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# Pelvic girdle reduction and asymmetry in threespine stickleback from Wallace Lake, Alaska

Emily A. Lescak[1], Frank A. von Hippel[1],
Richard R. Bernhardt[1] and Michael A. Bell[2]

*[1]Department of Biological Sciences, University of Alaska Anchorage, Anchorage, Alaska, USA and [2]Department of Ecology and Evolution, Stony Brook University, Stony Brook, New York USA*

## ABSTRACT

**Questions:** Can a bimodal frequency distribution of phenotypes persist over multiple generations despite ecological changes? Can an organism's environment elicit fitness trade-offs between armour development and somatic growth?

**Background:** Wallace Lake, located in south-central Alaska, contains a population of threespine stickleback (*Gasterosteus aculeatus*) exhibiting a bimodal distribution of pelvic phenotypes with modes at both highly reduced and fully developed pelvic armour. The lake has low ion availability, abundant macroinvertebrate predators, and introduced piscivorous fish.

**Methods:** Analyse temporal variability of the bimodal frequency distribution of pelvic phenotypes, direction and degree of asymmetry in bilateral armour traits, and whether extent of pelvic girdle development is inversely related to body size.

**Conclusions:** Distributions of pelvic phenotypes and of individuals with asymmetrical pelvic girdles persist over a 20-year time span. Individuals with greater pelvic expression exhibit more symmetrical anterior processes and ascending branches than those with pelvic reduction. Both directional and fluctuating asymmetry are present in armour traits. Stickleback with complete pelvic structures do not appear to experience reduced somatic growth compared with those with reduced pelvic girdles.

*Keywords*: directional asymmetry, disruptive selection, fluctuating asymmetry, frequency-dependent selection, stickleback.

## INTRODUCTION

A core problem in evolutionary biology concerns the origin and maintenance of intraspecific divergence, a process that is crucial to intraspecific polymorphisms, speciation, and adaptive radiation (Mayr, 1963; Schluter, 2000, 2003; Nosil and Crespi, 2006; Grant and Grant, 2008). Traditionally, natural selection has been viewed to reduce intraspecific variation through elimination of unfit individuals (Fisher, 1930). However, both frequency-dependent and disruptive selection give rise to and maintain intraspecific variation.

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Correspondence: E.A. Lescak, Department of Biological Sciences, University of Alaska Anchorage, 3211 Providence Dr., Anchorage, AK 99508, USA. e-mail: elescak@alaska.edu

Disruptive selection has historically been viewed as relatively rare, since the mean phenotype of the population resides in an adaptive valley (Endler, 1986). However, it may actually be as common as stabilizing selection (Kingsolver *et al.*, 2001). Disruptive selection maintains phenotypic variation within populations because individuals with extreme phenotypes experience higher fitness than those with intermediate phenotypes. Possible outcomes include sympatric species pairs, sexual dimorphism, and plasticity (Rueffler *et al.*, 2006). Disruptive selection has been recognized across taxa; it maintains bill polymorphisms in the African finch [*Pyrenestes* (Smith, 1993)] as well as a population of Darwin's finches [*Geospiza fortis* (Hendry *et al.*, 2009)], and has also been implicated in the maintenance of trophic morphology polymorphisms in threespine stickleback [*Gasterosteus aculeatus* (Bolnick, 2004; Bolnick and Lau, 2008)].

The threespine stickleback is an important model species for the study of evolutionary diversification, particularly with regard to armour evolution (e.g. Moodie, 1972; Bell, 1984, 2001; Reimchen, 1994), owing to the dramatic nature of adaptive divergence within this species complex and resulting phenotypic variability within and among populations. Stickleback have three major defence structures against predatory vertebrates: the pelvic girdle, dorsal spines, and lateral plates. The pelvic girdle is a bilateral structure located on the ventral and lateral surfaces of the body that comprises four major elements per side: the anterior process, ascending branch, posterior process, and pelvic spine (Nelson, 1971; Bell, 1988) (see Fig. 1). The pelvic and dorsal spines impede ingestion by predatory vertebrates by increasing effective size (Reimchen, 1983, 1994) and piercing the soft tissues of the predator's mouth (Hoogland *et al.*, 1957). The anterior bony lateral plates form a row along each side of the body, covering soft tissues and providing structural support for the pelvic and dorsal spines (Reimchen, 1983, 1992, 1994). Together, the pelvic girdle, lateral plates, and dorsal support bones form a ring around the abdominal cavity that resists compression by the jaws of predatory vertebrates, thereby increasing the likelihood of post-capture escape and survival (e.g. Hoogland *et al.*, 1957; Hagen and Gilbertson, 1972; Moodie, 1972; Reimchen, 1983, 1994; Bell, 1987, 1988).

Lateral plate phenotypes include three major morphs: complete (a continuous row of modally 33 plates from head to tail), partial (anterior and posterior plates with an intervening unplated gap), and low (less than 10 anterior plates) (Hagen and Gilbertson, 1972, 1973). Oceanic (marine and anadromous) stickleback are nearly always completely plated with robust pelvic structures, including the four elements described above, and three dorsal spines. In contrast, most freshwater populations are monomorphically low plated (e.g. Hagen and Gilbertson, 1972; Bell, 1984, 2001), but rarely have reduced numbers of dorsal spines and vestigial pelvic structures (Bell, 1974, 1987; Moodie and Reimchen, 1976; Campbell, 1985; Bell and Ortí, 1994; Klepaker *et al.*, 2013). Pelvic reduction usually evolves in a stereotyped sequence, with the pelvic spines lost first, followed by the posterior process, ascending branch, and anterior process (Bell, 1987; Bell *et al.*, 1993; Bell and Ortí, 1994).

Many independently derived populations with armour reduction have evolved in habitats with low ion availability (e.g. Bell, 1974, 1987, 1988, 2001; Giles, 1983; Chan *et al.*, 2010). Freshwater teleosts use energetically expensive active transport mechanisms to import cations (Dacke, 1979; Durham, 1991; Woods and Shuttleworth, 1995; Verbost *et al.*, 1997). Therefore, low concentrations of dissolved ions in ambient water may increase the cost of importation to mineralize armoured structures, reduce the energy available for investment in growth and other phenotypic traits, and increase the fitness of fish with reduced armour (Giles, 1983). Surveys of lake populations from Cook Inlet, Alaska revealed associations between ionic concentrations in ambient water and reduction of the pelvis (Bell *et al.*, 1993) and other armoured structures (Bourgeois *et al.*, 1994). Furthermore, growth rates are typically slower in completely plated stickleback reared in water of low ion strength (Marchinko and Schluter, 2007; Barrett *et al.*, 2008).

Predatory vertebrates and insects are also selective agents for the presence/reduction of bony armour. The presence of piscivorous fishes is a selective agent for robust armour, including a fully formed pelvic girdle with spines (Hoogland *et al.*, 1957; Hagen and Gilbertson, 1972; Moodie and Reimchen, 1976; Reimchen, 1980, 1983, 1994; Bell, 1987; Bell *et al.*, 1993; Lescak and von Hippel, 2011). Similarly, lateral plate number is subject to strong selection that depends directly or indirectly on the presence or absence of predatory fishes (e.g. Hagen and Gilbertson, 1972, 1973; Reimchen, 1980, 1983, 1994, 1995; for a review, see Bell, 2001). Reimchen (1980) hypothesized that stickleback with pelvic spines are more susceptible to predation by insects that grasp spines to facilitate capture and manipulation. Several experiments have demonstrated increased survival of stickleback with shorter pelvic girdles and spines (Hagen and Gilbertson, 1972; Reimchen, 1994; Marchinko, 2009). However, this phenomenon does not appear to hold true across all study systems (Zeller *et al.*, 2012; Mobley *et al.*, 2013). In the Wallace Lake population, located in the Cook Inlet Basin of Alaska, both body size and degree of bony armour appear to influence susceptibility to predation by macroinvertebrates (Lescak *et al.*, 2012).

Symmetry of bilateral armoured traits may also be subject to selection. Fluctuating asymmetry is sometimes associated with fitness and refers to random deviations from equal size or number of elements on the right and left sides of generally bilaterally symmetrical individuals (Mather, 1953; Reeve, 1960; van Valen, 1962; Soulé, 1967; Møller, 1994; Palmer and Strobeck, 1997). For example, Moodie and Reimchen (1976) found a negative relationship between the relative frequency of stickleback asymmetrical for lateral plates and the presence of predatory fishes, suggesting that asymmetry is associated with vulnerability to fish predation. However, lateral plate asymmetry also may have a range of possible benefits (Reimchen, 2010); for example, asymmetry of the posterior plates may lead to increased flexibility, allowing for greater acceleration and angle of the startle response (Bergstrom, 2002).

While many bilateral traits show fluctuating asymmetry, some show directional asymmetry, which refers to a consistent bias towards one side (van Valen, 1962). For example, most threespine stickleback populations with pelvic reduction in the Cook Inlet Basin tend to have more individuals with larger pelvic vestiges on the left side than on the right ['left-biased' (Bell *et al.*, 1985, 2006, 2007)]. Across taxa, directional asymmetry often has fitness consequences, as is the case in dentition in predatory snakes that prey upon snails (Hoso *et al.*, 2007).

Wallace Lake contains a threespine stickleback population with a strongly bimodal distribution of pelvic phenotypes (Bell and Ortí, 1994; Lescak, 2010). Bell and Ortí (1994) reported that 43.6% of specimens either lacked a pelvic girdle or had a small pelvic vestige (pelvic score ≤2), and 26.8% had a fully expressed pelvic girdle (pelvic score = 8). In this paper, we present a retrospective morphological analysis to determine stability of the bimodal frequency distribution over time and directionality of asymmetry in armoured traits. Asymmetry is analysed across individuals with and without regard to pelvic score to determine whether the extent of pelvic expression relates to degree of asymmetry. We hypothesize that stickleback with greater external bony armour experience reduced somatic growth compared with armour-reduced individuals due to the low ion availability of Wallace Lake.

## MATERIALS AND METHODS

### Study population and sampling procedures

Wallace Lake is located in the Matanuska-Susitna Valley of south-central Alaska (61°34′27.9″N, 149°34′27.3″W), has a surface area of 27 ha, and is at an elevation of 104 m. It had low conductivity in 1990 (19 $\mu\text{S}\cdot\text{cm}^{-1}$), with nearly identical values obtained in 2009 (20 $\mu\text{S}\cdot\text{cm}^{-1}$) using a YSI 85 Dissolved Oxygen/Conductivity Meter. This level of conductivity indicates a low concentration of dissolved ions (see, for example, Haught and von Hippel, 2011). In 1990, the lake had neutral pH (7.0) and relatively high values of chlorophyll A and pheophyton, indicating high productivity. More recent data for these variables are unavailable. No inlets or outlets are present, but the lake experiences intermittent seepage into a nearby creek. No native or legally stocked fish species are present, but residents have reported illegally planted northern pike (*Esox lucius*) over the past 20 years.

Stickleback were trapped near shore in fall and/or spring from 2001 through 2004 and 2008 (Table 1) in 0.32 cm and 0.64 cm unbaited mesh minnow traps set overnight. Specimens were euthanized with an overdose of MS-222 anaesthetic, fixed in 10% neutral buffered formalin, preserved in 70% ethanol, and stained with Alizarin Red S to visualize bony structures.

### Morphological analyses

One person (E.A.L.) scored the samples in random order to maximize measurement consistency and eliminate the potential for a shifting observer bias that could produce spurious temporal trends. The first 100 stickleback from each sample were scored for the complete suite of traits, and only standard length (SL) and pelvic score (PS) were recorded in the remaining specimens (Table 1).

Mass was obtained using a laboratory scale (Ohaus Scout 11 SCA210) with precision to the nearest 0.01 g. Length measurements were made using digital calipers to the nearest

**Table 1.** Means (and standard deviations) for each trait in adults from each sampling year

| Year | *N* | SL | Mass | AP (L) | AP (R) | AB (L) | AB (R) | PSL (L) | PSL (R) | LP (L) | LP (R) | PDD (L) | PDD (R) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2001 | 97 | 39.89 | 0.73 | 2.41 | 2.27 | 1.82 | 1.56 | 0.56 | 0.47 | 3.84 | 3.69 | 8.24 | 8.09 |
| | | (3.85) | (0.26) | (0.81) | (0.90) | (1.98) | (1.93) | (0.97) | (0.94) | (0.53) | (0.55) | (1.92) | (1.79) |
| 2002 | 670 | 37.55 | 0.64 | 2.23 | 2.02 | 1.66 | 1.36 | 0.55 | 0.46 | 3.92 | 3.82 | 7.69 | 7.52 |
| | | (3.82) | (0.27) | (0.87) | (0.99) | (1.81) | (1.73) | (0.91) | (0.91) | (0.55) | (0.61) | (2.02) | (1.94) |
| 2003 | 198 | 42.20 | 1.07 | 2.43 | 2.26 | 2.01 | 1.66 | 0.59 | 0.47 | 3.82 | 3.81 | 8.99 | 8.73 |
| | | (4.94) | (0.45) | (0.97) | (1.06) | (2.16) | (2.07) | (0.98) | (0.94) | (0.58) | (0.62) | (2.28) | (2.12) |
| 2004 | 100 | 43.54 | 1.23 | 2.41 | 2.25 | 1.83 | 1.52 | 0.46 | 0.51 | 4.04 | 4.00 | 9.12 | 9.12 |
| | | (4.39) | (0.46) | (0.98) | (1.07) | (2.31) | (2.17) | (0.94) | (1.07) | (0.53) | (0.53) | (1.80) | (1.83) |
| 2008 | 157 | 38.55 | 0.73 | 2.37 | 2.21 | 2.04 | 1.66 | 0.47 | 0.45 | 3.90 | 3.96 | 7.48 | 7.38 |
| | | (4.71) | (0.31) | (0.72) | (0.90) | (2.03) | (1.85) | (0.80) | (0.84) | (0.63) | (0.57) | (1.90) | (1.92) |

*Note*: All values are millimetres except for mass, which is measured in grams and LP, which is a count. SL = standard length, AP = anterior process length, AB = ascending branch length, PSL = pelvic spine length, LP = lateral plate number, PDD = distance between pelvic and second dorsal spines. L = left side, R = right side.

0.01 mm. Standard length was measured from the anterior tip of the upper jaw to the posterior end of the hypural plate. Three elements of the pelvic girdle – anterior process, ascending branch, and pelvic spine (Nelson, 1971) – were measured on both left and right sides (Fig. 1). The pelvic–dorsal distance (PDD; Fig. 1) of the fish was determined by measuring the distance between the tip of the erect pelvic spine and the tip of the erect second dorsal spine on each side of the fish. If a pelvic spine was absent, the ventro-lateral edge where the spine would have articulated was used. Pelvic–dorsal distance was measured using calipers; the fish was maintained in a stationary position by fitting it into a hole drilled into a board that accommodated the body and allowed for the spines to remain locked in an outward position. Measurements conducted in this manner yield high precision [mean coefficient of variation across measurements = 0.01 mm (J. Colgren *et al.*, unpublished data)].

Lateral plate number was counted for both sides under an illuminated magnifier (10×). Pelvic score (PS) was quantified using the scoring system described by Bell *et al.* (1993) for each side of the pelvis: 4 = all pelvic elements present; 3 = only pelvic spine and fin ray absent; 2 = pelvic spine and posterior process absent (anterior process and ascending branch remain); 1 = only anterior process present; 0 = all elements absent. However, this system was modified to include half scores that represent incomplete formation of an element; for example, a pelvis with a short posterior process was scored as 2.5. Each side of the pelvic structure was scored separately, and the two scores summed to obtain the specimen's total PS, which could vary from 0 to 8.

Pelvic score asymmetry was determined for PS ranging from 0.5 to 7.5 without regard to the actual length of any pelvic element by subtracting the PS of the left side from that of the right side. Thus, a positive asymmetry score means that the pelvis has more elements on the right side than on the left. Similarly, the number of lateral plates on the left side was subtracted from the number on the right side. Asymmetry was quantified for morphometric traits by subtracting the value of the trait on the left side from that of the right side and dividing the difference by the size of the right trait plus the left trait. This calculation was performed for the anterior process, ascending branch, pelvic spine, and pelvic–dorsal distance.

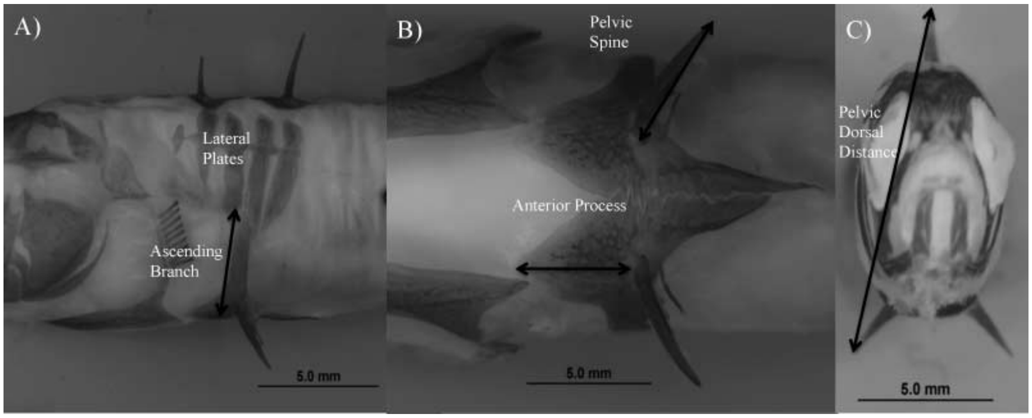

**Fig. 1.** Armour traits measured. (A) Lateral view, with anterior to the left. (B) Ventral view of the pelvic girdle, with anterior to the left. (C) Anterior view. Photo credit: M. Currey.

### Temporal stability of the bimodal distribution and pelvic score asymmetry

Generation time in Cook Inlet stickleback is usually 2 years (Baker *et al.*, 2008). Fish from each sample were divided into juvenile (1+ year) and adult (2+ years) groups based on frequency distributions of standard length. Asymmetry was not analysed for juvenile fish due to low sample sizes.

We used the multinomial logit model to examine whether the frequency distribution of pelvic score and asymmetry changed throughout the period of study with and without data from individuals collected during the summer of 1990 (Bell and Ortí, 1994; Bell *et al.*, 1993, 2007). Models were fitted using the 'vglm' function in the VGAM library (Yee, 2010) in R (R Development Core Team, 2010). The null model (intercept only) was compared with a model including PS or asymmetry and year as an explanatory variable ($\log(P_{i,j}/P_{i,\mathrm{ref}}) = \alpha_j + \beta_j(\mathrm{Year}_i)$) using the Aikaike Information Criterion (AIC).

Binomial regression models compared frequencies of PS = 2, PS = 8, and left-biased pelvic girdles over the entire study period (1990, 2001–2004, 2008) to examine consistency of the bimodal PS distribution and left-biased directional asymmetry. If significant differences among years were found (indicated by $P < 0.05$), pairwise comparisons were conducted between years. Model fitting and comparisons were performed using the statistical program R v.2.13.1.

### Directional and fluctuating asymmetry

Analysis of covariance (ANCOVA) and Pearson product–moment correlations were calculated in SPSS v.18 to analyse relationships between pelvic score and asymmetry in the anterior process and ascending branch in individuals with total PS ≥ 2 and 4 respectively. These subsets of individuals were used because the anterior process is present only in individuals with total PS ≥ 2 and the ascending branch is present only in individuals with total PS ≥ 4. One-sample two-tailed *t*-tests were used to determine whether mean asymmetry for each trait in each year (2001–2004, 2008) differed significantly from zero. Asymmetry scores that do not differ significantly from zero indicate fluctuating asymmetry, while scores that do differ significantly from zero indicate directional asymmetry.

### Relationship between pelvic score and somatic size

Independent-samples two-tailed *t*-tests were used to compare standard length, mass, and condition within years separately for individuals with reduced (PS = 0–7.5) versus complete (PS = 8) pelvic girdles, and individuals with extreme pelvic reduction (PS = 0–2) versus those with nearly complete or complete pelvic girdles (PS = 6–8). Condition refers to fish weight per unit body length and has been found to affect growth and survival (Paul, 1997; Boldt and Haldorson, 2004). We calculated condition using log-transformed length–weight residuals, since they are indicators of somatic growth (Brodeur *et al.*, 2004). To obtain the residuals, log-transformed mass was regressed against log-transformed standard length. The predicted log-transformed mass was then calculated and subtracted from measured log-transformed mass.

## RESULTS

### Temporal stability of the bimodal distribution and pelvic score asymmetry

When comparing data from 1990 and 2001–2008, models including year were better fits than null models for both pelvic score and pelvic asymmetry, indicating that frequency distributions differ among years. However, when the 1990 data were excluded, null models yielded lower AIC scores, indicating that proportions did not change by year for the 2001–2008 samples and that the 1990 cohort differs from those from 2001–2008 (Table 2; Figs. 2, 3).

Binomial models revealed that frequencies of PS = 2 individuals did not vary significantly among years ($P = 0.900$). However, frequencies did vary significantly by year for PS = 8

**Table 2.** Multinomial model comparison

| **Asymmetry**<br>*Model with 1990* | ΔAIC | *Model without 1990* | ΔAIC |
|---|---|---|---|
| Year | 0 | Year | 9.299 |
| Null | 17.5596 | Null | 0 |
| **Pelvic Score**<br>*Model with 1990* | ΔAIC | *Model without 1990* | ΔAIC |
| Year | 0 | Year | 20.756 |
| Null | 11.9431 | Null | 0 |

*Note*: ΔAIC represents the difference in AIC between that model and the best model (indicated by ΔAIC = 0).

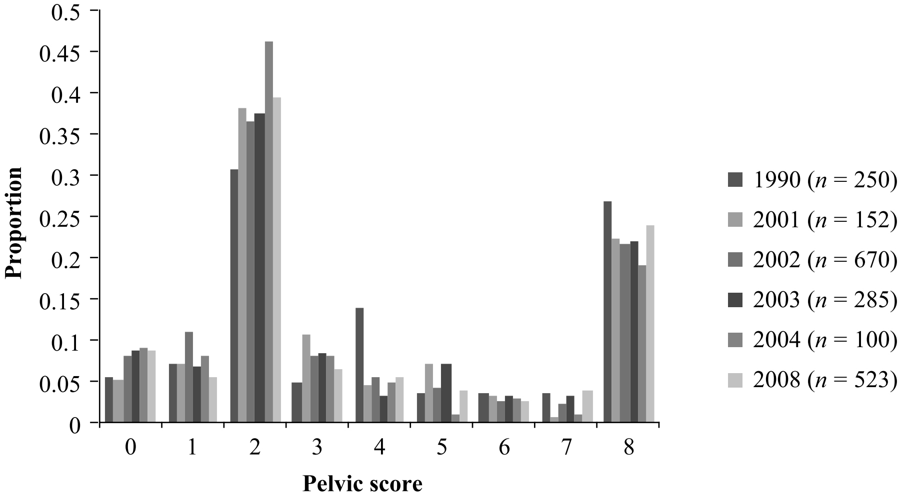

**Fig. 2.** Proportions of threespine stickleback with a pelvic score of 0–8 over 19 years in Wallace Lake.

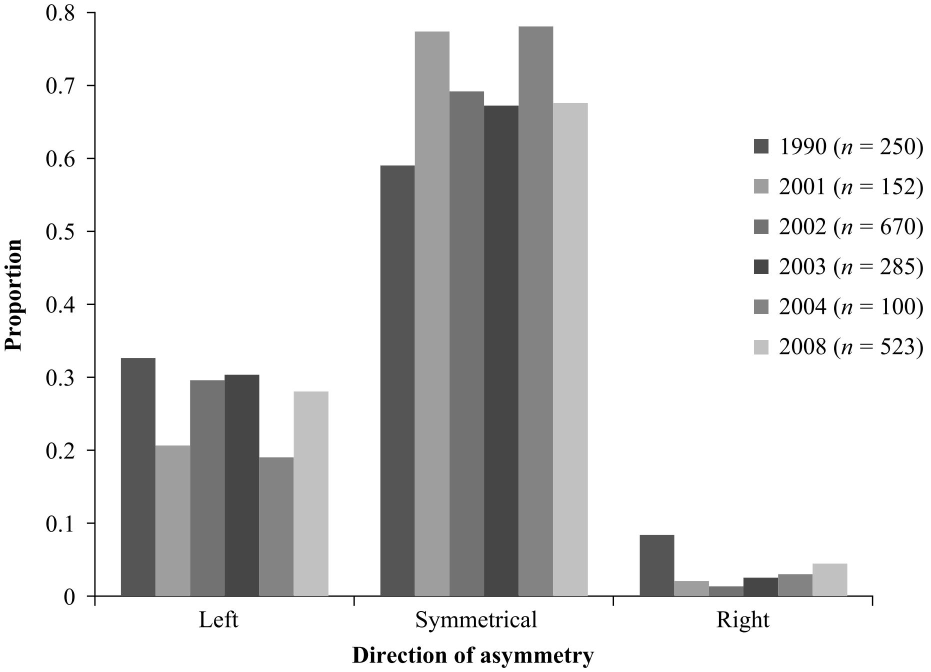

**Fig. 3.** Proportions of threespine stickleback with left-biased, symmetrical, and right-biased pelvic scores over 19 years in Wallace Lake.

($P = 0.037$) and left-biased pelvic girdles ($P = 0.013$). Pairwise binomial comparisons revealed that there were significantly more PS = 8 individuals in 1990 than in 2002 ($P = 0.033$) and significantly more left-biased individuals in 1990 than in 2001 ($P = 0.006$), 2002 ($P = 0.049$), and 2004 ($P = 0.003$).

## Directional and fluctuating asymmetry

Individuals with asymmetrical pelvic girdles tend to have larger elements on the left side than the right side (Figs. 3 and 4). Mean asymmetry of lateral plate number, anterior process length, pelvic spine length, and pelvic–dorsal distance did not differ consistently from zero (all $P > 0.050$), indicating an absence of directional asymmetry. However, mean asymmetry of PS and ascending branch length differed consistently from zero in all years ($P < 0.001$ and $P < 0.020$, respectively), indicating left-biased directional asymmetry.

Degree of asymmetry differed significantly in relation to pelvic score ($F_{32,2406} = 87.04$, $P < 0.001$), with robustly armoured individuals also having more symmetrical anterior processes and ascending branches than those with pelvic reduction. Pearson correlations revealed significant relationships between extent of pelvic reduction and asymmetry of the anterior process ($r = 0.069$, $P = 0.027$) for individuals with at least a PS of 2 and asymmetry of the ascending branch ($r = 0.080$, $P = 0.011$) for individuals with a PS $\geq 4$.

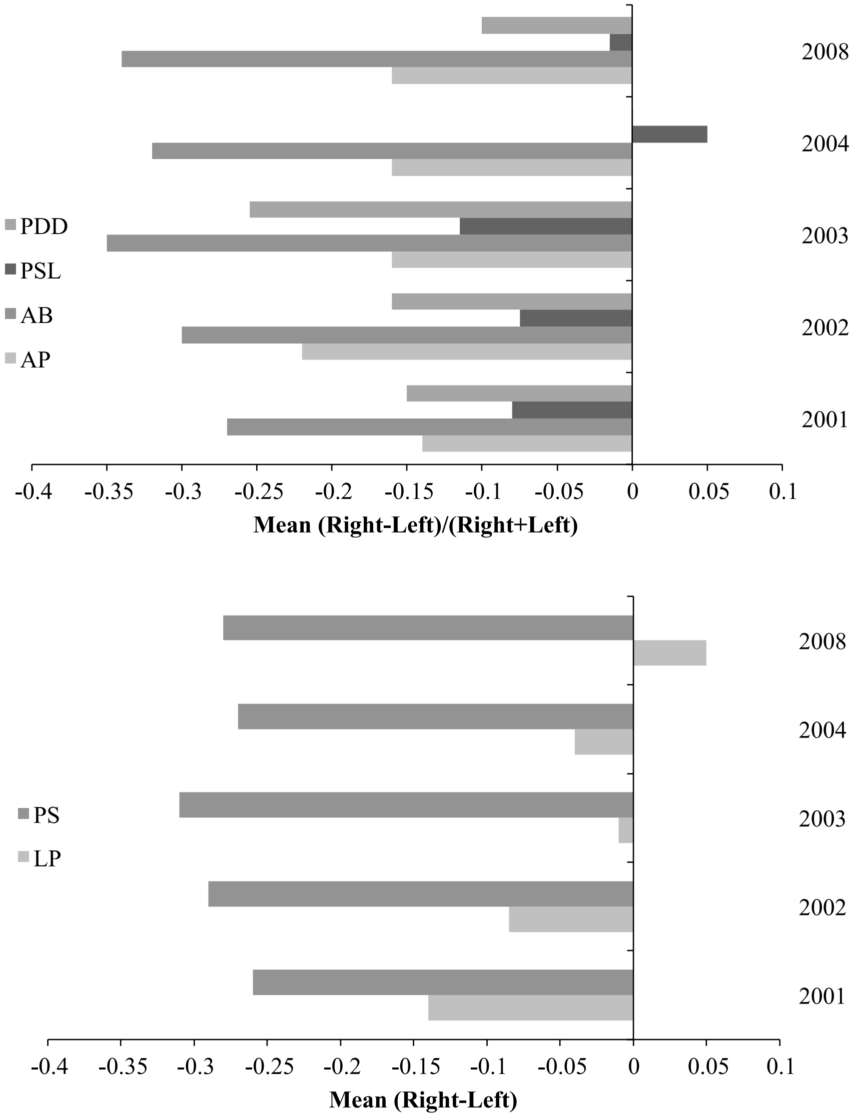

**Fig. 4.** Temporal variation of asymmetry. (Top) mean asymmetry values for morphometric traits in adults from each sample. (Bottom) Mean asymmetry values for meristic traits in adults from each sample. Positive values indicate that the trait is larger on the right side. Negative values indicate that the trait is larger on the left side. PDD = distance between pelvic and dorsal spines, PSL = pelvic spine length, AB = ascending branch length, AP = anterior process length, PS = pelvic score, LP = lateral plate number. Sample sizes for each year can be found in Table 1.

### Relationship between pelvic score and somatic size

Standard length, mass, and condition did not differ significantly within years for individuals with PS = 0–7.5 versus PS = 8 (SL: $t$ ranges from 0.20 to 0.95, $P > 0.050$; mass: $t$ ranges from 0.30 to 0.79, $P > 0.050$; condition: $t$ ranges from −0.426 to 1.091, $P > 0.050$) or individuals with PS = 0–2 versus PS = 6–8 (SL: $t$ ranges from 0.20 to 0.88, $P > 0.050$; mass: $t$ ranges from 0.28 to 0.72, $P > 0.050$; condition: $t$ ranges from −0.873 to 0.885, $P > 0.050$).

## DISCUSSION

Wallace Lake stickleback displayed a bimodal frequency distribution of pelvic scores over two decades, with modes at highly reduced (PS = 2) and fully formed (PS = 8; Fig. 2) pelvic girdles. The stability of this phenotype frequency distribution may appear to be trivial given the short time frame. However, threespine stickleback can exhibit dramatic contemporary evolution, especially when environmental factors such as predation regime or water chemistry change (Bell, 2001; Bell *et al.*, 2004; Kitano *et al.*, 2008). Northern pike, which prey upon stickleback (Hoogland *et al.*, 1957; Patankar *et al.*, 2006; Haught and von Hippel, 2011), appeared in Wallace Lake during the course of this study (first noted by residents in the summer of 2002). If the population of pike flourishes, one would expect a future increase in the proportion of individuals with complete pelvic girdles.

Although a significant difference in PS frequency among years was found between the 1990 sample and subsequent samples, the bimodal distribution was maintained. The 1990 sample was collected in the same manner as those from 2001 to 2008. However, it was collected during the summer, while subsequent samples were collected during fall and spring. Although seasonal differences in spine number have been detected in other stickleback populations (Reimchen and Nosil, 2002), consistent seasonal differences in morphology in the Wallace population were not found (Lescak, 2010). Therefore, the significant reduction in PS = 8 individuals in the 2002 sample compared with the 1990 sample could be attributed to unequal sample sizes or short-lived variation followed by phenotypic stability.

Pelvic reduction is recessive to full pelvic expression (Cresko *et al.*, 2004; Shapiro *et al.*, 2004). Chan *et al.* (2010) identified the regulatory region that controls the expression of the *Pitx1* gene in the pelvis during development. They also showed that a $Pitx1^{-}$ (recessive) allele that is silenced in the pelvis during development is responsible for pelvic reduction in the Wallace Lake population. Thus, stability of the frequency of reduced pelvic phenotypes likely reflects stable frequencies of homozygotes of the recessive allele of *Pitx1* (see Chan *et al.*, 2010).

Most individuals in this population have symmetrical pelvic girdles. When asymmetry is demonstrated, the left side tends to be larger than the right side (Fig. 3). A tendency towards left-biased pelvic girdles has been observed in pelvic-reduced populations in this region (Bell *et al.*, 1985, 2006, 2007) and elsewhere (Reimchen and Nosil, 2001a; Cole *et al.*, 2003; Shapiro *et al.*, 2004; Klepaker *et al.*, 2012) and is probably due to functional compensation of the *Pitx2* gene, which is expressed preferentially on the left side of the pelvis during development (Campione *et al.*, 1999; Marcil *et al.*, 2003; Palmer, 2004).

Shapiro *et al.* (2004) observed that left-biased directional asymmetry of the pelvis occurs only in $Pitx1^{-}/Pitx1^{-}$ homozygotes. We found that individuals with a low pelvic score had greater left-biased asymmetry in the anterior process and ascending branch than individuals with a high pelvic score. Furthermore, it appears that both directional asymmetry (pelvic score and ascending branch length) and fluctuating asymmetry (lateral plate number, anterior process length, pelvic spine length, and pelvic–dorsal distance) are present in this population. It is not clear whether this asymmetry is an incidental consequence of selection for reduced skeletal expression or if asymmetry is a target of selection, and if it is, whether the asymmetrical relationships are a correlated effect of selection on one or more traits.

The left-biased directional asymmetry and bimodal frequency distribution of pelvic scores suggest that Wallace Lake individuals with reduced pelvic structures are $Pitx1^{-}/Pitx1^{-}$ homozygotes and those with PS = 8 are either $Pitx1^{+}/Pitx1^{+}$ homozygotes or $Pitx1^{+}/Pitx1^{-}$ heterozygotes. The low frequency of intermediate pelvic phenotypes suggests that there is limited segregation at loci with minor effects on pelvic expression, some of which were mapped by Shapiro *et al.* (2004) and inferred from complementation crosses by Cresko *et al.* (2004).

Since pelvic phenotypes are strongly genetically determined (Cresko *et al.*, 2004; Shapiro *et al.*, 2004; Chan *et al.*, 2010), the stable distribution of pelvic scores probably represents temporal stability of environmental factors that select on pelvic morphology. One environmental factor affecting pelvic phenotypic expression could be the reduced availability of dissolved ions (Giles, 1983; Bell *et al.*, 1993). Possession of robust armour morphology in the water of low ionic strength of Wallace Lake was not associated with a reduction in standard length, mass, or condition, suggesting the lack of a trade-off between pelvic development and growth. This is a surprising finding in light of the association between pelvic reduction and water of low ionic strength in Cook Inlet lakes (Bell *et al.*, 1993) and the finding (Marchinko and Schluter, 2007; Barrett *et al.*, 2008) that greater lateral plate armour imposes a large growth cost on stickleback reared in fresh water. However, size may not be an appropriate surrogate for fitness; research on other fish species has demonstrated selection against fast growth, large size, and high condition in some situations (Carlson *et al.*, 2004, 2007; DiBattista *et al.*, 2007).

Another important environmental factor associated with pelvic reduction is predation regime. In experimental trials, trout preyed selectively on pelvic-reduced stickleback from Wallace Lake (Lescak and von Hippel, 2011), but this begs the question of what factor selects against full pelvic expression. Differential insect predation may be responsible; experimental trials with dragonfly naiads and Wallace Lake stickleback showed that small PS = 8 and large PS = 2 individuals are more susceptible to predation than large PS = 8 and small PS = 2 individuals (Lescak *et al.*, 2012). Heightened susceptibility of PS = 8 stickleback to predation by macroinvertebrates is also supported by Marchinko's (2009) predation experiment.

Selection appears to drive bimodality and asymmetry in pelvic phenotypes in other stickleback populations. In the population from Lake Storvatnet, Norway (Klepaker *et al.*, 2012), the bimodal distribution of pelvic phenotypes was hypothesized to be due to divergent selection throughout ontogeny. In stickleback from Boulton Lake, which is similar to Wallace Lake with regard to size and predation regime, individuals exhibit pelvic asymmetry, although *Pitx1* is not responsible for pelvic reduction (Chan *et al.*, 2010). Individuals with asymmetric pelvic girdles had lower incidences of parasitism and occupied different niches than those with complete pelvic girdles (Reimchen, 1997; Reimchen and Nosil, 2001a, 2001b).

The origin and maintenance of the bimodal distribution of pelvic phenotypes within the Wallace Lake stickleback population could be due to frequency-dependent or disruptive selection, which have been implicated in maintaining morphological diversity in other taxa (Smith, 1993; Hendry *et al.*, 2009) and trophic morphology polymorphisms in other stickleback populations (Bolnick, 2004; Bolnick and Lau, 2008). Additional work is necessary to understand the interplay among a complex predation regime, water chemistry, and behaviour of the fish in determining the phenotypic distributions of pelvic scores and armour asymmetry in this population. Wallace Lake has the most strongly bimodal distribution of pelvic scores of any lake in Cook Inlet surveyed to date (Bell and Ortí, 1994), giving it special importance as a model research population. Although northern pike recently appeared in the lake and should select against pelvic reduction, residents report that pike are not abundant. Since the introduction of exotic pike into a nearby lake containing threespine stickleback with pelvic reduction caused that population to go extinct (Patankar *et al.*, 2006) and pelvic-reduced populations may also experience heightened risk of extirpation due to natural stochasticity (Baker *et al.*, 2013), preventing future sport fish introductions into Wallace Lake should be a priority.

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