A comparative analysis of experimental selection on the stickleback pelvis

S. E. MILLER*,† (iD), M. BARRUETO* & D. SCHLUTER*

*Department of Zoology, University of British Columbia, Vancouver, BC, Canada
†Department of Neurobiology and Behavior, Cornell University, Ithaca, NY, USA

Abstract

Mechanisms of natural selection can be identified using experimental approaches. However, such experiments often yield nonsignificant effects and imprecise estimates of selection due to low power and small sample sizes. Combining results from multiple experimental studies might produce an aggregate estimate of selection that is more revealing than individual studies. For example, bony pelvic armour varies conspicuously among stickleback populations, and predation by vertebrate and insect predators has been hypothesized to be the main driver of this variation. Yet experimental selection studies testing these hypotheses frequently fail to find a significant effect. We experimentally manipulated length of threespine stickleback (*Gasterosteus aculeatus*) pelvic spines in a mesocosm experiment to test whether prickly sculpin (*Cottus asper*), an intraguild predator of stickleback, favours longer spines. The probability of survival was greater for stickleback with unclipped pelvic spines, but this effect was noisy and not significant. We used meta-analysis to combine the results of our mesocosm experiment with previously published experimental studies of selection on pelvic armour. We found evidence that fish predation indeed favours increased pelvic armour, with a moderate effect size. The same approach found little evidence that insect predation favours reduced pelvic armour. The causes of reduced pelvic armour in many stickleback populations remain uncertain.

Introduction

Measurements of natural selection in the wild have become commonplace (Kingsolver *et al.*, 2001). However, identifying the mechanisms of natural selection, which requires demonstrating links among the agent of selection, differential fitness and a change in trait distribution, presents a greater challenge. Furthermore, to ensure that the trait of interest is the direct target of selection requires manipulation of the trait. Observational studies of natural selection in the wild can help to identify agents of natural selection, but by themselves are correlational and provide only indirect evidence that the putative agent of selection is the cause of the change in trait distribution (Wade & Kalisz, 1990).

Correspondence: Sara E. Miller, Department of Neurobiology and
Behavior, Cornell University, Ithaca, NY, USA.
Tel.: +1 607-254-4377; Fax: +1 607-254-1303;
e-mail: miller@zoology.ubc.ca

Experimental studies of selection, in which a focal population varying in the trait of interest is exposed to a putative agent of selection, can be a valuable tool for testing the mechanisms of selection. However, selection experiments are notoriously difficult to perform, and as a result, often lack the sample size required to generate a precise estimate of the effect size. Measurements made in the wild typically report that selection on morphological traits is weak to moderate (Hoekstra *et al.*, 2001; Kingsolver *et al.*, 2001) and may fluctuate over time (Siepielski *et al.*, 2009; Morrissey & Hadfield, 2012); therefore, the effect of selection may be difficult to detect experimentally. Even if an effect is detected, wide confidence intervals for the estimate of selection on the trait of interest hinder assessment of the putative agent's importance.

Combining results from multiple experimental studies of the same or a similar agent of selection might offer a solution to these difficulties by producing an aggregate estimate of the mean selection coefficient that is more precise than any individual study (Arnqvist & Wooster,

1995; Hersch & Phillips, 2004; MacColl, 2011). Meta-analysis has been used to estimate effect size of selection on plant growth in response to agents of selection such as increased temperature, defoliation and stress (e.g. Koricheva *et al.*, 1998; Arft *et al.*, 1999; Ferraro & Oesterheld, 2002). This technique has not been widely applied to experimental measures of selection in animals (but see Møller & Thornhill, 1998; Riessen, 1999).

Here, we use this approach to evaluate the role of different predators on armour evolution in several species of stickleback fishes. Threespine and related species of stickleback display a wide range of differences among populations in bony armour and numerous other traits (Bell & Foster, 1994). Stickleback populations inhabiting similar environments frequently show parallel changes in the same traits under similar environmental conditions, suggesting that parallel trait variation may be caused by ecological factors (e.g. McKinnon & Rundle, 2002; Kaeuffer *et al.*, 2012). This link between phenotypic variation and environmental variation has made the stickleback a model organism for investigating parallel adaptive evolution.

Our study examines the mechanisms of selection on variation in pelvic armour – one of the most conspicuous phenotypic differences among stickleback populations (Bell & Foster, 1994). The stickleback pelvis is a bony structure consisting of a pelvic girdle and two hinged pelvic spines (Bell, 1988). When extended, the pelvic spines brace against the pelvic girdle, allowing them to remain 'locked' open, even after death (Reimchen, 1983). Marine and many freshwater populations have a robust pelvis with long spines. Complete loss of the pelvic structure has occurred independently in multiple freshwater populations of threespine stickleback (*Gasterosteus aculeatus*), ninespine stickleback (*Pungitius pungitius*) and brook stickleback (*Culaea inconstans*) (Nelson, 1969; Nelson & Atton, 1971; Klepaker *et al.*, 2013). Pelvic loss has a genetic basis that may have repeatedly evolved in *Gasterosteus* (Shapiro *et al.*, 2004; Chan *et al.*, 2010) and *Pungitius* (Shapiro *et al.*, 2009; Shikano *et al.*, 2013). Some populations are polymorphic in pelvic armour (Bell, 1988; Lescak *et al.*, 2013) with frequencies stable over multiple generations (Lescak *et al.*, 2013) or associated with different size classes or sexes (Reimchen & Nosil, 2002; Kitano *et al.*, 2007).

Predation by vertebrates and by insects has been hypothesized to be the main driver of variation in pelvic armour. It is theorized that long pelvic spines increase the probability of stickleback escape from attack by predatory fish and birds because they pierce the mouthparts of these vertebrate predators and increase the effective diameter of the stickleback, thereby making it more difficult for the stickleback to be swallowed (Hoogland *et al.*, 1956; Hagen & Gilbertson, 1972). Several lines of observational evidence support the hypothesis that longer pelvic spines provide protection from fish predators. In laboratory feeding

trials, pike (*Esox lucius*) preferentially consumed despined stickleback (Hoogland *et al.*, 1956). In the wild, stickleback sampled from the stomachs of trout (*Oncorhynchus clarkii*) have shorter spines than live stickleback collected using seine nets (Moodie, 1972). Lastly, increased armour and longer pelvic spines are positively correlated with density or number of species of piscivorous predatory fish (Moodie, 1972; Vamosi, 2003; Kitano *et al.*, 2008; Marchinko, 2009; Miller *et al.*, 2015).

In contrast, predation on juvenile stickleback by large aquatic insects is thought to favour reduction or loss of pelvic armour (Hoogland *et al.*, 1956; Hagen & Gilbertson, 1972; Reimchen, 1980). Dragonfly nymphs (*Aeshna* sp.) can eat 1–2 small juvenile stickleback per day (Hoogland *et al.*, 1956; Reimchen, 1980). For example, stickleback from freshwater populations, where aquatic insects are present, typically have reduced pelvic armour compared with stickleback from the sea, which lacks aquatic insects (Klepaker *et al.*, 2013). One hypothesis is that spines provide a convenient 'handhold' for insect predators to capture and hold on to stickleback (Reimchen, 1980). Consequently, stickleback with shorter or absent spines should avoid capture more easily. An alternative hypothesis is that individual stickleback with more armour grow more slowly than fish with reduced armour because investment in armour traits requires resources that would otherwise be used for growth (Marchinko & Schluter, 2007). Increased armour might thus prolong the length of time during which juvenile stickleback are small in size and most vulnerable to insect predation (Marchinko & Schluter, 2007). Lastly, more heavily armoured fish have a slower startle response, which may make them more likely to be captured by insects (Andraso & Barron, 1995).

Selection experiments testing the role of predators as agents of selection on pelvic spines have produced inconsistent results. Predatory fish more readily consume stickleback with shorter pelvic spines in some experiments (e.g. Reist, 1980a; Lescak & von Hippel, 2011), whereas other experiments show no significant effect (e.g. Reist, 1980a; MacColl & Chapman, 2011). Similarly, some experimental studies have shown that insect predators preferentially consume stickleback with longer pelvic spines (Reist, 1980b; Marchinko, 2009), whereas other experiments report nonsignificant effects or effects in the opposite direction (e.g. Lescak *et al.*, 2012; Zeller *et al.*, 2012; Mobley *et al.*, 2013). In all cases, estimates of selection on pelvic armour in these experiments are based on a small number of trials, yielding highly uncertain estimates of the effect size.

In this study, we examine the mechanism of selection on stickleback pelvic armour with an experiment and a meta-analysis. Our experiment focuses on selection on pelvic spine length resulting from predation by prickly sculpin (*Cottus asper*), an intraguild predator that

both eats stickleback and competes with stickleback for benthic prey. Stickleback populations sympatric with sculpin consistently have longer pelvic spines than stickleback from lakes in which prickly sculpin are absent (Miller *et al.*, 2015), and this variation in pelvic spine length among populations has a genetic basis (Rogers *et al.*, 2012; Miller *et al.*, 2015). However, causation cannot be inferred from this pattern alone. A previous mesocosm experiment found stickleback from a lake without sculpin had higher mortality rates from sculpin predation than stickleback from a lake with sculpin (Ingram *et al.*, 2012). However, it is not known which traits decreased mortality of fish from the sculpin-sympatric population. Although longer pelvic spines might have contributed, stickleback sympatric with sculpin possess other adaptations including genetically based differences in behaviour and body shape (Ingram *et al.*, 2012; Miller *et al.*, 2015). We carried out an experimental manipulation to test whether sculpin are a direct agent of selection on stickleback pelvic spine length.

To address the problem of low power and wide confidence intervals in selection experiments, we combined the results of our mesocosm experiment with previously published experimental studies of selection on pelvic armour. Using a meta-analysis, we determined the mean magnitude and direction of selection on pelvic armour by both fish and insect predators.

Materials and methods

Mesocosm experiment

In May 2013, experimental mesocosms were established in twenty 1136-L plastic cattle tanks 1 m deep by 2 m wide. Mesocosms were filled with water and seeded with benthic mud and zooplankton collected from nearby experimental ponds. To stimulate primary production, 0.05 g KH_2PO_4 and 1.0 g KNO_3 were added to each mesocosm. A 25-cm-diameter open-ended cylinder constructed from stiff black 7-mm plastic mesh was attached to the side of each cattle tank and suspended horizontally 0.5 m above the bottom to provide shade and a refuge from predation. Mesocosms were allowed to settle for 2 weeks prior to the addition of fish.

Adult stickleback were collected from Paq Lake and Ambrose Lake and sculpin were collected from Paq Lake using minnow traps and by dipnet. Fish were transported to 100-L holding tanks in the aquatic facility at the University of British Columbia and allowed to recover for several days. Paq and Ambrose lakes are in separate watersheds in the Sechelt Peninsula. Both lakes contain a simple fish community composed of threespine stickleback, prickly sculpin and coastal cutthroat trout.

To create variation in the length of the pelvic spine, stickleback were briefly anaesthetized in MS-222

(1 g L^{-1}) and pelvic spines were clipped to 2.5 mm [the average length of stickleback pelvic spines from lakes without sculpin (Miller *et al.*, 2015); Fig. S1]. Control stickleback were anaesthetized and handled in a similar manner but pelvic spines were not modified. Stickleback were returned to the 100-L tanks for 24 h of observation. There was no mortality following spine clipping.

The standard length of each stickleback was measured prior to introduction (36.5–60.6 mm). Four of each size-matched clipped and unclipped stickleback were added to each mesocosm (eight in total). Paq Lake stickleback were used for 10 mesocosms and Ambrose Lake stickleback were used for the remaining 10 mesocosms. Following the first set of trials, sufficient Paq Lake stickleback were available for six additional trials ($N = 26$ trials in total). A single sculpin (95–105 mm) was added to each mesocosm 2 days after the stickleback introduction. A visual survey of the number of stickleback in each mesocosm was conducted daily. Stickleback that died from causes other than sculpin predation were replaced with a similar-sized individual with the same pelvic phenotype. A trial was considered complete when half of the stickleback were consumed. At that time, the sculpin was removed and the remaining stickleback were collected. We carefully examined each stickleback for signs of injury and recorded standard length and pelvic phenotype. Over the course of the experiment, visibility in mesocosms decreased. As a result, several trials were stopped when greater or fewer than four stickleback remained. To ensure that all surviving stickleback were collected, each mesocosm was trapped with minnow traps for 48 h.

Paired *t*-tests were performed separately for Paq and Ambrose Lake mesocosms to compare the frequency of surviving clipped and unclipped stickleback. Standard length among surviving clipped and unclipped stickleback was also compared with paired *t*-tests. To avoid zero values, a 0.5 correction was added to each category (clipped survived, clipped died, unclipped survived and unclipped died) and the log odds ratio was calculated for each trial. A summary log odds ratio was estimated using the Mantel–Haenszel method (Borenstein *et al.*, 2009). A positive log odds ratio indicates stickleback with unclipped spines were more likely to survive, and a negative log odds ratio indicates increased survival of stickleback with clipped spines. We tested the relationship between sculpin body size and log odds ratio using a linear mixed-effects model with Lake as a random effect.

Comparison with other selection studies

We conducted a meta-analysis by searching the literature for experimental studies measuring selection on stickleback pelvic armour by insectivorous or piscivorous predators. We surveyed all known studies carried

out on threespine, ninespine and brook stickleback. The set of studies included four species of insect predators and four species of fish predators. Experimental studies were conducted by adding stickleback with variation in pelvic armour to a mesocosm containing a predator. Some of the studies included both predation and control treatments. However, most studies included only predator-present trials, but these were carried out in artificial settings in which the authors could assume that predation was the only source of selective mortality on the phenotype. Each study measured survival of stickleback varying in pelvic phenotype. Variation in pelvic armour was achieved by using study populations having naturally occurring variation in pelvic spine presence/absence, by physical modification of pelvic spine length or by using F2 or backcross hybrids between individuals from populations having divergent pelvic phenotypes. All experiments were relatively short-term, which limited the amount of fish growth during experimental trials. Thus, insect predation experiments tested the direct effects of armour on survival but not indirect effects of armour on survival via changes in growth.

Studies were only included in the meta-analysis if sufficient information was available to calculate the standard error of the effect size, which required multiple independent trials. Using these criteria, we excluded Ziuganov & Zotin (1995) because the study had a single replicate. We were also forced to leave out Reimchen (1980) because results from multiple replicates were pooled, which loses all information on the variance between trial outcomes. Although Reist (1980a, b) presented pooled data across replicates, the results for most trials were available in Reist (1978). When data for individual trials were not available, we contacted the authors of the original study. The authors of the studies Leinonen *et al.* (2011a), MacColl & Chapman (2011) and Mobley *et al.* (2013) generously provided raw data for individual trials. Trial data from Reimchen (1980) were no longer available.

As our measure of effect size, we used standardized mean difference in trait values, *d*, between treatments (predation – control) or after selection (survivors – before). This metric is very similar to the standardized selection differential (*i*) (equation 6.1 in Endler, 1986) except that *d* uses the pooled standard deviation across groups, whereas *i* uses the standard deviation from only the control treatment. The values of the two measures were always similar. For studies reporting a continuous measure of pelvic spine length, *d* was calculated using the formula for independent groups (Borenstein *et al.*, 2009). For studies measuring selection on the presence/absence of the pelvic structure, a log odds ratio was calculated from the proportion of survivors with and without pelvic spines/girdles in the two treatments for each trial. A correction of 0.5 was added to all experiments when studies reported zero values for any category. An overall summary log odds ratio was calculated for each experiment using the Mantel–Haenszel method (Borenstein *et al.*, 2009). Summary log odds ratios were converted to *d* to facilitate comparisons across studies (Hasselblad & Hedges, 1995). A limited number of studies also measured selection on fish body size (standard length). We computed effect size of selection in these studies using the same methods described above.

We used a random-effect meta-analysis model because experimental design, target population and stickleback species varied among studies (Borenstein *et al.*, 2009). The summary effect for the meta-analysis was calculated separately for insect and piscivorous predator experiments. Effect sizes were weighted using the inverse of the sampling variance of the experiment (Borenstein *et al.*, 2009). The random-effects model assumes that the true effect size varies from study to study (Borenstein *et al.*, 2009). The summary effect is therefore an estimate of the mean of the distribution of true effect sizes of pelvic armour. To minimize bias from the inclusion of multiple experiments from a single study, we calculated a second summary effect for each predator type using a single estimate for each study. A fixed-effects model was used to estimate the summary effect for each study. As before, a random-effect model was then used to calculate an overall summary effect across studies. A summary effect for standard length was similarly calculated with a random-effect model separately for insect and piscivorous predators. All summary effects were calculated using the 'meta' package (Schwarzer *et al.*, 2015) in the R statistical environment (R Development Core Team, 2015, v3.1.2).

Results

Mesocosm experiment

Trials took 15–50 days to reach 50% stickleback mortality. None of the surviving stickleback exhibited evidence of wounds from unsuccessful predation attempts. We found no significant difference in survival of stickleback with clipped and unclipped pelvic spines (Fig. 1, Table S1; Paq: $t = 0.75$, d.f. = 15, $P = 0.47$; Ambrose: $t = 0$, d.f. = 9, $P = 1$). The summary log odds ratio for all trials was 0.118 (95% CI: −0.358, 0.594) representing a nonsignificant 11.1% increase in survival probability for stickleback with unclipped pelvic spines, but the confidence intervals were wide. Results were similar when comparing each lake individually (Paq Lake: 0.189, 95% CI: −0.414, 0.793; Ambrose Lake: 0.00, 95% CI: −0.775, 0.775). Surviving clipped and unclipped stickleback did not differ significantly in standard length (Paq: $t = -0.09$, d.f. = 15, $P = 0.93$; Ambrose: $t = -1.45$, d.f. = 9, $P = 0.18$). There was no difference between mean standard length at the start of the experiment and mean standard length of the
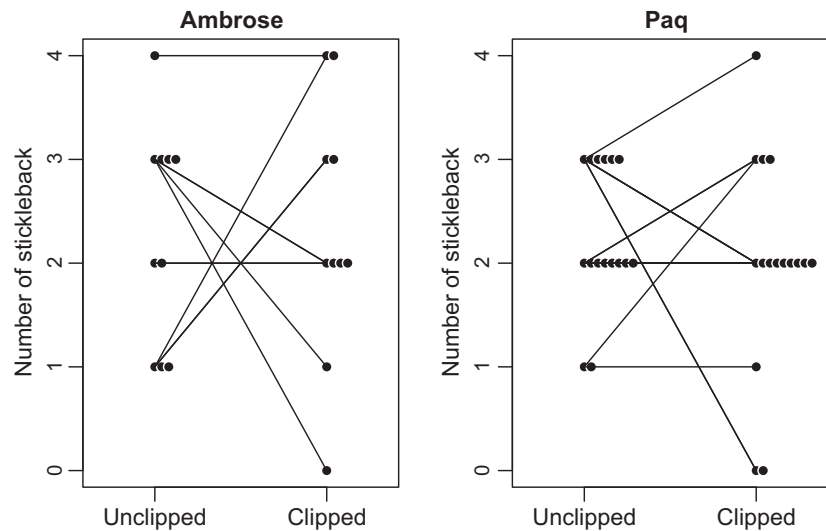
Fig. 1 Number of surviving threespine stickleback having clipped and unclipped pelvic spine treatment at the end of the mesocosm experiment (this study). Lines connect points from the same mesocosm. Trials were started with four stickleback of each phenotype.

survivors (Paq: $t = -1.19$, d.f. = 15, $P = 0.25$; Ambrose: $t = -0.48$, d.f. = 9, $P = 0.65$). Sculpin length did not affect the log odds ratio ($F_{1,23} = 0.67$, $P = 0.42$).

Meta-analysis of selection studies

We identified 25 published and unpublished experiments that met our criteria. Combined, these experiments represented 213 independent trials measuring selection on pelvic armour by fish or insect predators. A funnel plot of the distribution of effect sizes found no evidence of publication bias in insect or fish predation experiments (Fig. S2). The variance in the effect size among experiments was higher for insect predation experiments than for fish predation experiments (Fig. S2). Standard length data were available for three fish predation experiments and five insect predation experiments. Details and effect sizes for all studies are reported in Tables 1 and 2.

In fish predation experiments, longer pelvic spines significantly increased stickleback survival (Fig. 2), with a mean effect size of 0.12 (95% CI: 0.02, 0.23; $P = 0.02$; $n = 14$ experiments). This represents an increase in the mean pelvic spine length by 0.12 standard deviations in the presence of fish predators, with a confidence interval of moderate width (95% CI 0.02–0.23). Only one of the 14 individual experiments was statistically significant (Table 1; Fig. 2) suggesting that low power is a chronic issue in such selection experiments. Using a single estimate for each study ($n = 5$ studies) yielded similar results, with a summary mean effect of 0.14 (Fig. S3; 95% CI: 0.003, 0.27; $P = 0.05$).

The estimated mean effect of insect predation on pelvic spines was weak (-0.06; 95% CI: -0.31, 0.19; $P = 0.63$; $n = 11$ experiments) and not statistically significant (Fig. 3). Two of the 11 individual experiments reported a statistically significant effect, but one of

them was in the opposite direction of the summary effect. Even if an effect is real, a high proportion of significant outcomes are predicted to be in the wrong direction when experiments have low power (Gelman, 2014). The summary mean effect was 0.04 (95% CI: -0.19, 0.28; $P = 0.71$) when we use the single estimate for each study (Fig. S4; $n = 6$ studies).

In populations with natural trait variation, pelvic spine length is correlated with variation in other traits, and consequently, it is impossible to distinguish between direct selection for pelvic spines and indirect selection on a correlated trait such as body size in these experiments. For studies reporting standard length, only Zeller *et al.* (2012) used natural populations, whereas the remaining studies used physical modification or artificial crosses. Fish predators had no significant effect on standard length in these experiments (Fig. S5). The summary mean effect of fish predation on standard length was 0.09 (95% CI: -0.72, 0.90; $P = 0.83$). Insect predators preferentially consumed smaller fish (Fig. S5), with a summary mean effect of 0.27 (95% CI: -0.14, 0.67; $P = 0.20$), but the estimate was highly uncertain and the effect was not significant.

Discussion

Experiments that manipulate putative agents and targets of selection are one of the best methods for understanding the mechanisms of selection. When selection experiments are limited by low power and yield imprecise estimates of selection, meta-analysis can be useful to provide an overall test of the aggregate estimate of selection. The results of our experiment and meta-analysis of predation's effect on pelvic armour in stickleback suggest that fish predation favours longer pelvic spines in stickleback. The magnitude of this selection was small to moderate. At this time, we are unable to

Table 1 Experimental studies of selection on stickleback pelvic armour and body size (standard length) by piscivorous predators. Variation in the length of pelvic spines (source of variation) was obtained from populations with naturally occurring variation, by creating crosses between populations with divergent pelvic spine lengths, or by experimentally modifying spines. All effect sizes were converted to the standardized mean difference (d).

Author (year)	Experiment	Species	Source	Population	Predator	Traits	Size (mm)	Trials	Fish	Log odds	95% CI	d	d 95% CI	d length	d 95% CI length
Reist (1980a)	A	Culea inconstans	Wild caught	Wakomao Lake, AB	Esox lucius	PS	20–29.9	7	205	0.264	−0.292, 0.820	0.146	−0.161, 0.452		
	B	Culea inconstans	Wild caught	Wakomao Lake, AB	Esox lucius	PS	30–39.9	6	170	−0.321	−0.929, 0.287	−0.177	−0.512, 0.158		
	C	Culea inconstans	Wild caught	Wakomao Lake, AB	Esox lucius	PS	40–49.9	6	171	0.484	−0.116, 1.083	0.267	−0.064, 0.597		
	D	Culea inconstans	Wild caught	Wakomao Lake, AB	Esox lucius	PS	20–29.9	4	115	0.298	−0.422, 1.018	0.165	−0.232, 0.561		
	E	Culea inconstans	Wild caught	Wakomao Lake, AB	Esox lucius	PS	30–39.9	5	150	−0.053	−0.689, 0.584	−0.029	−0.380, 0.322		
	F	Culea inconstans	Wild caught	Wakomao Lake, AB	Esox lucius	PS	40–49.9	4	115	−0.178	−0.910, 0.554	−0.098	−0.502, 0.306		
	G	Culea inconstans	Modified wild caught	Wakomao Lake, AB	Esox lucius	PS	30–39.9	2	60	0.811	−0.221, 1.844	0.447	−0.123, 1.017		
	H	Culea inconstans	Modified wild caught	Wakomao Lake, AB	Esox lucius	PS	40–49.9	2	60	−0.132	−1.14, 0.876	−0.073	−0.629, 0.483		
Ziuganov & Zotin (1995)	Fish	Pungitius pungitius	Wild caught	Levin Navolok, Russia	Perca fluviatilis	PG	51–62	1*	200	1.800		0.993			
Lescak & von Hippel (2011)	Fish	Gasterosteus aculeatus	Wild caught	Wallace Lake, AK	Oncorhynchus mykiss	PG	37–45	26	260	0.651	**0.221, 1.081**	0.359	**0.122, 0.596**		
Leinonen et al. (2011a)	No refuge	Gasterosteus aculeatus	Half-sib crosses	Baltic Sea	Esox lucius	PS, SL	6 months	2	325			−0.053	−2.014, 1.907	0.014	−1.945, 1.974
	Refuge	Gasterosteus aculeatus	Half-sib crosses	Baltic Sea	Esox lucius	PS, SL	6 months	2	325			−0.017	−1.977, 1.943	0.694	−1.324, 2.712
MacColl & Chapman (2011)		Gasterosteus aculeatus	F2	Marine × Hoggan, BC	Cottus asper	PS, SL	33.2–43.3	16	160			−0.037	−1.025, 0.950	−0.034	−1.022, 0.954
This study	Paq	Gasterosteus aculeatus	Modified wild caught	Paq Lake, BC	Cottus asper	PS	36–51	16	128	0.197	−0.418, 0.812	0.108	−0.231, 0.447		
	Ambrose	Gasterosteus aculeatus	Modified wild caught	Ambrose Lake, BC	Cottus asper	PS	41–63	10	80	0.00	−0.766, 0.766	0.00	−0.422, 0.422		

Trait abbreviations are as follows: PG, presence/absence of pelvic girdle; PS, pelvic spine length; SL, standard length. Significant effect sizes are given in bold. Unreplicated experiments were excluded from the meta-analysis (*).

Table 2 Experimental studies of selection on stickleback pelvic armour and body size (standard length) by insect predators. Variation in the length of pelvic spines (source of variation) was obtained from populations with naturally occurring variation, by creating crosses between populations with divergent pelvic spine lengths, or by experimentally modifying spines. All effect sizes were converted to the standardized mean difference (d).

Author (year)	Experiment	Species	Source	Population	Predator	Traits	Size (mm)	Trials	Fish	Log odds	95% CI	d	d 95% CI	d length	d 95% CI length
Reist (1980b)	I	*Culea inconstans*	Wild caught	Wakomao Lake, AB	*Lethocerus americanus*	PS	20–29.9	11	149	0.302	−0.322, 0.927	0.166	−0.178, 0.511		
	J	*Culea inconstans*	Wild caught	Wakomao Lake, AB	*Lethocerus americanus*	PS	30–39.9	4	53	−0.282	−1.326, 0.762	−0.155	−0.731, 0.420		
	K	*Culea inconstans*	Wild caught	Wakomao Lake, AB	*Dysticus* spp.	PS	20–29.9	7	98	−0.999	**−1.816, −0.181**	−0.551	**−1.001, −0.100**		
	L	*Culea inconstans*	Wild caught	Wakomao Lake, AB	*Dysticus* spp.	PS	20–29.9	5	69	−0.391	−1.337, 0.555	−0.216	−0.737, 0.306		
	M	*Culea inconstans*	Wild caught	Wakomao Lake, AB	*Dysticus* spp.	PS	30–39.9	1*	14	0.575		0.317			
	N	*Culea inconstans*	Wild caught	Wakomao Lake, AB	*Aeshna* spp.	PS	20–29.9	1*	11	2.99		1.646			
	O	*Culea inconstans*	Wild caught	Wakomao Lake, AB	*Aeshna* spp.	PS	20–29.9	7†	91	−0.308		−0.170			
Reimchen (1980)		*Gasterosteus aculeatus*	Wild caught	Boulton Lake, BC	*Aeshna* spp.	PG	15–25	7†	408	−0.159		−0.088			
Ziuganov & Zotin (1995)	Insect	*Pungitius pungitius*	Wild caught	Levin Navolok, Russia	*Odonata* spp. *Dysticus* spp.	PG	51–62	1*	200	−1.520		−0.838			
Marchinko (2009)	Paxton	*Gasterosteus aculeatus*	F2 hybrids	Paxton × Marine	*Aeshna* spp. *Notonecta* spp.	PS, SL	10–18	6	477			−0.100	−1.232, 1.032	0.209	−0.67, 1.09
	McKay	*Gasterosteus aculeatus*	F2 hybrids	McKay × Marine	*Aeshna* spp. *Notonecta* spp.	PS, SL	10–23	10	767			−0.842	−1.757, 0.073	0.689	−0.476, 1.85
Barrueto (2009)	1	*Gasterosteus aculeatus*	Modified lab raised	Salmon River	*Notonecta* spp.	PS, SL	11–22	7	423			−0.888	−1.986, 0.210	−0.061	−1.11, 0.987
	2	*Gasterosteus aculeatus*	Backcrosses	Paxton Lake, BC	*Notonecta* spp.	PG, SL	9–18	8	573			0.538	−0.460, 1.536	0.642	−0.305, 1.59
Lescak et al. (2012)		*Gasterosteus aculeatus*	Wild caught	Wallace Lake, AK	*Aeshna* spp.	PG	23–57	11	220	0.637	**0.111, 1.163**	0.351	**0.061, 0.641**		
Zeller et al. (2012)		*Gasterosteus aculeatus*	Wild caught	Bern, Switzerland	*Aeshna* spp.	PS, SL	Adults	16	960			0.272	−0.424, 0.968	0.093	−0.242, 0.818
Mobley et al. (2013)		*Pungitius pungitius*	Modified wild caught	Bothnian Bay, Sweden	*Aeshna* spp.	PS	20–29.9	20	200	−0.028	−0.497, 0.442	−0.015	−0.274, 0.243		

Trait abbreviations are as follows: PG, presence/absence of pelvic girdle; PS, pelvic spine length; SL, standard length. Significant effect sizes are given in bold. Experiments were excluded from the meta-analysis when unreplicated (*) or when results from multiple trials were presented as pooled data (†).
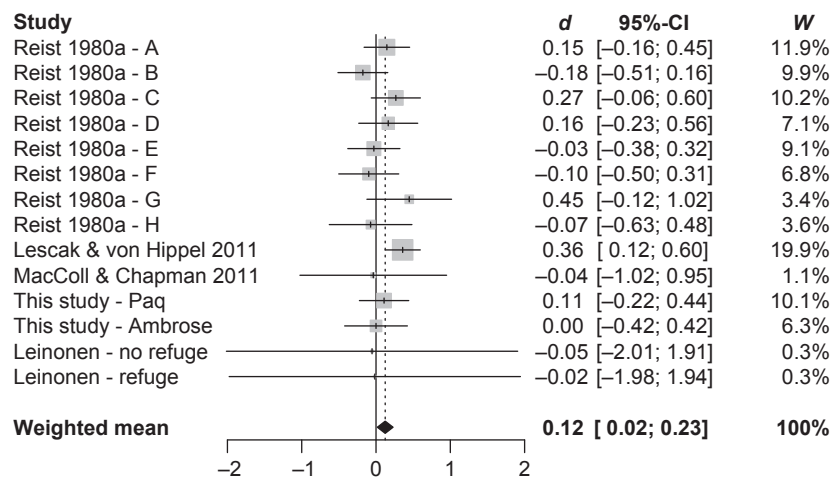
Fig. 2 Forest plot of the effect size of all fish predation experiments on pelvic armour. The centre of the grey box indicates the mean of the effect size (*d*) for each experiment, and the area of the grey box is proportional to the weight of that study in the meta-analysis. The horizontal lines span the 95% confidence interval of the effect. The weighted mean was calculated using a random-effects model. *W* is the weight of the study in the model. The weighted mean is indicated by the vertical dotted line, and the 95% confidence interval of this estimate is contained within the black diamond.
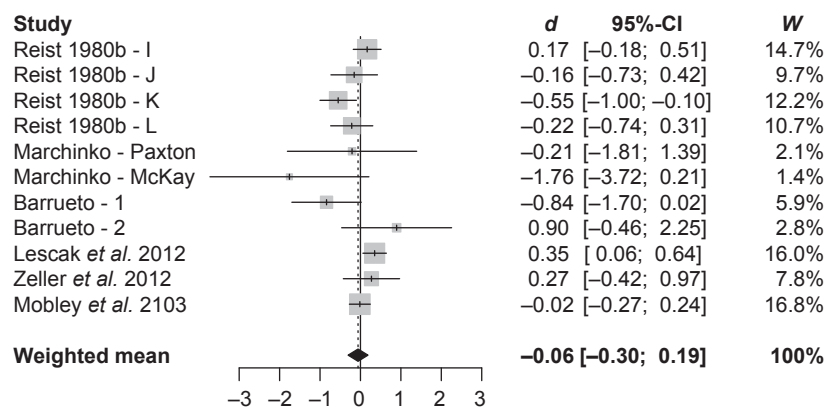

Fig. 3 Forest plot of the effect size of all insect predation experiments on pelvic armour. The centre of the grey box indicates the mean of the effect size (*d*) for each experiment, and the area of the grey box is proportional to the weight of that study in the meta-analysis. The horizontal lines span the 95% confidence interval of the effect. The weighted mean was calculated using a random-effects model. *W* is the weight of the study in the model. The weighted mean is indicated by the vertical dotted line, and the 95% confidence interval of this estimate is contained within the black diamond.

support or reject the hypothesis that insect predators favour reduced pelvic armour.

Prickly sculpin have been hypothesized as an agent of selection for longer pelvic spines. We tested this hypothesis by experimentally modifying the length of pelvic spines and measuring differential mortality between stickleback with clipped and unmodified pelvic spines. We observed an 11% increase in the probability of survival for stickleback with unclipped pelvic spines. However, the confidence intervals for this estimate overlapped with zero and this effect was not statistically significant. From these data alone, we were unable to

conclude that prickly sculpin preferentially consumed stickleback with shortened pelvic spines.

There are three possible reasons for the failure to detect selection on pelvic spine length from prickly sculpin predation. (i) Sculpin are not an agent of selection on this trait. In this case, perhaps other predators, such as coastal cutthroat trout or birds, could favour longer pelvic spines in lakes containing sculpin. Lakes without sculpin also contain these predators, but it is conceivable that sculpin predation indirectly increases predation and selection on stickleback by these other predators by apparent competition or by habitat shift

(Miller *et al.*, 2015). (ii) The experiment was flawed. A challenge of selection experiments is choosing the correct size class of both the agent and target of selection (Endler, 1986). Natural selection could favour longer pelvic spines only when sculpin and stickleback belong to specific size classes other than those used in the experiment. The current experiment used adult sculpin near the upper limit of the size range of sculpin in Paq Lake (personal observation), whereas smaller sculpin might be more gape-limited and hence a more effective agent of selection on armour in the size class of stickleback used here. Additionally, spine manipulations may not have been successful because spines were all clipped to the same length rather than scaled to body size. Lastly, mesocosms may not effectively replicate predation in a realistic environmental setting. (iii) Natural selection favoured longer pelvic spines, but we were unable to detect an effect because our experiment was underpowered (type II error). We observed a trend towards increased survival of stickleback with unclipped pelvic spines, but as in most other experiments of this kind (Fig. 2), this result was not statistically significant and confidence intervals for treatment effects were large.

Partly to overcome the lower power of individual studies, we compiled a meta-analysis of experimental studies of selection on pelvic armour from insect and fish predators. We found that fish predators indeed selected for longer pelvic spines, with a summary effect size of 0.12 units of a standard deviation, even though only one of 14 individual experiments (including our own) yielded an effect size that excluded zero, and despite the heterogeneity among studies in species and design. This value represents a small-to-moderate effect on fitness and is comparable to 0.14, the mean absolute value for the standardized linear selection differential obtained by Kingsolver *et al.* (2001). This effect size is nevertheless potentially evolutionarily significant. If we assume that pelvic spine length has a heritability of 0.38 (Leinonen *et al.*, 2011b) and that selection acts consistently with a coefficient somewhere between the lower and upper limit of our 95% confidence interval, we predict that the mean pelvic spine length would increase by one standard deviation in 19–165 generations. The effect size was similar when experiments were combined into a single estimate for each study. On the basis of our meta-analysis, and in agreement with observational studies, we conclude that fish predators are an agent of selection favouring increased pelvic armour. However, due to the heterogeneity of study designs, we cannot rule out the possibility that selection is acting upon a correlated trait rather than direct selection for increased pelvic spines.

In contrast, it is still unclear whether insect predators are an agent of direct selection favouring reduced stickleback pelvic spines. The summary effect size indicated a very small increase in survival for stickleback with shorter pelvic spines. However, the large confidence interval for this estimate ranges from −0.31 to 0.19, preventing us from ruling out selection for either increased or decreased pelvic armour by insects. Although Reimchen's (1980) hypothesis has been frequently cited, there is as of yet no convincing experimental evidence in support of insect predators selecting for reduced pelvic armour by the 'handhold' or other mechanisms.

Studies included in the meta-analysis measured selection at a range of body sizes in multiple stickleback species for several species of insect predator. This variation in methodology may obscure the effect of insect predation. For example, Lescak *et al.* (2012) observed that dragonfly naiads preferentially consumed stickleback with the most pelvic armour when the fish were smaller than the dragonfly but preferred stickleback without pelvic armour when the fish was larger than the dragonfly. Different species of insect predators may also vary in the strength or direction of selection upon pelvic spine length. This meta-analysis lacked sufficient experimental studies of each insect species to test for variation in selection among insect species or among stickleback of different size classes.

These uncertainties leave open the question of what is the selective mechanism underlying loss or reduction of the pelvic armour in many stickleback populations. Previously stated hypotheses may be correct, but tests of these hypotheses remain underpowered. Several alternative hypotheses may explain reduction in pelvic spine length. Indirect selection against pelvic spines may occur because investment in armour reduces availability of energy and minerals such as calcium or phosphorus for growth (Giles, 1983). Stickleback are primarily eaten by insects when they are small; therefore, a slower growth rate increases the length of time in which stickleback are vulnerable to insect predation. Direct selection for increased growth rate could lead to indirect selection for decreased armour. However, support for this 'ion limitation hypothesis' is mixed. The summary effect of insect predation on standard length revealed that insects preferred to consume smaller stickleback. This effect was not significant, but due to the reduced number of studies measuring this trait, our power to detect an effect was limited. Marchinko & Schluter (2007) raised stickleback with differing numbers of lateral plates, another type of bony armour, in freshwater and saltwater. Supporting the ion limitation hypothesis, in freshwater, stickleback with more lateral plates grew more slowly compared to stickleback with fewer lateral plates. However, a later study by Rollins *et al.* (2014) did not detect higher growth rate of stickleback with decreased pelvic armour in the laboratory or in the wild, suggesting that ion limitation may vary among traits or that the effect was too small to detect in that study. In an observational study in Alaskan lakes, Bell *et al.* (1993) found that pelvic reduction was associated with low calcium concentrations when

predatory fish were absent from the lakes, but not when native piscivorous predators were present. However, pelvic reduction has been observed in Canadian lakes with high concentrations of calcium (Klepaker *et al.*, 2013). Other hypotheses have proposed that reduced pelvic armour increases buoyancy (Myhre & Klepaker, 2009) and manoeuvrability (Reimchen, 2000). Investigation of these potential alternative mechanisms of selection will require future experimental studies.

Meta-analysis has the potential to be a powerful method for analysing experimental studies of selection in other organisms. We have demonstrated that combining multiple studies can yield a significant summary estimate of the effect of selection even when individual studies lack power and precision. A single experimental selection study produces an effect size for a given agent of selection under specific environmental conditions. Such estimates are often noisy with large confidence intervals. In contrast, a summary effect estimates the mean of the distribution of the true effect sizes. Consequently, the summary effect may have lower sampling error than individual studies, and is useful as an overall estimate of selection. For a selection study to be included in a meta-analysis, it must be possible to calculate the standard error of the effect size for the study. We encourage future selection experimenters to include multiple independent trials in their experiments, to report experiments with nonsignificant results and to make the results of each individual trial available in the paper or in online data repositories.

Acknowledgments

References

Andraso, G.M. & Barron, J.N. 1995. Evidence for a trade-off between defensive morphology and startle-response performance in the brook stickleback (*Culaea inconstans*). *Can. J. Zool.* **73**: 1147–1153.

Arft, A.M., Walker, M.D., Gurevitch, J.E.A., Alatalo, J.M., Bret-Harte, M.S., Dale, M. *et al.* 1999. Responses of tundra plants to experimental warming: meta-analysis of the international tundra experiment. *Ecol. Monogr.* **69**: 491–511.

Arnqvist, G. & Wooster, D. 1995. Meta-analysis: synthesizing research findings in ecology and evolution. *Trends Ecol. Evol.* **10**: 236–240.

Barrueto, M. 2009. Adaptive significance of pelvic girdle loss in threespine stickleback. (M.S. Thesis). University of British Columbia, Vancouver, BC.

Bell, M.A. 1988. Stickleback fishes: bridging the gap between population biology and paleobiology. *Trends Ecol. Evol.* **3**: 320–324.

Bell, M.A., Foster, S.A., eds) 1994. *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press, Oxford, UK.

Bell, M.A., Orti, G., Walker, J.A. & Koenings, J.P. 1993. Evolution of pelvic reduction in threespine stickleback fish: a test of competing hypotheses. *Evolution* 906–914.

Borenstein, M., Hedges, L.V., Higgins, J.P.T. & Rothstein, H.R. 2009. *Introduction to Meta-Analysis*. Wiley and Sons Ltd, Chichester, UK.

Chan, Y.F., Marks, M.E., Jones, F.C., Villarreal, G., Shapiro, M.D., Brady, S.D. *et al.* 2010. Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. *Science* **327**: 302–305.

Endler, J.A. 1986. *Natural Selection in the Wild*. Princeton University Press, Princeton, NJ.

Ferraro, D.O. & Oesterheld, M. 2002. Effect of defoliation on grass growth. A quantitative review. *Oikos* **98**: 125–133.

Gelman, A. 2014. "This is what 'power=0.06' looks like. Get used to it." http://andrewgelman.com/2014/11/17/power-06-looks-like-get-used/. Accessed January 11, 2017.

Giles, N. 1983. The possible role of environmental calcum levels during the evolution of phenotypic diversity in Outer Hebridean populations of the Three-spined stickleback, Gasterosteus aculeatus. *J. Zool.* **199**: 535–544.

Hagen, D. & Gilbertson, L. 1972. Geographic variation and environmental selection in *Gasterosteus aculeatus* L. in the Pacific Northwest, America. *Evolution* **199**: 32–51.

Hasselblad, V. & Hedges, L.V. 1995. Meta-analysis of screening and diagnostic tests. *Psychol. Bull.* **117**: 167–178.

Hersch, E.I. & Phillips, P.C. 2004. Power and potential bias in field studies of natural selection. *Evolution* **58**: 479–485.

Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hoang, A., Hill, C.E. *et al.* 2001. Strength and tempo of directional selection in the wild. *Proc. Natl. Acad. Sci. USA* **98**: 9157–9160.

Hoogland, R., Morris, D. & Tinbergen, N. 1956. The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as means of defence against predators (*Perca* and *Esox*). *Behaviour* **10**: 205–236.

Ingram, T., Svanbäck, R., Kraft, N.J.B., Kratina, P., Southcott, L. & Schluter, D. 2012. Intraguild predation drives evolutionary niche shift in threespine stickleback. *Evolution* **66**: 1819–1832.

Kaeuffer, R., Peichel, C.L., Bolnick, D.I. & Hendry, A.P. 2012. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. *Evolution* **66**: 402–418.

Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hill, C.E. *et al.* 2001. The strength of phenotypic selection in natural populations. *Am. Nat.* **157**: 245–261.

Kitano, J., Mori, S. & Peichel, C.L. 2007. Sexual dimorphism in the external morphology of the threespine stickleback (*Gasterosteus aculeatus*). *Copeia* **2007**: 336–349.

Kitano, J., Bolnick, D.I., Beauchamp, D.A., Mazur, M.M., Mori, S., Nakano, T. *et al.* 2008. Reverse evolution of armor plates in the threespine stickleback. *Curr. Biol.* **18**: 769–774.

Klepaker, T., Østbye, K. & Bell, M.A. 2013. Regressive evolution of the pelvic complex in stickleback fishes: a study of convergent evolution. *Evol. Ecol. Res.* **15**: 413–435.

Koricheva, J., Larsson, S. & Haukioja, E. 1998. Insect performance on experimentally stressed woody plants: a meta-analysis. *Annu. Rev. Entomol.* **43**: 195–216.

Leinonen, T., Herczeg, G., Cano, J.M. & Merilä, J. 2011a. Predation-imposed selection on threespine stickleback (*Gasterosteus aculeatus*) morphology: a test of the refuge use hypothesis. *Evolution* **65**: 2916–2926.

Leinonen, T., Cano, J.M. & Merilä, J. 2011b. Genetics of body shape and armour variation in threespine sticklebacks. *J. Evol. Biol.* **24**: 206–218.

Lescak, E.A. & von Hippel, F. 2011. Selective predation of threespine stickleback by rainbow trout. *Ecol. Freshw. Fish.* **20**: 308–314.

Lescak, E.A., von Hippel, F., Lohman, B.K. & Sherbick, M.L. 2012. Predation of threespine stickleback by dragonfly naiads. *Ecol. Freshw. Fish.* **21**: 581–587.

Lescak, E.A., von Hippel, F., Bernhardt, R.R. & Bell, M.A. 2013. Pelvic girdle reduction and asymmetry in threespine stickleback from Wallace Lake, Alaska. *Evol. Ecol. Res.* **15**: 155–170.

MacColl, A.D.C. 2011. The ecological causes of evolution. *Trends Ecol. Evol.* **26**: 514–522.

MacColl, A.D. & Chapman, S.M. 2011. A benthic predatory fish does not cause selection on armour traits in three-spined stickleback *Gasterosteus aculeatus* (Gasterosteiformes: Gasterosteidae). *Biol. J. Linn. Soc.* **104**: 877–885.

Marchinko, K.B. 2009. Predation's role in repeated phenotypic and genetic divergence of armor in threespine stickleback. *Evolution* **63**: 127–138.

Marchinko, K.B. & Schluter, D. 2007. Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. *Evolution* **61**: 1084–1090.

McKinnon, J. & Rundle, H.D. 2002. Speciation in nature: the threespine stickleback model systems. *Trends Ecol. Evol.* **17**: 480–488.

Miller, S.E., Metcalf, D. & Schluter, D. 2015. Intraguild predation leads to genetically based character shifts in the threespine stickleback. *Evolution* **69**: 3194–3203.

Mobley, K.B., Ruiz, R.C., Johansson, F., Englund, G. & Bokma, F. 2013. No evidence that stickleback spines directly increase risk of predation by an invertebrate predator. *Evol. Ecol. Res.* **15**: 189–198.

Møller, A.P. & Thornhill, R. 1998. Bilateral symmetry and sexual selection: a meta-analysis. *Am. Nat.* **151**: 174–192.

Moodie, G. 1972. Predation, natural selection and adaptation in an unusual threespine stickleback. *Heredity* **28**: 155–167.

Morrissey, M.B. & Hadfield, J.D. 2012. Directional selection in temporally replicated studies is remarkably consistent. *Evolution* **66**: 435–442.

Myhre, F. & Klepaker, T. 2009. Body armour and lateral-plate reduction in freshwater three-spined stickleback *Gasterosteus aculeatus*: adaptations to a different buoyancy regime? *J. Fish Biol.* **75**: 2062–2074.

Nelson, J.S. 1969. Geographic variation in the brook stickleback, *Culaea inconstans*, and notes on nomenclature and distribution. *J. Fish. Board Can.* **26**: 2431–2447.

Nelson, J.S. & Atton, F.M. 1971. Geographic and morphological variation in the presence and absence of the pelvic skeleton in the brook stickleback, *Culaea inconstans* (Kirtland), in Alberta and Saskatchewan. *Can. J. Zool.* **49**: 343–352.

R Core Team 2015. *R: A Language and Environment for Statistical Computing*. R foundation for statistical computing, Vienna, Austria.

Reimchen, T.E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators? *Can. J. Zool.* **58**: 1232–1244.

Reimchen, T.E. 1983. Structural relationships between spines and lateral plates in threespine stickleback (*Gasterosteus aculeatus*). *Evolution* **37**: 931–946.

Reimchen, T.E. 2000. Predator handling failures of lateral plate morphs in *Gasterosteus aculeatus*: functional implications for the ancestral plate condition. *Behaviour* **137**: 1081–1096.

Reimchen, T.E. & Nosil, P. 2002. Temporal variation in divergent selection on spine number in threespine stickleback. *Evolution* **56**: 2472–2483.

Reist, J.D. 1978. Predation as a factor in maintaining the pelvic polymorphism in a central Alberta population of *Culaea inconstans* (Kirtland), (Pisces: Gasterosteidae). (M.S. Thesis). University of Alberta, Calgary, AB.

Reist, J.D. 1980a. Selective predation upon pelvic phenotypes of brook stickleback, *Culaea inconstans*, by northern pike, *Esox lucius*. *Can. J. Zool.* **58**: 1245–1252.

Reist, J.D. 1980b. Predation upon pelvic phenotypes of brook stickleback, culaea-inconstans, by selected invertebrates. *Can. J. Zool.* **58**: 1253–1258.

Riessen, H.P. 1999. Predator-induced life history shifts in Daphnia: a synthesis of studies using meta-analysis. *Can. J. Fish Aquat. Sci.* **56**: 2487–2494.

Rogers, S.M., Tamkee, P., Summers, B., Balabahadra, S., Marks, M., Kingsley, D.M. *et al.* 2012. Genetic signature of adaptive peak shift in threespine stickleback. *Evolution* **66**: 2439–2450.

Rollins, J.L., Lohman, B.K. & Bell, M.A. 2014. Does ion limitation select for pelvic reduction in threespine stickleback (*Gasterosteus aculeatus*)? *Evol. Ecol.* **16**: 101–120.

Schwarzer, G., Carpenter, J.R. & Rücker, G. 2015. Meta-analysis with R. Springer International Publishing, Switzerland.

Shapiro, M.D., Marks, M.E., Peichel, C.L., Blackman, B.K., Nereng, K.S., Jónsson, B. *et al.* 2004. Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature* **428**: 717–723.

Shapiro, M.D., Summers, B.R., Balabhadra, S., Aldenhoven, J.T., Miller, A.L., Cunningham, C.B. *et al.* 2009. The genetic architecture of skeletal convergence and sex determination in ninespine sticklebacks. *Curr. Biol.* **19**: 1140–1145.

Shikano, T., Laine, V.N., Herczeg, G., Vilkki, J. & Merilä, J. 2013. Genetic architecture of parallel pelvic reduction in ninespine sticklebacks. *G3 (Bethesda)* **3**: 1833–1842.

Siepielski, A.M., DiBattista, J.D. & Carlson, S.M. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecol. Lett.* **12**: 1261–1276.

Vamosi, S. 2003. The presence of other fish species affects speciation in threespine sticklebacks. *Evol. Ecol. Res.* **5**: 717–730.

Wade, M.J. & Kalisz, S. 1990. The causes of natural selection. *Evolution* **26**: 44: 1947.

Zeller, M., Lucek, K., Haesler, M., Seehausen, O. & Sivasundar, A. 2012. Little evidence for a selective advantage of armour-reduced threespined stickleback individuals in an invertebrate predation experiment. *Evol. Ecol.* **26**: 1293–1309.

Ziuganov, V.V. & Zotin, A.A. 1995. Pelvic girdle polymorphism and reproductive barriers in the ninespine stickleback *Pungitius pungitius* (L.) from northwest Russia. *Behaviour* **132**: 1095–1105.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Length of size corrected pelvic spine length for wild fish from Paq and Ambrose lakes.

Figure S2 Funnel plot of standard error and the effect size of (a) fish predation experiments and (b) insect predation experiments used in the meta-analysis.

Figure S3 Forest plot of the effect size of all fish predation experiments on pelvic armor.

Figure S4 Forest plot of the effect size of all insect predation studies on pelvic armor.

Figure S5 Forest plot of the effect size of all predation experiments on body size (standard length).

Table S1 Data from mesocosm experimental trials.