



A benthic predatory fish does not cause selection on armour traits in three-spined stickleback *Gasterosteus aculeatus* (Gasterosteiformes: Gasterosteidae)

ANDREW D. C. MACCOLL^{1,2*} and SONIA M. CHAPMAN^{2†}

¹Department of Zoology, 6270 University Boulevard, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

²School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD, UK

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Predation can promote divergence between prey populations and contribute to ecological speciation. In theory, predators can also constrain prey population divergence. In coastal British Columbia, Canada, *Gasterosteus aculeatus* (three-spined stickleback) species pairs only occur in lakes with a single species of predatory fish: *Oncorhynchus clarkii* (the cutthroat trout). Similar lakes containing additional predatory fish species (*Cottus asper*, prickly sculpins; *Oncorhynchus mykiss*, rainbow trout) contain only single species of morphologically intermediate stickleback, suggesting that these predators prevent the coexistence of stickleback species pairs. We conducted a mesocosm experiment to investigate how prickly sculpins might constrain divergence, by quantifying their impact on survival and natural selection on antipredator (armour) traits in F₂ stickleback from a cross between ecologically divergent populations. We tested three hypotheses: (1) sculpin predation on sticklebacks reduces survival in a way that could result in their exclusion from certain niches; (2) sculpins compete with stickleback; (3) sculpins respond to prey vulnerabilities in similar ways to cutthroat trout, tending to constrain rather than to enhance divergence. We found that sculpins significantly reduce stickleback survival, that their presence per se does not reduce growth in stickleback, and that predation did not result in selection on any of the armour traits measured, or on gill raker length, which is an important trophic trait. These results tend to refute hypotheses (2) and (3), while supporting hypothesis (1). © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, 104, 877–885.

ADDITIONAL KEYWORDS: adaptive radiation – *Cottus asper* – mesocosm experiment – natural enemies – natural selection – predation – sculpin – selection differential.

INTRODUCTION

The ecological theory of adaptive radiation states that phenotypic differentiation between populations results from differences in natural selection between environments (Schluter, 2000). Traditionally the ecological theory has concentrated on the role of competition, and the associated evolution of trophic traits, as the predominant cause and form of divergence between populations. Recently there has been a reinvigoration of interest in predation as an agent of

divergent selection (Vamosi, 2005). In an early reference, Worthington (1937, 1940) suggested that predators might prevent evolutionary transitions between niches, and therefore circumscribe adaptive radiation. Fryer & Iles (1955) and Fryer (1959) rejected this, and empirical work since then has focused on how varying levels of predation in different environments promote divergence (McPhail, 1969; Nosil & Crespi, 2006; Marchinko, 2009). However, theory suggests that predation can also cause convergence of closely related prey species, leading to a reduction of diversity (Abrams, 2000).

In *Gasterosteus aculeatus* L. (three-spined stickleback) it has been repeatedly suggested that variation in predation between populations can contribute to variation in antipredator (armour) traits (Moodie,

*Corresponding author.

E-mail: andrew.maccoll@nottingham.ac.uk

†Present address: School of Psychology, Washington Singer Laboratories, Perry Road, Exeter, EX4 4QG, UK

1972; Gross, 1978; Reimchen, 1992, 1994). It has also been suggested that predation can play a direct role in strengthening the divergence of sympatric species pairs of sticklebacks (Vamosi & Schluter, 2002, 2004; Rundle, Vamosi & Schluter, 2003). However, Vamosi (2003) identified an apparent anomaly in the role of predation when he showed that sympatric species pairs only occur in lakes of coastal British Columbia (BC) that contain *Oncorhynchus clarki* (cutthroat trout) as the only other fish species. Ecologically matched lakes that contain additional predatory fish species (*Cottus asper*, prickly sculpin; *Oncorhynchus mykiss*, rainbow trout) only support a single species of morphologically intermediate three-spined stickleback.

This result is anomalous, because the most relevant theory (Abrams, 2000) suggests that additional predators should increase the probability of divergence between closely related prey species. Possible reasons why an increase in the number of predators might restrict divergence of stickleback are suggested by theory and by Vamosi (2003, 2005), and are outlined below.

1. Worthington's (1937) original suggestion was that predation might prevent the diversification of prey, by suppressing the population growth rate, resulting in exclusion from certain niches or reduced divergent selection (Abrams, 1986, 2000). The 'double invasion' model for the evolution of the stickleback species pairs in BC (Rundle & Schluter, 2004) proposes that the lakes were invaded by two separate waves of ancestral anadromous fish. Between invasions it is suggested that the first wave had begun to evolve into the benthic form by adaptation to the (more profitable) littoral niche. In lakes with prickly sculpin, occupation of the littoral habitat by three-spined stickleback may have been prevented if sculpins are effective predators of sticklebacks.
2. Sculpins prevented sticklebacks occupying the littoral niche by competition rather than predation (Vamosi, 2003).
3. Sculpins respond to prey vulnerabilities in similar ways to cutthroat trout, tending to produce convergence, rather than divergence, of prey species (Abrams, 2000).
4. Additional fish species changed the ecology or evolution of the original predator so that its impacts on stickleback were changed (Abrams, 2000).

Here we take a reductionist approach to examine the impact that prickly sculpins might have on divergence in three-spined stickleback, as a first experimental step to understanding the role of additional fish species on stickleback divergence.

Stickleback F₂ offspring, from crosses between an anadromous (heavily armoured) and a benthic-like (lightly armoured) population, were reared in mesocosms in the presence or absence of prickly sculpin. F₂ progeny provided great phenotypic diversity in traits for selection to act on, and recreated the kind of situation that might have existed in BC lakes following a double invasion (Rundle & Schluter, 2004). We used prickly sculpins as putative predators because they are one of the species that seem to inhibit the coexistence of stickleback species pairs, and because the extent to which they prey on stickleback, or might act as selective agents on antipredator traits, is poorly known (although see Moodie, 1972; Pressley, 1981). Although the latter two references clearly show that sculpins are capable of preying upon both stickleback eggs and adults, sculpins feed mainly on benthic invertebrates (Scott & Crossman, 1973). Our simple experimental design with one predator species and one phenotypically variable prey species sheds light on what happens in more complex communities by improving our knowledge of one key interaction.

Our aim was to explore hypotheses 1–3, listed above. Hypothesis 1 would be falsified if sculpins had no impact on the survival of stickleback, but would be supported if they reduced the survival of stickleback. The hypothesis would be strongly supported if there was evidence that sculpins cause selection against morphology that is associated with the exploitation of the littoral (benthic) niche, e.g. against reduced armour traits and short gill rakers (Schluter & McPhail, 1992). Hypothesis 2 would be falsified if growth rates of stickleback were unaffected by the presence of sculpins. Abrams' (2000) theoretical work suggests that additional predators are less likely to drive divergence if they respond in similar ways to antipredator traits of the prey. As substantial direct and indirect evidence suggests that trout do prey selectively on stickleback according to their armour traits, hypothesis 3 would be falsified if sculpin predation causes different selection on armour traits than trout predation. In general we would expect that the presence of predators should lead to directional selection (higher survival) on sticklebacks, favouring better armour traits: longer spines, stronger pelvic girdles, and more bony lateral plates. The present experiment cannot shed light on hypothesis 4. In addition to testing hypotheses 1–3, we provide data on the relationship between the extent of development of antipredator traits and the growth rate of individuals, as this could further the development of theory on the role of predation in divergence, which assumes the existence of trade-offs between traits and growth (Abrams, 2000; Bowers *et al.*, 2003).

MATERIAL AND METHODS

Wild-caught anadromous stickleback from Little Campbell River (49°01'N, 122°46'W) and freshwater stickleback from Hoggan Lake (49°09'N, 123°50'W), both in BC, were crossed to make two unrelated F₁ families. Anadromous stickleback from Little Campbell River are fully armoured marine-type stickleback, with large spines, well-developed pelvic girdle, and a complete row of bony lateral plates (Marchinko, 2009). Freshwater stickleback from Hoggan Lake are benthic-like, with small spines, reduced pelvic girdles, and few (five or six) plates. Hoggan lake has populations of both cutthroat and rainbow trout, but no sculpins. When the F₁s reached maturity, sibling–sibling matings were used to produce six F₂ families (4 : 2 split between F₁ families). Fish in these families were individually marked with visible implant elastomer (Northwest Marine Technology, WA, USA) at about 3 months of age, when they were approximately 25–40 mm long, and were kept in tanks in the lab for a further 2 weeks. There were no deaths during this period. Prickly sculpins were caught in Sakinaw Lake, BC (123°58'W, 49°42'N, where a single species of three-spined stickleback is also present) using minnow traps, and returned to the lab in Vancouver in aerated polystyrene boxes. They were maintained in a 300-litre tank, on a diet of defrosted frozen Chironomid larvae for 1 week, before being transferred to experimental mesocosms. The sculpins used in the experiment were big enough to swallow the sticklebacks used here (A.D.C. MacColl, pers. observ.).

Eighteen Rubbermaid plastic cattle tanks (diameter at base 145 cm; diameter at rim 167 cm; depth 61 cm; volume 1136 litres) were used as experimental mesocosms. They were positioned in the shade of trees, filled with mains water, and left to stand for 1 week. They were each fertilized with 2.46 g NaNO₃ and 0.18 g NaH₂PO₄ to kick start primary productivity (Harmon *et al.*, 2009), and were then left to stand for another week. Ten litres of mud and decaying vegetation were added to each mesocosm, from the benthos of the established experimental ponds at the University of British Columbia (UBC) (Schluter, 1994). This material was rich in invertebrate life. The mesocosms were allowed to settle before 1600 mL of zooplankton was added to each mesocosm. This had been collected by plankton net tows of a fishless experimental pond at UBC and Paxton Lake, Texada Island (49°42'N, 124°31'W), and contained approximately three organisms per mL of *Daphnia*, copepods, ostracods, and *Chaoborus*. The next day ten stickleback, chosen haphazardly from among those marked, were added to each mesocosm in random order. Each fish had its standard length

(‘initial length’) recorded, along with its elastomer marks, as it was added to its destination mesocosm. We did not photograph fish at this stage because gill rakers and most armour traits cannot be measured accurately from photographs of live fish, and the stress caused to the fish might have resulted in elevated mortality. Next day, one sculpin (standard length 92–106 mm) was added to nine of the mesocosms (‘predation mesocosms’) chosen randomly. This resulted in a sculpin density (0.61 m⁻² of benthic area) similar to that found in nature (White & Harvey, 2001). The other nine mesocosms were ‘control mesocosms’. A further 1300 mL of zooplankton was added to each mesocosm after 1 week. Five weeks after the stickleback were added, all mesocosms were emptied through 5-mm netting by a drain hole low on their side. Surviving stickleback were netted as they were encountered, the standard length was measured (‘final length’), and stickleback euthanized by overdose of MS222 and stored in 70% ethanol. ‘Growth’ was calculated for all surviving fish as the final length minus the initial length.

Preserved stickleback were transferred to 10% formalin for 2 weeks and stained with alizarin red to allow the visualization of armour traits. The following morphometric traits were recorded from digital photographs (Nikon D80 with 60 mm macro lens): length of first dorsal, second dorsal, and left pelvic spines; length of pelvic girdle (all following Marchinko, 2009); height of pelvic girdle (measured from a lateral view as the distance from the insertion point of the pelvic spine to the distal tip of the vertical process of the girdle); and number of lateral plates on the left side. The first gill arch on the left side was removed from each fish, the length of the bony part of the three longest rakers were measured at 10× using a graticule, and their average was taken to give the ‘gill raker length’.

The standard way to estimate (directional) selection differentials (S) in an experiment like ours is to subtract the trait mean before selection from that after selection. For example, in the control treatment:

$S_c = \bar{z}_{c,after} - \bar{z}_{c,before}$, where S_c is the selection differential, $\bar{z}_{c,after}$ is the trait mean in control mesocosms at the end of the experiment, after selection, and $\bar{z}_{c,before}$ is the trait mean in control mesocosms at the start of the experiment, before selection.

Similarly, in the predation treatment:

$S_p = \bar{z}_{p,after} - \bar{z}_{p,before}$, where \bar{z}_p indexes trait means in predation mesocosms, as above.

However, measurements of traits at the start of the experiment were not available. In any case, we were not interested in selection per se, but in selection caused by the presence of sculpins. Because any selection in control tanks caused by any other selective agent might result in S_c being non-zero, it would be

necessary to estimate the component of selection resulting from sculpins alone, S_s , as:

$$S_s = S_p - S_c$$

if $\bar{z}_{c,before} = \bar{z}_{p,before}$, which we can assume given the random allocation of stickleback at the start, then:

$$S_s = \bar{z}_{p,after} - \bar{z}_{c,after}$$

Therefore, to test for selection on each trait, appropriate selection differentials were quantified (Brodie, 1992) from the differences in phenotypic distributions between predation and control mesocosms at the end of the experiment (see also Marchinko, 2009). Before calculating selection differentials, all traits that were correlated with overall fish size (length) were standardized by taking residuals from the regression of the trait on final length. Directional selection differentials were estimated directly from the difference in mean trait values between predation and control mesocosms. To estimate selection on the second moment of trait distributions (i.e. stabilizing, disruptive, and correlational selection), cross products (including the squares of individual traits) were calculated for all pairs of morphometric traits, and the value in control mesocosms subtracted from that in predation mesocosms (Brodie, 1992). All selection differentials were then standardized to facilitate comparison, by dividing estimates of S by the variation (standard deviation) in the trait to give ' i ' values (Brodie, 1992). Significances of selection differentials were tested by computing a Student's t -statistic (Endler, 1986).

The number of fish used to calculate the selection differentials in this study (104) was not very large, and therefore there is some possibility that the negative results we report (see below) are the result of type-II statistical error. To assess this possibility, we compared the selection differentials calculated in this study with those in a large database of empirical estimates of selection differentials from a wide range of natural populations: the 'Kingsolver' database (Kingsolver *et al.*, 2001). We used the absolute (unsigned) values of significant ($P < 0.05$) estimates of i in that database to examine the distribution of selection differentials in natural populations, and to compare them with our own data set. For each of our own traits (or trait covariances), we also calculated our power to detect a biologically significant selection differential (Thomas, 1997), which we defined as being equivalent to the mean absolute value of i in the Kingsolver database.

Data were analysed in GENSTAT 10. Variation in growth and survival between mesocosm tanks was analysed with linear mixed models (LMMs) with appropriate fitted terms (see the Results). The F_2 'family' that individuals originated from and the mesocosm 'tank' in which the individual fish had been

allocated to were fitted as random effects (Galwey, 2006). Binomial errors and a logit link function were used when analysing survival data. Normal errors and an identity link function were used when analysing variation in growth. Denominator degrees of freedom in LMMs were calculated in GENSTAT using the Kenward–Rogers algorithm that adjusts for the inclusion of random terms in the model. Relationships between growth and trait values (including length) were analysed with generalized linear models (GLMs) with normal errors and identity link functions. The significance of terms in all models was tested by dropping them from the final model. Power calculations were also carried out in GENSTAT, using variances (or covariances) of traits estimated from the data (Thomas, 1997).

RESULTS

One of the predation mesocosms experienced heavy leaf fall from an over-hanging tree. As a result, conditions probably became anoxic during the experiment and no fish survived. There was no indication that this problem afflicted other mesocosms. In another predation mesocosm the sculpin died. Eight stickleback survived in this mesocosm, so it is likely that the sculpin had died early in the experiment. Both of these mesocosms were excluded from the analysis. Thirty stickleback were recovered from the remaining seven predation mesocosms, and 74 were recovered from the nine control mesocosms. There was substantial variation in armour traits among the stickleback collected at the end of the experiment, in both control and predation mesocosms (Table 1). The mean growth rate of sticklebacks in the experiment (mean \pm SE; 0.18 ± 0.007 mm day⁻¹) was almost exactly the same as that recorded for the morphologically intermediate Cranby Lake population in the wild by Pritchard & Schluter (2001).

We present the remaining results from the perspective of our three hypotheses.

1. Sculpin greatly reduced the survival of sticklebacks. The survival in control mesocosms (mean \pm SE; 0.81 ± 0.31) was almost double that in predation mesocosms (0.41 ± 0.29 ; Table 2). Survival of individual stickleback was not related to their initial length, or to the square of their initial length (Table 2). The final lengths of sticklebacks did not vary between treatments: Wald $F_{1,15} = 0.00$; $P = 0.95$; LMM, with family and tank as random effects (variance components \pm SE: 3.65 ± 3.25 and 1.15 ± 1.14 , respectively), normal errors, and identity link function.
2. The presence of sculpin did not affect the growth of sticklebacks (Table 3). The growth of individual

Table 1. Variation in morphometric traits among 74 F₂ sticklebacks collected from nine control mesocosms (no predation) and 30 F₂ sticklebacks from seven predation (sculpin) mesocosms at the end of the experiment

Morphometric trait	Coefficient of variation (%)		Range	
	Control	Predation	Control	Predation
Standard length	9.7	10.1	33.2–43.3 mm	36.3–42.2 mm
First dorsal spine	15.4	17.7	2.22–3.51 mm	2.54–3.49 mm
Second dorsal spine	12.9	15.0	2.74–4.07 mm	3.08–4.03 mm
Pelvic spine	13.8	15.3	4.01–6.12 mm	4.38–5.75 mm
Girdle height	14.7	15.2	3.36–5.04 mm	3.68–4.66 mm
Girdle length	13.1	15.4	5.72–8.45 mm	6.13–8.24 mm
Plate number	33.9	41.1	12.4–33.9	15.4–32.4
Gill raker length	12.9	12.4	0.693–0.990 mm	0.779–0.980 mm

'Coefficient of variation' is for all fish from control and predation mesocosms. 'Range' gives the average minimum and maximum trait values across control and predation mesocosms.

Table 2. Results of a linear mixed model (LMM) analysis of survival of all sticklebacks in mesocosms in relation to treatment (sculpin predation treatment or control) and initial length

	Wald <i>F</i>	d.f.	<i>P</i>
Treatment	24.80	1, 155	< 0.001
Length	1.15	1, 157	0.28
Length ²	0.38	1, 156	0.54
Treatment × length	0.67	1, 155	0.42
Treatment × length ²	0.30	1, 154	0.59

The model included the random terms family (variance component ± SE, 0.08 ± 0.19) and tank (0.00 ± 0.00), and had binomial errors and a logit link function.

sicklebacks was negatively related to initial length (Table 3), and tended to increase with density in control mesocosms (slope = 0.87 ± 0.32), and to decrease with density in predation mesocosms (slope = -0.29 ± 0.18).

- The mortality caused by sculpin did not result in directional, stabilizing, disruptive or correlational selection on any trait (or trait combination). The distribution of selection differentials is shown in Figure 1. Not one selection differential was significant at $P < 0.05$ among the 35 that were tested. All of the morphometric traits were significantly related to the final length of the sticklebacks (Table 4), so all traits were standardized for final length before being used in the calculation of selection differentials. The distribution of selection differentials in our data set was significantly different to that in the Kingsolver database (Fig. 1; Mann–Whitney $U_{35,239} = 5.81$; $P < 0.001$). The mean absolute (unsigned) value of i in the Kingsolver

Table 3. Results of a linear mixed model (LMM) analysis of growth (final length minus initial length, mm) of surviving sticklebacks in relation to treatment (predation or control), initial length ('length'), and density at the end of the experiment (number of surviving stickleback)

	Wald <i>F</i>	d.f.	<i>P</i>
Treatment	1.16	1, 12	0.30
Length	32.39	1, 89	< 0.001
Density	0.78	1, 15	0.39
Density × treatment	7.00	1, 13	0.02
Density × length	0.20	1, 93	0.66
Treatment × length	0.18	1, 85	0.67

The model included the random terms family and tank (variance components ± SE, 1.23 ± 1.04 and 1.45 ± 0.75, respectively), and had normal errors and an identity link function.

database was 0.42. The mean power to detect an effect of that size in this study was 0.59 (range 0.30–0.90). Only 16% of significant selection differentials in the Kingsolver database had an absolute value of less than the mean absolute value recorded in this study (0.148).

Lastly, the development of antipredator traits was not associated with a reduction in the growth rate, as assumed by theory. Growth tended to be greater among individual stickleback with larger values of armour traits for their size (Table 5), although these relationships were only significant for spine lengths. In general this pattern was unaffected by treatment, except that individuals with large (standardized) girdle heights grew more in the predation than control treatment mesocosms.

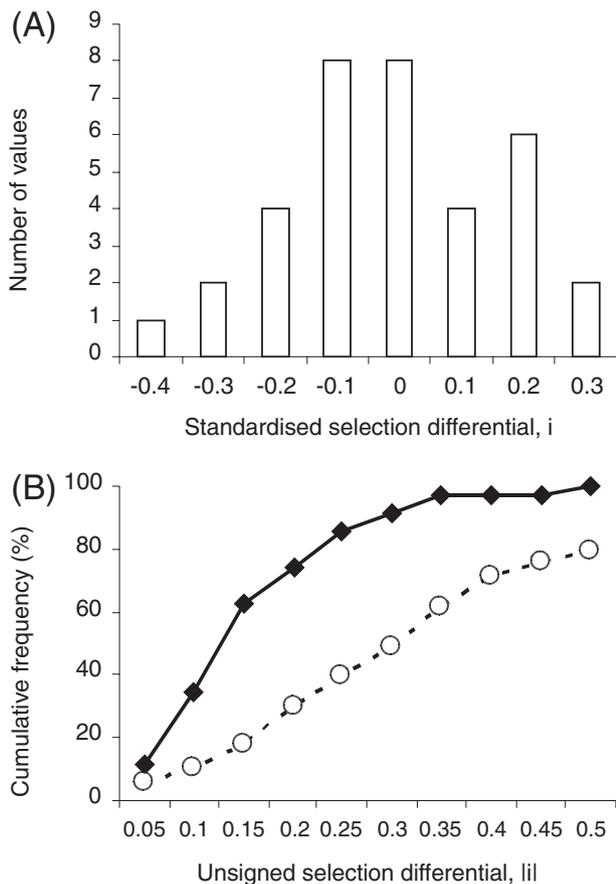


Figure 1. A, the distribution of standardized selection differentials ' i ' for individual traits of *Gasterosteus aculeatus* (three-spined sticklebacks) and all 28 pairwise cross products. The traits were standard length, length of gill rakers, and six armour traits, including spine lengths, pelvic girdle dimensions, and number of lateral plates. Categories on the x -axis are for the number of values up to the value of i . None of the differentials was significant at $P < 0.05$. B, the cumulative frequency distribution of absolute (unsigned) selection differentials in our study (filled symbols, solid line), and for 239 estimates in the Kingsolver database (open symbols, dashed line).

DISCUSSION

Prickly sculpins had a large impact on the survival of three-spined stickleback under the conditions of this experiment, suggesting that sculpin are effective predators of stickleback, which is consistent with previous anecdotal evidence (Moodie, 1972; Pressley, 1981). However, the presence of sculpin was not directly associated with any reduction in growth of stickleback, which would have suggested competition or an impact of the presence of predators on feeding behaviour. There was no suggestion in this experiment that sculpins caused any directional, stabilizing, disruptive or correlational selection on armour traits

Table 4. Relationships between morphometric traits and final length among fish that survived in all mesocosms, from generalized linear models (GLMs) with normal errors and identity link function

Morphometric trait	Parameter			
	estimate	SE	t_{102}	P
First dorsal spine	0.078	0.009	8.19	< 0.001
Second dorsal spine	0.074	0.019	7.44	< 0.001
Pelvic spine	0.146	0.012	11.73	< 0.001
Girdle height	0.108	0.012	8.75	< 0.001
Girdle length	0.208	0.016	13.32	< 0.001
Plate number	0.555	0.229	2.42	0.017
Gill raker length	0.019	0.002	8.47	< 0.001

in stickleback, despite the very substantial variation in such traits in the experimental populations and the strong impact of sculpins on survival, which should have created a large opportunity for selection.

Our results contradict our initial hypothesis 2 because stickleback growth was not directly affected by the presence of sculpins. They also contradict hypothesis 3 because sculpins imposed no selection on armour, and their selective impact on sticklebacks is therefore likely to be different from that of cutthroat trout, which are known to cause selection (Rundle *et al.*, 2003; Vamosi & Schluter, 2004). By contrast, our results support hypothesis 1 to some extent, as sculpins clearly had an effect on stickleback survival. However, if sculpin predation was worse in a certain niche (e.g. benthic), then we might expect it to cause selection against specific morphologies associated with the use of that niche (such as short gill rakers; Schluter, 2000). There was no evidence for this, but this may be a result of the limited opportunity for niche differentiation in our experiment. Our mesocosms lacked a clear separation between littoral and pelagic zones, which would only be achievable in larger pond experiments. Nevertheless, although it is difficult to predict how the stickleback mortality inflicted by sculpins in this experiment would scale to natural conditions, it might be enough to exclude sticklebacks from the littoral habitat favoured by sculpins, and hence prevent the evolution of the benthic ecotype, especially given additional sculpin predation on stickleback eggs (Moodie, 1972; Pressley, 1981).

The lack of selection on stickleback traits caused by sculpin predation is surprising. Although we cannot definitively rule out the possibility that type-II error accounts for this negative result, we can say with some certainty that selection, if it took place, was weak compared with previous estimates in the literature (Kingsolver *et al.*, 2001), as there is no reason to

Table 5. Relationships between growth in all mesocosms and size-standardized trait values, treatment, and their interaction for each morphometric trait

Morphometric trait			Standardised trait value		Treatment		Trait × treatment	
	Parameter	SE	$F_{1,101}$	P	$F_{1,100}$	P	$F_{1,99}$	P
First dorsal spine	1.28	0.64	4.03	0.047	0.51	0.48	0.50	0.48
Second dorsal spine	1.73	0.63	7.51	0.007	0.55	0.46	0.04	0.84
Pelvic spine	0.91	0.49	3.38	0.07	0.81	0.37	1.61	0.21
Girdle height	0.34*	0.54	2.90	0.09	0.57	0.45	5.34	0.02
	3.24†	1.17						
Girdle length	0.33	0.40	0.68	0.41	0.43	0.52	2.43	0.12
Plate number	0.002	0.027	0.00	0.95	0.42	0.52	0.08	0.78
Gill raker length	-1.43	2.87	0.25	0.62	0.45	0.51	0.01	0.91

The results come from generalized linear models (GLMs) with normal errors and the identity link function. Each 'parameter' value gives the slope of the relationship between that trait and growth.

For girdle height: *slope for control mesocosms; †slope for predation mesocosms.

believe that small sample sizes would lead to systematic underestimation of the magnitude of differentials. The comparison of differentials with the Kingsolver database (Kingsolver *et al.*, 2001) suggests that our result is unlikely to derive from low power. Indeed, our power to detect selection differentials typical of those in the literature was high for at least some traits. There are three alternative explanations to low power.

1. The relative size of prey and predators in this experiment were such that they rendered the defences of the stickleback ineffective against the sculpin (Werner, 1974; Reimchen, 1990). This seems unlikely given that the sculpins were relatively small, and the stickleback are approximately one-quarter to one-third of their length, even at the start of the experiment. This should have made the stickleback defences more effective against sculpins, and maximized the probability of selection.
2. Sculpins prey on stickleback in such a way that stickleback armour is generally an ineffective defence, no matter what the relative size of prey and predator. Sculpins are ambush predators, and as a result their captures may be fewer in number, but more certain than those of a pursuit predator such as a cutthroat trout. Testing this idea would require detailed observation of individual predation attempts, and the role that defensive structures might or might not play in escaping them (Reimchen, 1994).
3. The crossing of stickleback populations to produce F_2 fish resulted in a breakdown of co-adapted elements of stickleback armour, so that it was no longer effective: e.g. long dorsal spines are only effective against predators when present with long

pelvic spines. We cannot entirely reject this possibility, because we have no data on the covariance of armour elements in the parental populations; however, for the F_2 fish in this experiment there remained strong positive correlations between all elements of the armour (data not shown).

The density of sculpins and overall growth rates of stickleback were very similar to those in nature (Pritchard & Schluter, 2001; White & Harvey, 2001), suggesting that mesocosm conditions closely mimicked natural predation and resource availability. The mesocosms should have also compared well with natural conditions in other ways. Water depth and benthic habitat (mud and rotting vegetation) were certainly similar to those in the littoral zone of small lakes in coastal British Columbia, although less complex because of the absence of emergent vegetation.

Contrary to the assumptions of theoretical models (Abrams, 2000; Bowers *et al.*, 2003), there was no evidence in this experiment for a trade-off between growth and the extent of development of armour traits. There appeared to be no relationship between growth and lateral plates, which is surprising given recent evidence that their development tends to be associated with a growth cost in freshwater (Barrett, Rogers & Schluter, 2009). In other armour traits the data suggested the very opposite of a trade-off: there was a positive association between growth and the length of spines and size of the pelvic girdle. This raises the possibility of linkage either between armour traits and growth directly (Marchinko & Schluter, 2007) or between armour traits and some other aspect of phenotype that influences growth (Sinervo & Svensson, 2002; Blumstein, 2006). For example, this pattern would be consistent with more

heavily armoured sticklebacks being bolder in their acquisition of food. The pattern could also arise if growth is faster in the Little Campbell River population, and if underlying genetic loci are physically linked to those responsible for variation in armour traits.

In summary, prickly sculpins have a substantial effect on the survival of three-spined stickleback in mesocosm conditions, but they do not appear to cause selection on size, armour traits or gill raker length. This is partly consistent with the hypothesis that sculpins could constrain the divergence of stickleback into benthic–limnetic pairs by making the benthic niche evolutionarily unavailable (Vamosi, 2003), given that in the wild sculpins are found most commonly in the littoral zone. It may be time to re-examine Worthington's hypothesis (1937, 1940; see also Vamosi, 2005).

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REFERENCES

- Abrams PA. 1986.** Adaptive responses of predators to prey and prey to predators: the failure of the arms race analogy. *Evolution* **40**: 1229–1247.
- Abrams PA. 2000.** Character shifts of prey species that share predators. *American Naturalist* **156**: S45–S61.
- Barrett RDH, Rogers SM, Schluter D. 2009.** Environment specific pleiotropy facilitates divergence at the Ectodysplasin locus in threespine stickleback. *Evolution* **63**: 2831–2837.
- Blumstein DT. 2006.** The multipredator hypothesis and the evolutionary persistence of antipredator behavior. *Ethology: Formerly Zeitschrift fur Tierpsychologie* **112**: 209–217.
- Bowers RG, White A, Boots M, Geritz SAH, Kisdi E. 2003.** Evolutionary branching/speciation: contrasting results from systems with explicit or emergent carrying capacities. *Evolutionary Ecology Research* **5**: 883–891.
- Brodie ED. 1992.** Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. *Evolution* **46**: 1284–1298.
- Endler JA. 1986.** *Natural selection in the wild*. Princeton, NJ: Princeton University Press.
- Fryer G. 1959.** Some aspects of evolution in Lake Nyasa. *Evolution* **13**: 440–451.
- Fryer G, Iles TD. 1955.** Predation pressure and evolution in Lake Nyasa. *Nature* **176**: 470–470.
- Galwey NW. 2006.** *Introduction to mixed modelling: beyond regression and analysis of variance*. Chichester: Wiley.
- Gross HP. 1978.** Natural selection by predators on defensive apparatus of the three-spined stickleback, *Gasterosteus aculeatus* L. *Canadian Journal of Zoology* **56**: 398–413.
- Harmon LJ, Matthews B, Des Roches S, Chase JM, Shurin JB, Schluter D. 2009.** Evolutionary diversification in stickleback affects ecosystem functioning. *Nature* **458**: 1167–1170.
- Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE, Hoang A, Gibert P, Beerli P. 2001.** The strength of phenotypic selection in natural populations. *American Naturalist* **157**: 245–261.
- Marchinko KB. 2009.** Predation's role in repeated phenotypic and genetic divergence of armor in threespine stickleback. *Evolution* **63**: 127–138.
- Marchinko KB, Schluter D. 2007.** Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. *Evolution* **61**: 1084–1090.
- McPhail JD. 1969.** Predation and the evolution of a stickleback (*Gasterosteus*). *Journal of the Fisheries Research Board of Canada* **26**: 3183–3208.
- Moodie GEE. 1972.** Predation, natural selection and adaptation in an unusual threespine stickleback. *Heredity* **28**: 155.
- Nosil P, Crespi BJ. 2006.** Experimental evidence that predation promotes divergence in adaptive radiation. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 9090–9095.
- Pressley PH. 1981.** Parental effort and the evolution of nest-guarding tactics in the threespine stickleback *Gasterosteus aculeatus* L. *Evolution* **35**: 282–295.
- Pritchard JR, Schluter D. 2001.** Declining interspecific competition during character displacement: summoning the ghost of competition past. *Evolutionary Ecology Research* **3**: 209–220.
- Reimchen TE. 1990.** Size structured mortality in a threespine stickleback (*Gasterosteus aculeatus*) cutthroat trout (*Oncorhynchus clarki*) community. *Canadian Journal of Fisheries and Aquatic Sciences* **47**: 1194–1205.
- Reimchen TE. 1992.** Injuries on stickleback from attacks by a toothed predator (*Oncorhynchus*) and implications for the evolution of lateral plates. *Evolution* **46**: 1224–1230.
- Reimchen TE. 1994.** Predators and morphological evolution in threespine stickleback. In: Bell MA, Foster SA, eds. *The evolutionary biology of the threespine stickleback*. Oxford: Oxford University Press, 240–276.
- Rundle HD, Schluter D. 2004.** Natural selection and ecological speciation in sticklebacks. In: Dieckmann U, Doebeli M, Metz JAJ, Tautz D, eds. *Adaptive speciation*. Cambridge: Cambridge University Press, 192–209.
- Rundle HD, Vamosi SM, Schluter D. 2003.** Experimental test of predation's effect on divergent selection during character displacement in sticklebacks. *Proceedings of the*

- National Academy of Sciences of the United States of America* **100**: 14943–14948.
- Schluter D. 1994.** Experimental evidence that competition promotes divergence in adaptive radiation. *Science* **266**: 798–801.
- Schluter D. 2000.** *The ecology of adaptive radiation*. Oxford: OUP.
- Schluter D, McPhail JD. 1992.** Ecological character displacement and speciation in sticklebacks. *American Naturalist* **140**: 85–108.
- Scott WB, Crossman EJ. 1973.** Freshwater fishes of Canada. *Bulletin of the Fisheries Research Board of Canada* **184**: 1–966.
- Sinervo B, Svensson E. 2002.** Correlational selection and the evolution of genomic architecture. *Heredity* **89**: 329–338.
- Thomas L. 1997.** Retrospective power analysis. *Conservation Biology* **11**: 276–280.
- Vamosi SM. 2003.** The presence of other fish species affects speciation in threespine sticklebacks. *Evolutionary Ecology Research* **5**: 717–730.
- Vamosi SM. 2005.** On the role of enemies in divergence and diversification of prey: a review and synthesis. *Canadian Journal of Zoology* **83**: 894–910.
- Vamosi SM, Schluter D. 2002.** Impacts of trout predation on fitness of sympatric sticklebacks and their hybrids. *Proceedings of the Royal Society of London Series B* **269**: 923–930.
- Vamosi SM, Schluter D. 2004.** Character shifts in the defensive armor of sympatric sticklebacks. *Evolution* **58**: 376–385.
- Werner EE. 1974.** Fish size, prey size, handling time relation in several sunfishes and some implications. *Journal of the Fisheries Research Board of Canada* **31**: 1531–1536.
- White JL, Harvey BC. 2001.** Effects of an introduced piscivorous fish on native benthic fishes in a coastal river. *Freshwater Biology* **46**: 987–995.
- Worthington EB. 1937.** On the evolution of fish in the Great Lakes of Africa. *Internationale Revue der Gesamten Hydrobiologie* **35**: 304–317.
- Worthington EB. 1940.** Geographical differentiation in fresh waters with special reference to fish. In: Huxley J, ed. *The new systematics*. London: Oxford University Press, 287–302.