complex and site-dependent relationship with fitness. The ecological landscape of subtle asymmetry has rarely been investigated, including whether there are temporal or spatial differences in the distribution of asymmetric relative to symmetric phenotypes, or between left-biased and right-biased individuals.

Earlier studies addressing ecological components to fluctuating asymmetries focused on absolute rather than signed asymmetries. In threespine stickleback there are differences in nesting sites between fish that are symmetric and asymmetric for number of bony lateral plates (Moodie 1972), and in distances from shore for symmetric and asymmetric pelvic girdle expression

(Reimchen 1980). There is evidence that FA in the appendages of corixids (Hemiptera) results in reduced competitive ability relative to symmetric individuals (Nosil and Reimchen 2001). Other studies have looked at differences between left- and right-biased individuals, but only for conspicuous asymmetries that are presumably genetically determined (Van Valen 1962). Ecological and functional differences occur between left-and right-mouthed scale eating cichlids (Hori 1993), and between left-eyed and right-eyed starry flounder (Bergstrom 2007; Bergstrom and Palmer 2007). However, these ecological associations with the direction of bias have not involved subtle asymmetries, which are thought to arise due to errors made during development. There is a paucity of investigations that move beyond considering these slight departures from symmetry as nonheritable "errors" into considering them as new phenotypes with which to exploit new niche space (but see Seligmann 1998; Windig and Nylin 1999). Such information could identify ecological mechanisms that underlie the fitness and biomechanical consequences of subtle asymmetry in phenotype.

The threespine stickleback (*Gasterosteus aculeatus*) exhibits population variation in the asymmetry of defensive armor. The bony lateral plates, which buttress the dorsal and pelvic spines and provide integument protection from puncturing predators (Reimchen 1983, 1992), commonly exhibit bilaterally asymmetric counts (Hagen and Gilbertson 1972; Moodie 1972; Moodie and Reimchen 1976; Fig. 1). The frequency of these asymmetries is highly variable among freshwater populations (Bergstrom and Reimchen 2000), and is less frequent in populations subject to extensive fish predation (Moodie and Reimchen 1976). Asymmetrics also are infected by endoparasites more often than symmetrics (Reimchen and Nosil 2001a; Bergstrom and Reimchen 2005), implying reduced immunocompetence, but dietary data also indicate partial trophic partitioning between symmetrics and asymmetrics (Reimchen and Nosil 2001a). In contrast, during nesting, male stickleback with asymmetric plate counts have improved success raising clutches relative to symmetrical males (Moodie and Moodie 1996). The normally symmetric pelvic girdles of threespine stickleback exhibit directional asymmetries in several localities from western North America (Reimchen 1980; Bell et al. 1985). In one of these, stickleback with asymmetric pelvic condition are more common in pelagic than shallow habitats (Reimchen 1980), are infected by coelomic parasites less often than symmetrics (Reimchen 1997), and exhibit trophic niche partitioning relative to symmetric phenotypes (Reimchen and Nosil 2001b). These cumulative findings indicate the potential for microhabitat partitioning between symmetrical and asymmetrical phenotypes.

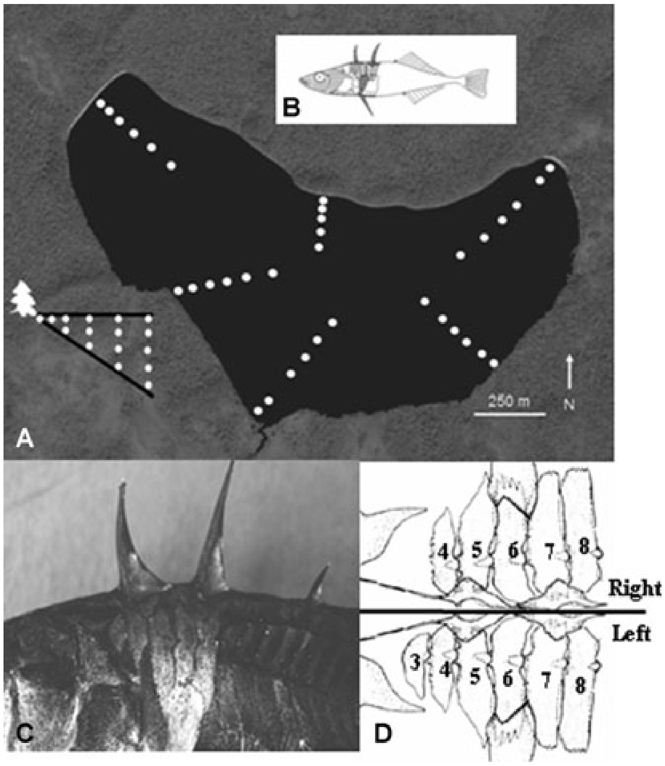

Figure 1. (A) Drizzle Lake and the horizontal and vertical sampling stations. Each of the six lake regions (W, NW, N, NE, SE, SW) show trap sites (white dots) positioned at 0.5 m, 1 m, 2 m, 3 m, 4 m, and ≥ 5 m depth contour lines and at vertical stations in the water column (A inset). (B) Representative sketch of stickleback from Drizzle Lake. (C) Close-up photograph of a stickleback shows positioning of dorsal spines and bony lateral plates. (D) Sketch showing an asymmetrical stickleback with lateral plate positions numbered, and with six lateral plates on the left side and five on the right side (head to the left).

In this article, we provide the first detailed intrapopulation evaluation of bilateral asymmetries in the number and position of lateral plates in relation to gender, age, and habitat. The data come from a study initiated in 1976 at a remote lake on the Haida Gwaii archipelago (Queen Charlotte Islands), western Canada, to examine variability in predation regime and defense morphology (Reimchen 1983, 1988, 1994). One component of the study involved 19 full-lake sampling transects of more than 11,000 sticklebacks to quantify spatial, temporal and ontogenetic trends in defense morphology, including lateral plate asymmetry. The lake (Drizzle Lake) has an oval perimeter (110 ha) and simple bathymetry (max depth 20 m) in which cutthroat trout (*Oncorhynchus clarki*) are the primary predators of juvenile and subadult stickleback in the littoral zone (Reimchen 1990) and avian piscivores—Common Loon (*Gavia immer*) and Red-necked Grebe (*Podiceps grisegena*)—are the primary predators of subadults and adults in the limnetic zone (Reimchen 1994). The spatial and temporal differences in predation regime are probably representative of many temperate lake habitats. We also consider the potential influence of behavioral laterality between predator and prey on the spatial distribution of plate asymmetries within the lake by examining diving bird behavior and predator-induced injuries on stickleback. Based on the general consensus that subtle asymmetries are a byproduct of developmental instabilities, we initially investigated if asymmetric sticklebacks have lower fitness relative to symmetrics by comparing age-specific frequencies and predicted a frequency reduction of asymmetrics among successive age classes. Second, we examined lake horizontal and vertical spatial distribution of asymmetrics predicting similar proportions throughout the lake. Third, we investigated if there were spatial and temporal differences in the distribution of fish differing in the direction of the asymmetry and predicted similar distribution between left- and right-biased asymmetries. Statistical departures from these predictions could have important implications to our understanding of the evolutionary and ecological significance of developmental instability.

Materials and Methods

Study area, general collection methodology, and scoring techniques are described elsewhere (Reimchen 1988, 1994, 1995). In summary, collections were made during 19 replicated lake transects from May 1980 through June 1983. We grouped monthly transects into spring (April to June), summer (July to September), winter (October to March). In each transect, 16 minnow traps were set for 48 h in a three-dimensional grid at six horizontal zones corresponding to depth contours (0.5 m, 1 m, 2 m, 3 m, 4, $\geq$ 5 m) and at 1 m successive depths in the water column from the surface to the bottom for each horizontal zone greater than 1-m depth (Fig. 1). For lake bottom depths greater than 4 m, traps were suspended at 1 m successive depths from the surface to 4 m with an additional single trap placed on the bottom. Over 14 days, this protocol was repeated in each of six regions of the lake (northwest, west, southwest, southeast, northeast, and north) yielding a total of 96 trap sites per transect (Fig. 1). Dominant lake surface conditions (smooth, wavelets, choppy waves) were recorded at dawn, midday, and dusk throughout the 14-day transect period corresponding to general wind categories of calm, breeze, and gale, respectively. Midday maximum air temperatures were recorded with a mercury thermometer at a field station 100 m from the lake.

Over the 19 transects, 11,263 stickleback were captured. The majority of fish in the collection were adults ($N = 9272$ versus subadults $N = 1991$) and were scored at the site of collection for standard length, position of lateral plates on each side of the body, dorsal and pelvic spine fractures, and body lacerations. All injured fish and all subadults, of which the latter were uncommon in the samples, in addition to a random sample of uninjured adults from each sample were euthanized and retained for more complete analyses in the laboratory. Sex was recorded on 2795 females and 1984 males.

Plate number and position was measured on each side of the fish. We scored asymmetry (Rside–Lside) in two methods: (1) total plate number asymmetry that was the difference in number of plates between sides and (2) asymmetry for each plate position (1 through 10). The latter was only assigned when at least one plate was present at that position. Position and number of the plates on each side of the body were scored twice (see Reimchen 1983) to check for repeatability. Inconsistency was present in about 1% of the fish and invariably occurred when the most anterior plate was very small. In such cases, these individuals were rescored by TER at least two additional times until full repeatability was achieved. All fish were of the low-plated morph (fewer than 10 plates per side, no keel).

Avian piscivores occur intermittently throughout the year on Drizzle Lake. (Reimchen 1988, 1994). From 1980 through 1985, foraging activity of these piscivores was videotaped to identify fish species and estimate body length of the fish (Reimchen and Douglas 1980, 1984). To ascertain the possibility for laterality (side-biased capture) of fish by diving birds, we have reexamined here these videotapes. Imaging resolution was insufficient on most to score orientation except for the close videos taken for nesting Red-throated Loons (*Gavia stellata*) which carry fish captured from other habitats to prefledged chicks (Reimchen and Douglas 1984). We examined video-playback of 122 separate feeding events and scored the species of fish and direction of the fish in the loon's bill into one of four categories (head to the left, head to the right) and dorsoventral alignment (ventral surface toward the back of the bill, ventral surface toward tip of bill). If this piscivore captures prey with no lateral bias, then there should be no statistical departures from equality among these four categories.

For assessment of demographic and spatial patterns of asymmetry within the lake, we used generalized linear mixed models (GLMM) for tests involving binomial dependents (asymmetric or not, left-biased or right-biased), and linear mixed models (LMM) for tests involving signed mean asymmetry, which is normally distributed. We used these mixed models to include transect as a random effect and therefore account for variation among replicate samples. The models were fit using maximum likelihood and then compared using likelihood-ratio tests for GLMM's and t- and F-tests for the LMM's. Statistical analyses were conducted in the software programs R and SPSS version13.0.

Results

DEMOGRAPHIC AND TEMPORAL PATTERNS

Absolute asymmetry

Among all fish examined, 55.2% were asymmetric for number of lateral plates. Absolute plate number asymmetry $|(R–L)|$ ranged from one to six, and among these, the majority (81%) had a

Table 1. Incidence of absolute (unsigned) lateral plate asymmetry among 11,263 Drizzle Lake threespine stickleback. The majority of stickleback were asymmetric and of these, more than 80% had one plate difference between left and right sides of the body.

Plate number asymmetry | (R—L) |	*N*	Total %	Asymmetric %
0	5041	44.8	–
1	5064	45.0	81.4
2	1056	9.4	17.0
3	87	7.7×10^{-1}	1.4
4	12	1.1×10^{-1}	1.9×10^{-1}
5	2	1.8×10^{-2}	3.2×10^{-2}
6	1	8.9×10^{-3}	1.6×10^{-2}

single plate difference between the sides (Table 1). For computational purposes, we grouped fish into two categories, symmetric and asymmetric. We tested for temporal stability in frequencies of asymmetric phenotypes over the 19 transects taken during the four-year sampling period. Total frequencies differed significantly among transects ($\chi^2 = 30.52$; df = 18; $P = 0.03$), ranging from 48% to 60% (Fig. 2). Absolute asymmetries tended to be more common in transects taken in winter months (October–March: 57.5%) and less common in transects taken in summer months (July–September: 53.1%; GLMM: Season $\chi^2 = 6.13$; $P = 0.01$). There were no significant differences in the frequency of asymmetric versus symmetric fish between sexes (total pooled male = 54.5%; female = 55.9%; GLMM: Sex $\chi^2 = 0.58$; $P = 0.45$) or between age classes (total pooled sub-adults = 54.9%, adults = 55.3%; GLMM: Age $\chi^2 = 0.01$; $P = 0.92$).

Signed asymmetry

Signed plate number asymmetry (R–L) ranged from −6 to +5 with a mode at zero. The distribution of signed asymmetries approximated a bell-shaped distribution (Fig. 3) but had a mean that was significantly different from zero and exhibited a left side bias (mean = −0.083; standard error = 0.009; $t = 9.2$; df = 11262; $P < 0.001$). The left-side bias persisted in all 19 transects, and although there was variability in the extent of bias, it did not differ significantly among transects ($F_{18} = 1.19$; $P = 0.26$; Fig. 4). Mean signed asymmetry did not differ between sexes or between subadults and adults (LMM: Sex $F = 2.08$; $P = 0.15$, Age $F = 1.65$; $P = 0.20$).

Directionality in plate asymmetry varied along the vertebral axis of the fish (Fig. 5). Positions one through seven had a left-side bias with significant departures from a mean of zero at positions two, three, four, and six. Asymmetry at plates eight, nine, and 10 did not differ significantly from zero, but exhibited various degrees of right side bias. The extent of directionality at these positions did not differ significantly among the temporal transects (proportion of left- versus right-biased plate positions × transect;

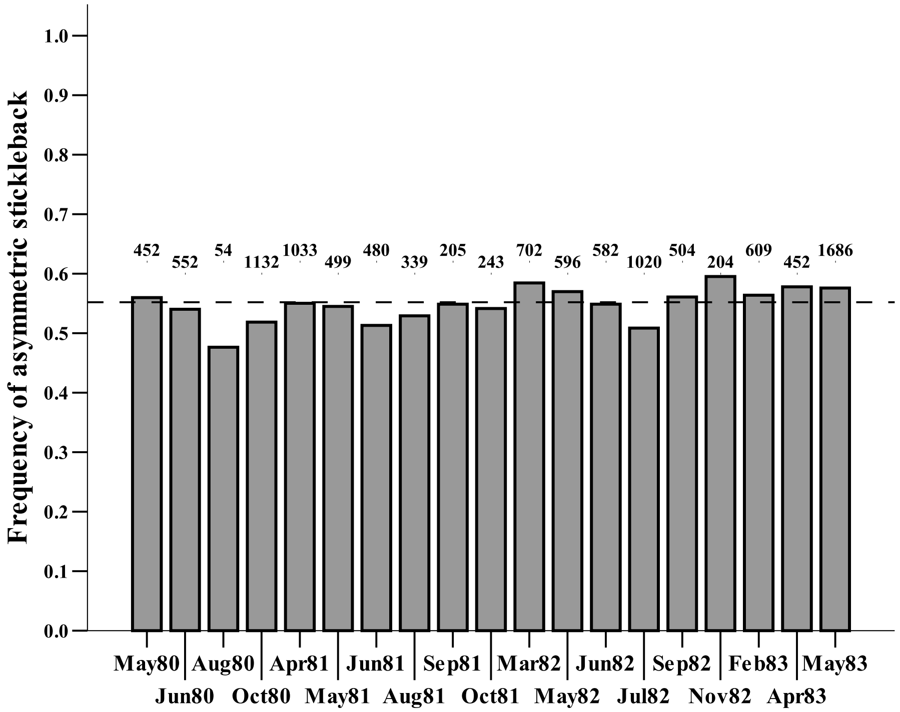

Figure 2. Frequencies of asymmetric stickleback among the 19 temporal sampling transects. Horizontal dashed line designates the total frequency of asymmetric fish across transects (55.2%). Numbers above bars are sample sizes for each transect.

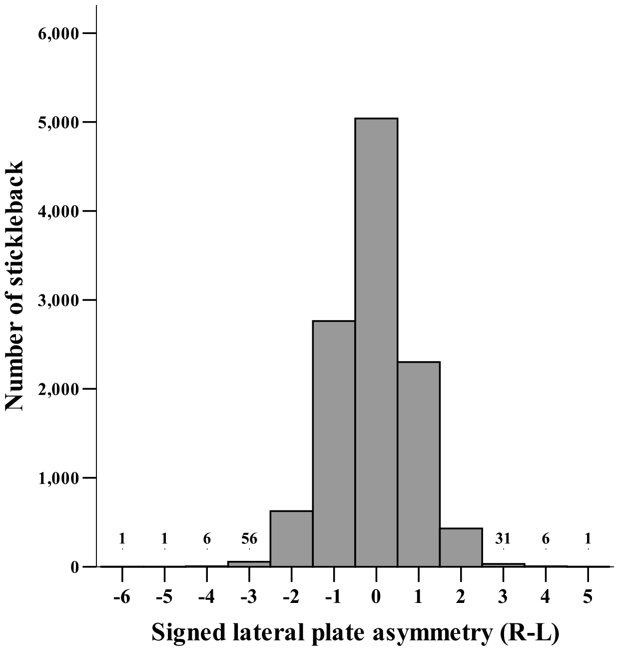

Figure 3. Frequency of signed lateral plate asymmetry (R–L) in Drizzle Lake stickleback. Numbers above bars are sample sizes for the rare asymmetry morphs.

all $G_{18} \leq 28.78$; all $P > 0.05$). The greatest number of asymmetries occurred at plate positions three (left-biased) and eight (fluctuating), followed by positions two (left-biased) and four (left-biased; Fig. 5).

LAKE SPATIAL PATTERNS

Absolute asymmetry

We examined the frequencies of absolute plate asymmetries in the lake. Among the six geographical regions around the perimeter of the lake, there were no statistically significant differences in absolute asymmetries (ranged from 53% to 56%; GLMM: Region $\chi^2 = 1.07$; $P = 0.30$). Across a littoral–limnetic transect, there was no significant change in frequencies with distance from shore (GLMM: Distance from shore $\chi^2 = 0.08$; $P = 0.77$), nor was there a difference between photic (within 2 m of surface) and aphotic samples (>2 m deep; GLMM: Water depth $\chi^2 = 0.53$; $P = 0.47$). There were no two-way or three-way interactions among region, depth contour, or water depth (GLMM: all $\chi^2 \leq 1.37$; all $P \geq 0.24$).

Signed asymmetry

We examined the mean lateral plate signed asymmetry of fish with respect to lake positions. There were no significant differences among the six geographical regions (LMM: Region $F = 0.57$; df = 5; $P = 0.72$), nor between the surface, photic depths and the deeper, aphotic depths (LMM: Depth $F = 0.04$; df = 1; $P = 0.85$). However, there were differences in mean signed asymmetry across the littoral–limnetic depth contour zones. In the littoral traps (<2 m depth contour), mean signed asymmetry was near-zero but with increased distance from shore, mean plate asymmetry became distinctly left-biased (Fig. 6; LMM: Depth Contour $F = 3.40$; df = 5; $P = 0.005$). This effect did not differ significantly between

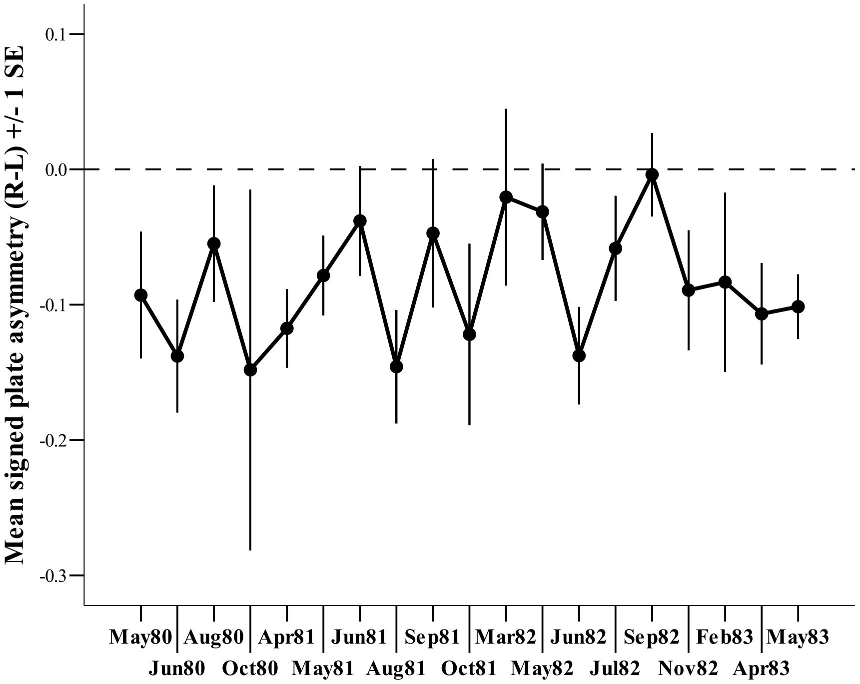

Figure 4. Temporal variability in mean signed lateral plate asymmetry (R–L) for Drizzle Lake stickleback (1980–1983). Horizontal dashed line represents the hypothetical signed asymmetry mean for a population that was perfectly symmetrical or exhibited true fluctuating asymmetry.

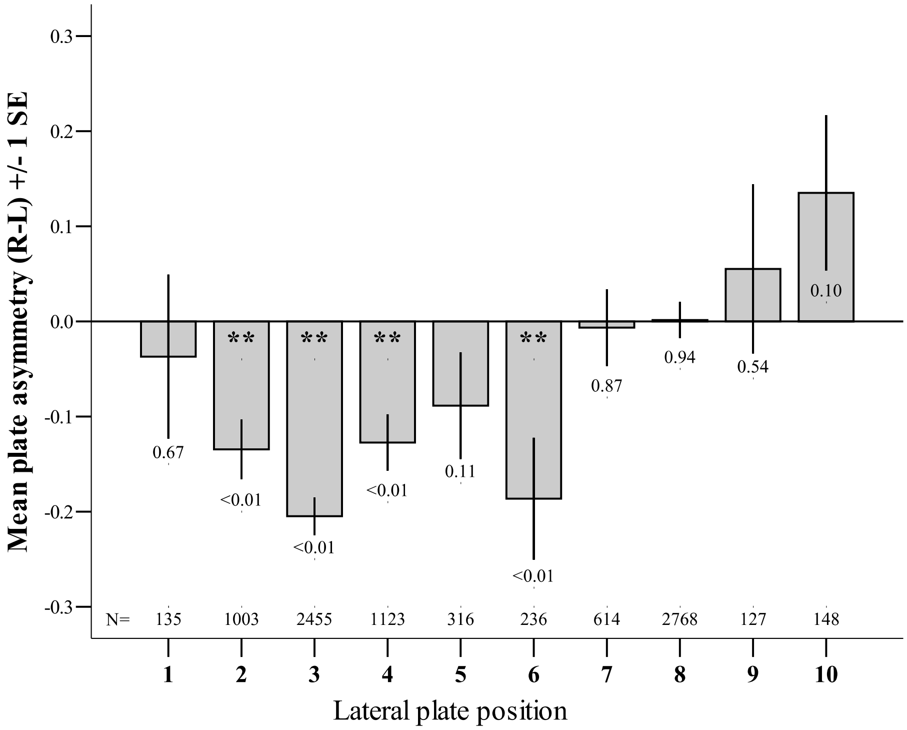

Figure 5. Frequency of signed asymmetry (R–L) at each plate position for all threespine stickleback in the study. *P*-values (numbers below error bars) result from *t*-tests of signed mean asymmetry for that plate position, including symmetric fish, against a mean of zero. Numbers of fish asymmetric for each plate position are given above the x-axis. The sum of these asymmetries is greater than that listed in Table 1 because many individuals are asymmetric at more than one plate position. $^{**}P < 0.01$.

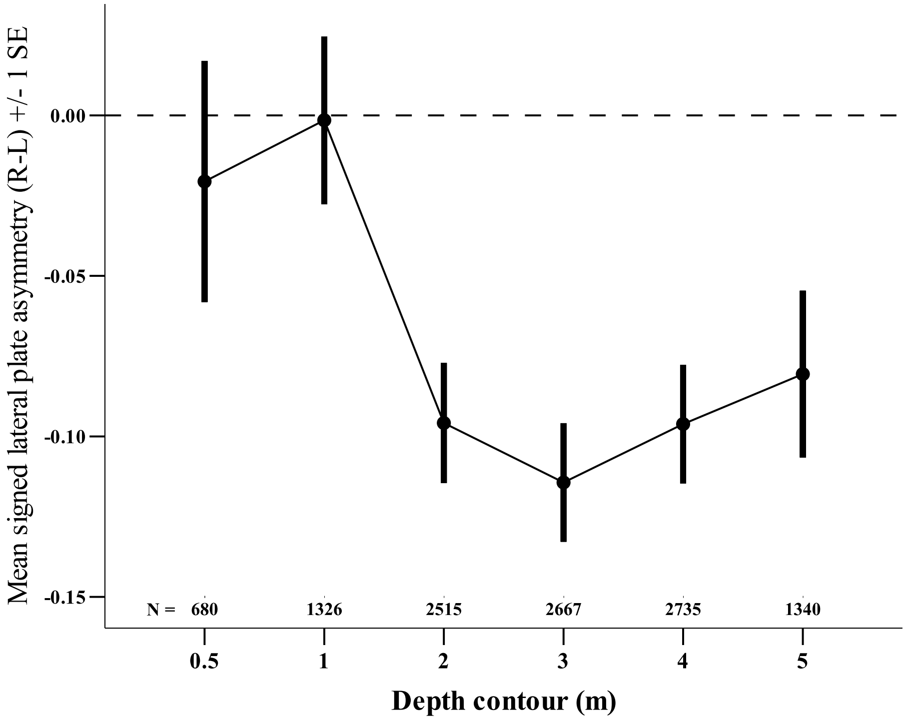

Figure 6. Mean signed lateral plate asymmetry as a function of depth contours. Shallowest contours (littoral zones inshore from 2 m depth contours) do not differ from an asymmetry mean of zero, but deeper contours (limnetic zones offshore from 2–5 m depth contours) are left-side biased. Horizontal dashed line represents the hypothetical signed asymmetry mean for a population that was perfectly symmetrical or exhibited true fluctuating asymmetry. N = number of threespine stickleback found at that depth contour, pooled across replicates and regions.

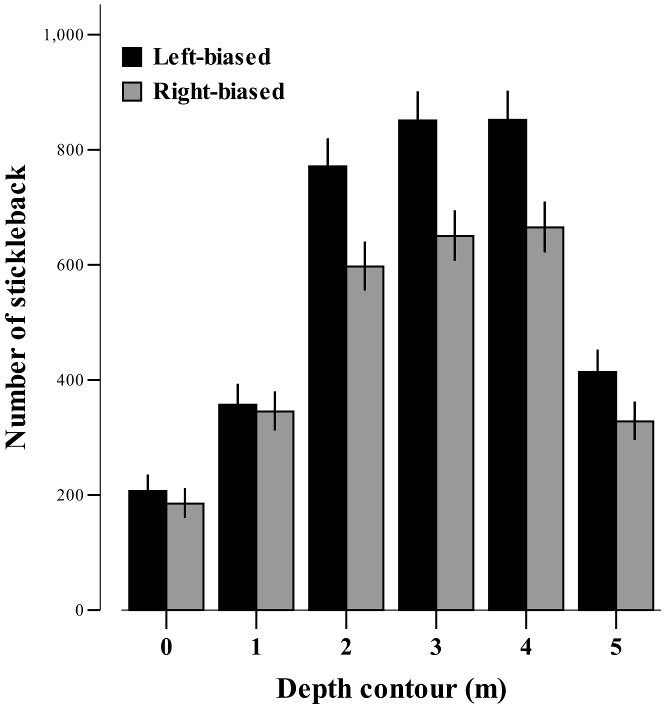

Figure 7. Number of left- and right-biased asymmetric stickleback among depth contours. Left- and right-biased asymmetric stickleback are found in similar numbers in the shallowest contours (littoral zones inshore from 2-m depth contours), but left-biased stickleback are significantly more common in the deeper contours (limnetic zones offshore from 2 to 5 m depth contours). Error bars are 95% confidence intervals.

juveniles and adults (LMM: Depth Contour × Age class $F = 0.52$; df = 5; $P = 0.76$), between males and females (LMM: Depth Contour × Sex $F = 1.08$; df = 5; $P = 0.37$), among seasons (Depth Contour × Season $F = 0.81$; df = 10; $P = 0.62$), years (Depth Contour × Year $F = 0.63$;df = 15; $P = 0.86$), regions (Depth Contour × Region $F = 0.53$; df = 25; $P = 0.97$), or water depths (Depth Contour × Water Depth $F = 1.29$; df = 2; $P = 0.28$). The absence of left-side bias in the shallowest contours was not due to an increase of symmetrical fish there, which would push the mean toward zero, but was rather due to a similar proportion of left- and right-biased fish in this area (Fig. 7). In contrast, a greater proportion of left- than right-biased fish was found in all of the deeper contours.

The shift in proportion of left- and right-biased fish across the littoral–limnetic depth contour zones was not ubiquitous among plate positions. Significant shifts in directionality were seen only for positions three (Fig. 8; GLMM: Depth Contour $\chi^2 = 4.73$; $P = 0.03$) and 10 (GLMM: Depth contour $\chi^2 = 10.58$; $P < 0.01$), whereas the other positions exhibit little change (all $\chi^2 < 0.43$; all $P > 0.47$).

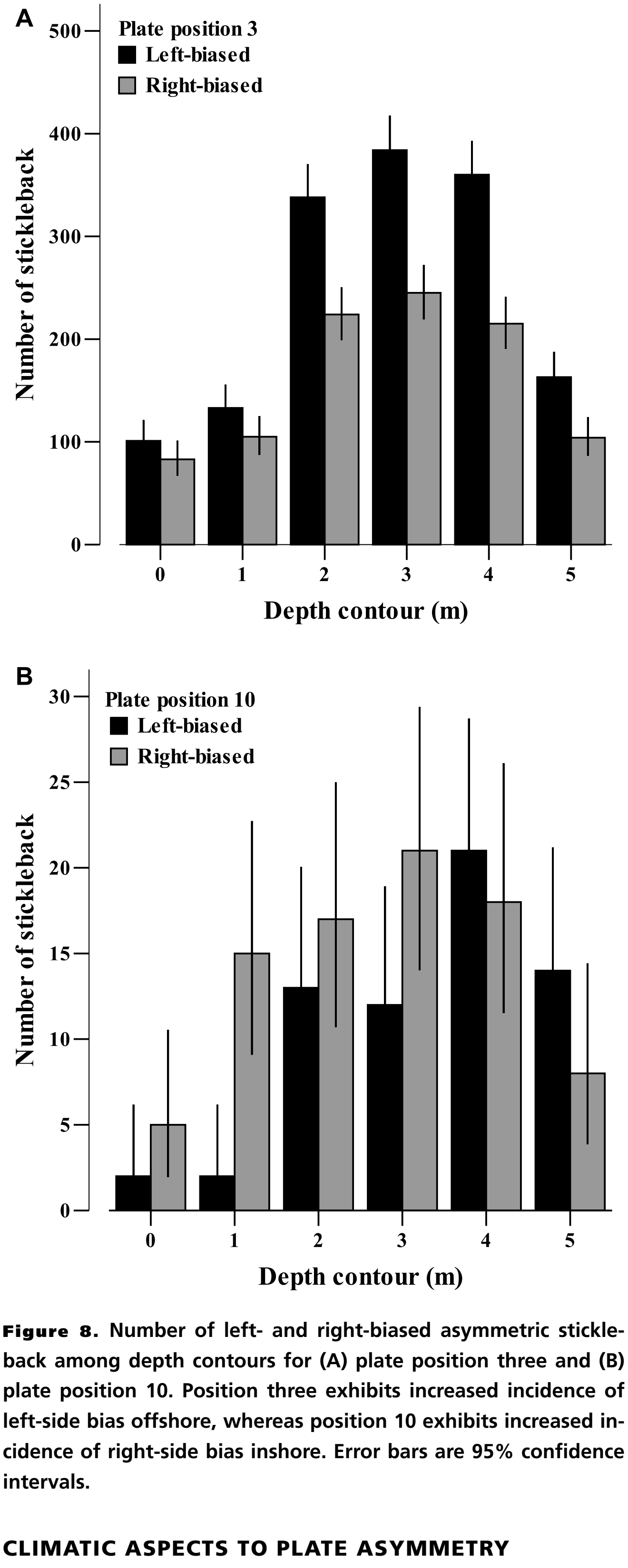

Figure 8. Number of left- and right-biased asymmetric stickleback among depth contours for (A) plate position three and (B) plate position 10. Position three exhibits increased incidence of left-side bias offshore, whereas position 10 exhibits increased incidence of right-side bias inshore. Error bars are 95% confidence intervals.

CLIMATIC ASPECTS TO PLATE ASYMMETRY

We examined plate asymmetries with respect to lake temperature and wind conditions during the 48-h trap sets among the 19 transects. Transect frequencies of absolute asymmetries were inversely related to the temperature at the time of sampling (Fig. 9;

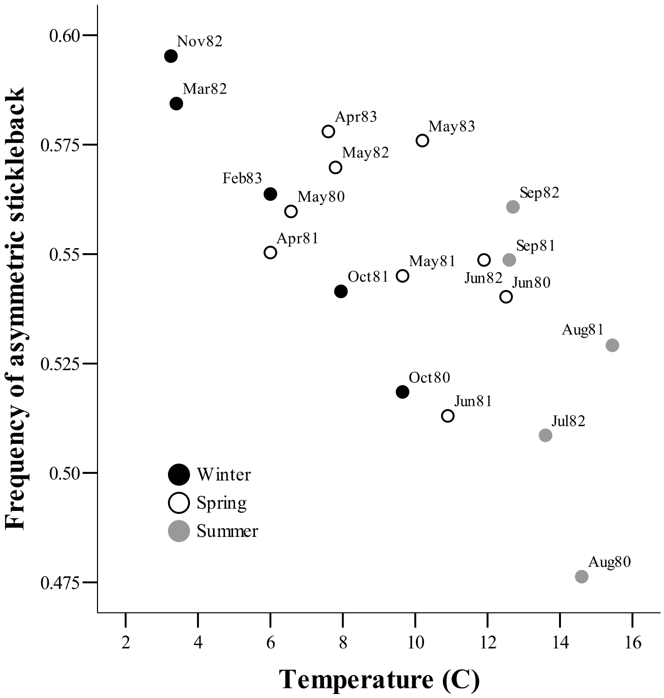

Figure 9. Frequency of asymmetric stickleback as a function of temperature and season. Each datapoint represents fish collected during a single transect and is labeled by transect sample date.

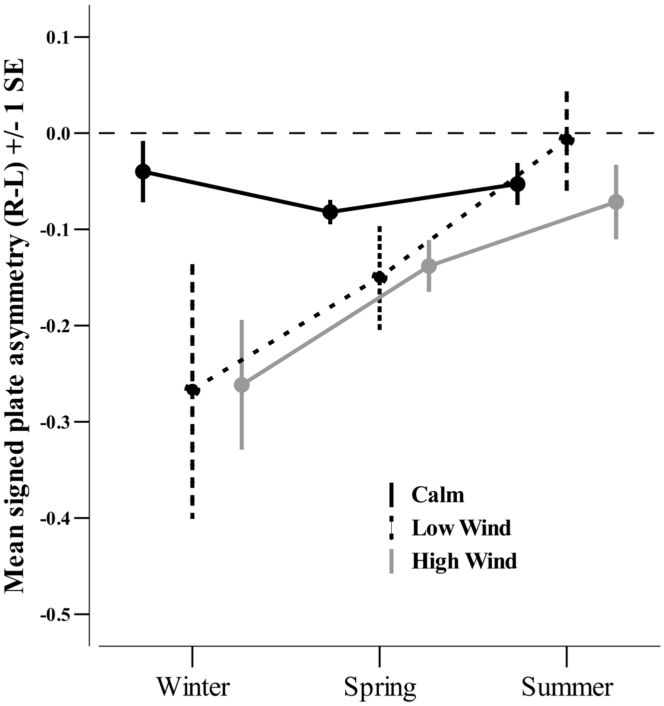

Figure 10. Mean signed (R–L) lateral plate asymmetry among wind conditions and seasons. Left-biased stickleback were more prevalent in windy, colder seasons.

$R^2 = 0.50$; $F = 17.25$; MS $= 0.01$; df $= 1$; $P = 0.001$). The regression slopes did not differ significantly among seasons (ANCOVA: Season × Temperature $F = 1.58$; MS < 0.01; df $= 2$; $P = 0.24$) and when these slopes were pooled, temperature had a larger effect on transect asymmetry frequencies than did season (Temperature $F = 10.09$; MS $= 0.004$; df $= 1$; $P = 0.007$, Season $F = 1.09$; MS < 0.001; df $= 2$; $P = 0.37$). In contrast, transect mean signed plate asymmetry was independent of lake temperature ($R^2 = 0.004$; $F = 0.08$; MS < 0.001; df $= 1$; $P = 0.79$). There were no associations with frequencies of asymmetric fish and wind conditions at the time of sampling (GLMM: Wind $\chi^2 = 0.01$; $P = 0.94$) nor was there an interaction with region (GLMM: Wind × Region $\chi^2 = 1.83$; $P = 0.17$) or season (GLMM: Wind × Season $\chi^2 = .08$; $P = 0.78$). In contrast, left-biased stickleback were captured more often during windy rather than calm conditions (LMM: Wind $F = 5.88$; df $= 2$; $P = 0.003$) although this effect was seen in winter and spring but not in summer (Fig. 10; LMM: Wind × Season $F = 2.34$; df $= 4$; $P = 0.05$). These associations were not affected by lake region (LMM: Wind × Season × Region $F = 0.54$; df $= 15$; $P = 0.92$, LMM: Wind × Region $F = 1.16$; df $= 4$; $P = 0.29$).

ASYMMETRY AND LATERALITY IN PREDATOR/PREY INTERACTIONS

Frequency and directionality of pelvic spine injuries

Of the multiple predator-induced injuries that were scored on these stickleback (Reimchen 1988), only injuries to the bilaterally paired pelvic spines provide data on potential laterality of predator–prey interactions because of the asymmetry of their incidence (a broken left or right spine). Among the 465 fish that had pelvic fractures, there was no overall side bias: 30 (6.5%) had injuries to both sides, 216 (46.5%) had injuries only to the left spine, and 219 (47.1%) had injuries only to the right spine ($\chi^2 = 0.02$; df $= 1$; $P = 0.89$). There were a significantly greater proportion of females with pelvic spine injuries (16.2%) than males (8.2%) (GLMM: Sex $\chi^2 = 35.59$; df $= 1$; $P < 0.001$). Although males had slightly more fractures on the left (54.7%) and females had slightly more fractures on the right (52.7%) the difference between them was not significant (GLMM: Sex $\chi^2 = 1.99$; df $= 1$; $P = 0.15$).

Numbers of fish with pelvic injuries were insufficient to allow GLMM analyses with transect as a random factor because it resulted in too many zero values. We therefore pooled fish by year and looked for spatial patterns in the distribution of fish with left and right pelvic injuries within each year. There was evidence for lake spatial differences in the distribution of left versus right pelvic fractures. There was a significant excess of right pelvic fractures in the southeast region in 1982 ($\chi^2 = 11.83$, df $= 5$; $P = 0.04$), and although this was nonsignificant in the other three years, the same trend was present in each of them. This effect did not differ between the sexes (log-linear:

region × sex × pelvic injury direction $G = 3.63$; df = 5; $P = 0.60$). There were no differences in the proportion of left and right pelvic fractures among the littoral–limnetic depth contours for any year (all $P \geq 0.62$), nor was there an interaction with sex (log-linear: depth contour × sex × pelvic injury direction $G = 2.28$; df = 4; $P = 0.68$).

Bill-orientation of fish carried by loons

Of 122 fish captures by Red-throated loons, there was evidence for laterality but alternate trends between species. For both prey species, loons preferentially attacked from the ventral side (herring: 77.1%, $N = 48$, $\chi^2 = 14.1$, $P = 0.001$; surfperch: 74.3%, $N = 74$, $\chi^2 = 17.5$, df = 1; $P = 0.001$). However, herring were positioned at a higher frequency with the head to the right (65%, $\chi^2 = 5.04$, df = 1; $P = 0.02$) and the surfperch with the head to the left (70%, $\chi^2 = 11.8$, df = 1; $P = 0.001$).

Discussion

Investigations of subtle bilateral asymmetries for the last five decades have focused on FA, which can be caused by genetic imbalance, physiological stress, and developmental instability (Mather 1953; Ames et al. 1979; Parsons 1990; Clarke 1993; Imasheva et al. 1997). Most single samples in previous studies of threespine stickleback indicate that the lateral plates conform to the statistical definition of FA (Hagen and Gilbertson 1972; Moodie and Reimchen 1976; Ziuganov 1995; Bergstrom and Reimchen 2000), and most data on asymmetry of lateral plates match predictions emerging from the FA literature (Moodie and Reimchen 1976; Bergstrom and Reimchen 2000, 2003, 2005; Reimchen and Nosil 2001a). However, there are conceptual and empirical ambiguities in the FA classification for armor of *Gasterosteus*. Although smaller, single samples of stickleback suggest that lateral plates exhibit FA, we observed in larger replicated within-lake samples a weak but consistent left-side bias to mean signed asymmetry of lateral plates in each size class and sex. Such directional asymmetry (DA) in lateral plates has been previously described from another stickleback population that differs dramatically from Drizzle Lake in showing major reduction in spine and lateral plate expression (Reimchen and Nosil 2001a). Left-side bias has also been observed in the pelvic girdle of *Gasterosteus* from British Columbia (Reimchen 1980) and Alaska (Bell et al. 1985), as well as in Miocene deposits of *Gasterosteus* (Bell et al. 2007). Slight left-side biases in otherwise symmetrical bilateral structures may reflect basic asymmetry in vertebrate developmental pathways. Among Cephalochordates and Urochordates, there is a left-side bias in internal organ development and protein expression (Yasui et al. 2000; Boorman and Shimeld 2002). If this is applicable to the development of the lateral plates of *Gasterosteus,* the slight left-side bias in the anterior plates may be influenced by this underlying asymmetry in gene expression during early ontogeny. Bilateral expression of a meristic trait such as lateral plates may default to left-side expression during developmental instability and would be consistent with increased parasitism and reduced immunocompetence of *Gasterosteus* morphs with DA in plate number (Reimchen and Nosil 2001a) and the pelvic girdle (Reimchen 1997).

Although fitness costs to asymmetry have been extensively demonstrated among numerous taxa, potential habitat or other ecological attributes to slight asymmetries remain poorly investigated. Microhabitat differences in asymmetric and symmetric phenotypes were first reported with *Gasterosteus* plate phenotypes and showed differences in nesting locations. Moodie (1972) demonstrated that asymmetric male stickleback nested in more protected microhabitats relative to symmetrical males and as a consequence incurred reduced mortality and increased reproductive success (Moodie and Moodie 1996). Previous studies showed an increased prevalence of higher plate counts close to shoreline relative to open water (Reimchen 1995) which may reflect an adaptation to greater puncturing predators (trout) near shore and compression piscivores (diving birds) in open water (Reimchen 1994). In our analysis of data from Drizzle Lake, we observed a corresponding shift in directional asymmetries of lateral plates with an increased left-side bias among fish collected at increased distances from shore. These habitat trends in the directionality of lateral asymmetry did not match any theoretical predictions although there are strong ecological correlates. Offshore habitats were characterized by extensive avian piscivory on the sticklebacks in contrast to salmonid piscivory in shallow water (Reimchen 1994).

Stable isotope analyses of tissues provide a time-integrated signature of dietary niche (Minagawa and Wada 1984; France 1995; Vander Zanden and Rasmussen 2001; Post 2002) and allow an independent test of these potential associations between plate asymmetries and microhabitat. In the Drizzle Lake population, there are directionally consistent nitrogen isotopic differences between left- and right-biased plate asymmetries independent of total plate number and yet no statistical differences between symmetric and asymmetric stickleback (Reimchen et al. 2008). Depleted δ^{15}N signatures in left-biased stickleback suggest increased planktivory relative to right-biased fish, consistent with the distributional data in the current study. That these trends with an independent niche proxy are concordant with our microhabitat data strengthens confidence that the observed spatial trends in signed asymmetry across the littoral–limnetic gradient are ecologically meaningful.

The shift in DA that we observed in adult fish might reflect a history of differential survivorship along a selective gradient between littoral and limnetic habitats of the lake. One selective regime influencing the extent of symmetry of defenses might relate to the geometry of predator–prey interactions with diving birds. If all spatial interactions between aquatic prey and an approaching predator were random in three-dimensional space, no directionality in defenses would be expected. However, if predators approach prey from a preferred side, or if prey track predators preferentially with one eye rather than the other, the potential for asymmetric responses increases. There is evidence that this may be the case for piscivorous birds and their prey fish. Some birds show right eye dominance when approaching a food item (Gunturkun 1997), and so prey may experience predation more often on their left side. In fish, the left eye is used at proportionately higher frequency than the right eye when tracking a threatening stimulus such as a predator (Bisazza et al. 1998). It is not clear to us how this would account for observations of Red-throated Loons (*G. stellata*), which captured surfperch and herring with the body of the fish aligned in the bill in opposite directions. However, these data show that *Gavia*, one of the major stickleback predators, exhibits laterality in prey capture. Additional evidence of laterality in handling of fish during parental feeding has been recently reported for terns (Grace and Craig 2008).

One might expect that such an asymmetry in capture orientation would be detectable among escaped fish with bird-induced injuries, which are prevalent in this population (Reimchen 1988). We detected no overall side bias based on pelvic spine injuries but found increased occurrence of right pelvic fractures in the south region of the lake, which has higher densities of stickleback than the north lake regions (T. E. Reimchen, unpubl. data) and found slightly more right pelvic fractures on females and more left pelvic injuries on males. Such a gender difference may relate to niche partitioning as males tend to be more littoral and more benthic whereas females tend to be limnetic (Reimchen 1980; Reimchen and Nelson 1987). This would alter the geometry of predator–prey interactions as a diving bird foraging in the shallow littoral zone would require a horizontal approach yet in limnetic zones, avian piscivores approach the fish from below the horizontal in which the fish is silhouetted against the surface.

Functionality of subtle directional asymmetries has occurred in other species and has been associated with both increased foraging success (Hoso et al. 2007) and increased escape success from predators (Seligmann 1998). In the present study, asymmetric left-biased stickleback may be enjoying a functional advantage in microhabitats of the lake that are dominated by avian piscivores, an hypothesis supported by the fact that we detected no decrease in the incidence of asymmetry with age, as we had originally anticipated. The increased incidence of stickleback with more plates on their left sides in areas of the lake in which they are more likely to be under attack by diving birds suggests there may be a functional benefit to this subtle DA. The shift to left-biased DA was driven primarily by plate position three, which is anterior to the dorsal spines and may provide protection to the integument in the head region of the stickleback. We also found greater numbers of right-biased fish for plate position 10 inshore (Fig. 8), and although this may be suggestive of a caudal axial shift in side bias, the sample sizes of these fish were sufficiently low to suspect a sampling effect. Although the biomechanical explanations of within-lake shifts in directionality are not clear at this time and we cannot exclude a nonadaptive mechanism, this trend is consistent with among-lake comparisons across the archipelago, which show a significant increase in left-biased plate number asymmetry in those localities (ponds) that are driven by bird predation (C. A. Bergstrom and T. E. Reimchen, unpubl. data).

The major spatial and temporal complexity in sources of predator-induced mortality in this and other populations on the archipelago (Reimchen 1994) suggests the potential for widespread functional coupling between behavior and asymmetric morphology. We consider it improbable that within-population correlations between asymmetry and habitat represent current selection differentials driven by predation, given the potential dispersal ability of stickleback throughout the lake, large shifts in frequencies of asymmetrical adults between sequential samples, and the low predation rates on the adult stickleback from Drizzle Lake (Reimchen 1990, 1995). Rather, we suspect that the correlation between the direction of asymmetry and habitat may be an indication of behavioral differences between asymmetry phenotypes resulting from a history of variable selection pressures in different parts of the lake. Correlations between conspicuous DA and behavior exist in other taxa (Hori 1993; Benkman 1996; Delehanty and O'Hearn 2005; Bergstrom and Palmer 2007). However, behavioral correlations with the direction of subtle directional asymmetries are quite rare (but see Seligmann 2002) and the genetic mechanisms that determine subtle directional and fluctuating asymmetries are controversial and not well understood (Roff and Reale 2004; Van Dongen 2007). Recently, Palmer (2004) proposes that subtle, nonheritable FA may lead to heritable DA via genetic assimilation. Regardless of the genetic architecture of asymmetry of Drizzle Lake stickleback, there is support for its functionality, suggesting these fish may be on an evolutionary trajectory from developmental instability to adaptive DA.

Supportive evidence for the correlations between behavior and asymmetry in threespine stickleback emerges from the microclimatic differences in the capture success of different phenotypes. Asymmetric fish were more prevalent in the traps in colder weather, even within seasons, and left-biased individuals were more prevalent during windy conditions, especially in winter and spring. We suspect these associations may be a function of a history of selection associated with these conditions. Trout predation, for example, has been identified as a potential selective agent against lateral plate asymmetries (Moodie 1972; Moodie et al. 1973; Moodie and Reimchen 1976; Reimchen 1983; Bergstrom and Reimchen 2003) and is reduced in colder conditions

(Reimchen 1990). This may have generated correlated selection between behavior and morphology, with asymmetric stickleback becoming more active in colder weather when trout activity is reduced.

Our data on lateral plate asymmetry in Drizzle Lake stickleback, which have emerged from the extended time frame of the study as well as the large dataset, suggest a novel ecological component to the occurrence, directionality, and spatial distribution of armor asymmetry in the lake. If this relatively simple habitat has evidence for microhabitat structuring of niche space among symmetric/asymmetric phenotypes, it seems that the prospects for such processes in other populations and other species, many of which will be from more complex communities, will be substantial. The expanding evidence of lateralization of behavior in vertebrates (Vallortigara and Rogers 2005) increases the potential for asymmetrical pursuit and capture phases of the predator–prey interaction. Differentiating biomechanical functionality of asymmetry from developmental instability becomes increasingly problematic without much more detailed insight on lateralized behavior in predator–prey encounters in natural populations.

ACKNOWLEDGMENT

We acknowledge Drs. J. B. Foster and J. S. Nelson for initial support and the Natural Sciences and Engineering Research Council of Canada for continued funding of this research program (operating grant NRC 2354 to TER). S. Douglas assisted extensively in field work and we thank her as well as G. E. Moodie, M.A. Bell, and J. Nelson for discussion.

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Associate Editor: B. Swalla