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Partitioning the relative fitness effects of diet and trophic morphology in the threespine stickleback

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ABSTRACT

Background: Numerous models show that if morphology and diet are correlated, frequency-dependent competition will lead to fitness differences among phenotypically dissimilar individuals within a species.

Hypothesis: Selection acts primarily on diet, and only indirectly on morphology via its correlation with diet.

Field sites and organism: British Columbia, Canada; 340 individual threespine stickleback (*Gasterosteus aculeatus*) from McNair Lake and 430 individuals from First Lake.

Measurements: Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$; a proxy for diet); trophic morphology (quantitative traits and geometric shape variables); and growth rates (RNA/DNA ratios; a proxy for the component of fitness arising from competitive or foraging ability).

Analysis: Linear and quadratic regression of growth rate on stable isotopes and morphological variables to calculate the relationship between growth (a fitness proxy) and diet and/or morphology. When both morphology and isotopes affected growth rates, we used a path analysis to separate their effects.

Conclusions: In the McNair Lake population, growth was dependent primarily on diet type and only indirectly on trophic morphology. In a second population, path analysis found that isotopes and body shape separately explain variation in growth rates. We infer that, in stickleback, selection on trophic morphology is often a correlated side-effect of selection on diet composition, rather than direct fitness effects of morphology *per se*.

Keywords: directional selection, frequency-dependent selection, fitness landscape, function-valued trait, *Gasterosteus aculeatus*, stabilizing selection, stable isotopes, trophic morphology.

INTRODUCTION

A number of adaptive dynamic and quantitative genetic models suggest that frequency-dependent competition for resources can generate stabilizing or disruptive selection (Roughgarden, 1972; Rosenzweig, 1978; Slatkin, 1980, 1984; Taper and Case, 1985; Dieckmann and Doebeli, 1999; Doebeli and

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Dieckmann, 2003; Ackermann and Doebeli, 2004; Doebeli *et al.*, 2007). Models of frequency-dependent competition typically assume that diet has a one-to-one relationship with a measurable morphological character (e.g. body size or gape width). In natural populations, morphological characters are indeed correlated with among-individual variation in resource use (Smith, 1987; Robinson *et al.*, 1996; Bolnick *et al.*, 2003; Svanbäck and Eklöv, 2003, 2004; Martin and Pfennig, 2009). For such normally distributed trophic traits, average phenotypes are more abundant and thus likely to experience higher competition, but may be adapted to the most abundant resources. The mode of selection (stabilizing or disruptive) therefore depends on the distribution of the trophic trait, the resource diversity, and the strength of competition. Strong intraspecific competition can generate disruptive selection on trophic morphology by disproportionately reducing the abundance of resources for the most abundant phenotypes in the population (Smith, 1987, 1993; Bolnick, 2004a; Bolnick and Lau, 2008; Martin and Pfennig, 2009; Svanbäck and Persson, 2009), particularly if resources are diverse (e.g. bimodal). In contrast, stabilizing selection could arise if the phenotypic variance is greater than the optimal variance determined by the resource distribution, for instance, if there is a single resource.

Models of competition-induced selection thus assume that selection acts directly on individuals' diets, and only indirectly generates selection on morphology. This indirect action of selection on morphology is often overlooked, because models assume a one-to-one relationship between diet and morphology. However, in most cases trophic morphology is only moderately or weakly correlated with diet [typically <0.5 (Price, 1987; Robinson, 2000; Bolnick and Paull, 2009)]. Consequently, it may be more appropriate to measure selection or variation in diet itself, rather than a morphological proxy (e.g. Bolnick *et al.*, 2007). Following the terminology introduced by Arnold (1983), we posit that there is a performance function in which morphology influences diet, and a fitness function relating diet to fitness, which results in an indirect morphology–fitness correlation. We thus view diet as an emergent trait that, much like other 'performance' traits, is determined by a combination of underlying phenotypes (e.g. morphology, behaviour, experience).

A weak correlation between diet and morphology should allow one to statistically separate the effects of selection on diet and morphology to test the hypothesis that selection acts directly on diet and indirectly on morphology. The alternative hypothesis, contrary to existing models of selection resulting from frequency-dependent competition, is that selection acts on morphology via some performance trait other than diet type. For instance, phenotypically different individuals might adopt the same diet but have different rates of prey capture or handling.

Here, we test these alternatives using quadratic regression and path analysis to evaluate the partial correlations between each phenotypic metric (morphology or diet) and a proxy for a component of selection that reflects competitive or foraging ability (hereafter, 'fitness'). Based on models of competition-induced selection, we expect that diet is the best predictor of our fitness proxy, with no additional contribution of morphology. If so, measures of selection on diet and morphology should yield similar qualitative trends. For instance, if diet is subject to disruptive selection, morphology should be under disruptive selection as well, but have no separate partial correlation with fitness. Note that there are reasonable scenarios under which these predictions can be falsified. While it may appear obvious that diet affects fitness more directly than does morphology, if fitness variation arises primarily via foraging efficiency rather than prey identity, then morphology is subject to selection for reasons independent of diet composition. In other words, the performance function relevant to selection may entail performance metrics other than diet.

We tested the above predictions by estimating selection gradients simultaneously for both resource use and morphology, in the threespine stickleback, *Gasterosteus aculeatus*, which has previously been shown to experience selection arising from frequency-dependent competition in some populations (Bolnick, 2004a; Bolnick and Lau, 2008). Revisiting two populations previously shown to experience disruptive selection, we instead found evidence for stabilizing and directional selection. This selection acted on both resource use and morphology, but in one lake the selection on morphology was simply a result of the diet–morphology correlation as predicted.

Study system

Threespine stickleback is a suitable model organism to test the theoretical assumptions regarding frequency-dependent competition. First, solitary natural populations show substantial diet variation among co-occurring individuals, which tend to specialize on either pelagic or benthic resources (Robinson, 2000; Nosil and Reimchen, 2005; Bolnick and Paull, 2009; Bolnick *et al.*, 2010; Matthews *et al.*, 2010). Second, this diet variation is correlated with morphology (Robinson, 2000; Bolnick *et al.*, 2008; Snowberg and Bolnick, 2008; Bolnick and Paull, 2009; Matthews *et al.*, 2010). As a result, within a single lake population, diet similarity between individuals declines as a function of morphological distance (Bolnick and Paull, 2009), as required for competition to be frequency-dependent. Third, there is empirical evidence that stickleback trophic morphology is subject to natural selection, ranging from disruptive in lakes with benthic/limnetic generalists, to stabilizing in very large lakes, and directional in lakes with large migration load (Schluter, 1994, 1995, 2003; Bolnick, 2004a; Bolnick and Lau, 2008; Bolnick *et al.*, 2008). Finally, diet and morphology are only moderately correlated (Robinson, 2000; Bolnick, 2004a; Bolnick *et al.*, 2008; Snowberg and Bolnick, 2008), making it possible to statistically separate selection on diet and morphology, to test the predictions listed above.

MATERIALS AND METHODS

Collection

In May 2008, we collected adult stickleback from McNair Lake and First Lake ($n = 340$ and 430, respectively) on northern Vancouver Island, British Columbia, Canada. The two lakes contain solitary stickleback populations, morphologically intermediate between the classic benthic and limnetic species pair forms. Within these solitary populations, individuals utilize various combinations of littoral and pelagic prey. We chose these two lakes because their stickleback populations were previously shown to be subject to natural selection on trophic morphology (Bolnick and Lau, 2008).

In each lake, fish were collected using about 50 unbaited minnow traps placed for ~4 h in a variety of microhabitats along ~0.5 km of shoreline in depths ranging from 0.1 to 10 m. The sampling scheme was intended to capture a representative sample of the phenotypic variation within lakes, since both benthic and pelagic phenotypes nest close to shore. The vast majority (>90%) of morphological and dietary variance within a lake occurs within small-scale samples [<10 m radius (L.K. Snowberg and D.I. Bolnick, unpublished results)], so we are confident that our samples reflect the variance within each lake. Any sampling bias due to selective trapping might affect trait means or variances, but should not bias our selection estimates.

Collected specimens were euthanized in MS 222 and kept on ice in the field after a caudal muscle tissue clip (~0.5 mg) was removed and preserved in 0.5 mL of RNALater (Ambion). Fish were quickly transferred to a −20°C freezer and the RNALater-preserved samples to a 4°C refrigerator for ~5 weeks. All samples were stored at −80°C thereafter. Collection and euthanasia were carried out in accordance with University of Texas institutional guidelines for the care of vertebrate animals (Institutional Animal Care and Use Committee 03120501).

Diet

Carbon and nitrogen stable isotope ratios are widely used in ecology as a measure of long-term feeding history (Peterson and Fry, 1987; Post, 2002; Dalerum and Angerbjörn, 2005; Newsome *et al.*, 2007). Within a lacustrine population of stickleback, stable isotope values are moderately to weakly correlated with individuals' trophic morphology, use of benthic versus limnetic prey (gut contents), and foraging microhabitat (Bolnick *et al.*, 2008; Matthews *et al.*, 2010). We dissected a piece of caudal muscle tissue from each stickleback. Samples were dried at 60°C for at least 48 h, ground with a mortar and pestle, and put into tin capsules (~1 mg of material). These were measured for $\delta^{13}C$ and $\delta^{15}N$ at the University of California at Davis Stable Isotope Facility. We did not perform any lipid extraction/normalization techniques, because lipid content in our samples was low, as indicated by the C:N ratios [MacNair Lake: 3.45 ± 0.88; First Lake: 3.27 ± 0.08; mean ± S.D. (Post *et al.*, 2007)], and not correlated with isotope signatures.

In stickleback, $\delta^{13}C$ reflects the proportion of benthic versus pelagic carbon in their diet, and $\delta^{15}N$ reflects relative trophic position (Vander Zanden and Rasmussen, 2001; Post, 2002). These interpretations can be made explicit by standardizing fish isotope ratios by the isotopes of basal benthic and limnetic consumers [snails and mussels, respectively (Post, 2002)]. However, in both lakes nearly half of stickleback had $\delta^{13}C$ exceeding snail $\delta^{13}C$ (maximum $\delta^{13}C$ for stickleback and snails was −19‰C and −25‰, respectively). This can occur if snails consume phytoplankton detritus, making their isotopes less than fully benthic. The more extreme values in fish leads to nonsensical estimates of percent benthic carbon (e.g. 250% benthic carbon) and relative trophic position. Consequently, we report raw isotope values, which we typically find are highly correlated with percent benthic carbon and trophic position within a given population [$r > 0.98$ (L.K. Snowberg and D.I. Bolnick unpublished)].

Morphology

We collected two types of morphological data, which were analysed separately. The first dataset was composed of univariate measurements including body mass (blotted dry and weighed to 0.001g), standard length, length of the lower jaw, body width at the pectoral fins, length of the two largest gill rakers (GRL1 and GRL2), and gill raker number (GRN). Body shape measurements were taken with a digital calliper accurate to 0.01 mm. Gill rakers were measured with an ocular micrometer on a dissecting microscope. Sex was determined by gonad inspection. Gill rakers were only measured on 244 of the 340 fish from McNair Lake. All measurements were performed by M.S. Araújo.

A second dataset of geometric morphometric shape was obtained using tpsDig2 (Rohlf, 2008) to digitize 23 homologous landmarks from the left side of each fish. We aligned the landmarks in tpsRelw (Rohlf, 2005). To measure overall body shape variation, we calculated

relative warps from the 23 photograph landmarks, using tpsRelw. The first two relative warps (RW_1 and RW_2; see Fig. A1 at evolutionary-ecology.com/data/2657Appendix.pdf) were used as measures of body shape in subsequent analyses. In McNair Lake, RW_1 and RW_2 explained 34.2% and 16.3% of the variance in position, respectively, of the 23 aligned landmarks (35.9% and 16.4%, respectively, in First Lake).

Selection estimate

Survivorship and lifetime fecundity are impossible to measure in natural populations of stickleback. Moreover, we are more interested in the component of selection arising from resource competition and foraging success, rather than total fitness variation. We therefore use growth rate as a measure of foraging success and thus a proxy for the component of fitness arising from competition. Growth rate is widely used as a fitness proxy in stickleback (Schluter, 1994, 1995, 2003; Hatfield and Schluter, 1999; Hendry *et al.*, 2002; Bolnick, 2004a; Bolnick and Lau, 2008; but see Taylor *et al.*, 2011). Better foragers (or competitors) grow faster (Wootton, 1976, 1994), and consequently are more fecund [number of eggs (Wootton, 1973, 1977; Ali and Wootton, 1999; Huntingford *et al.*, 2001; Bolnick, 2004a)] and may survive better (Reimchen, 1991); although some studies argue to the contrary (Bell and Haglund, 1978; Litvak and Leggett, 1992; Barber *et al.*, 2001). We acknowledge that measuring adult growth does not reveal selection on juveniles, measure other sources of selection (e.g. predation, parasitism, sexual selection), and that selection may not always favour the fastest growth rate.

To measure growth rate, we used the ratio of RNA to DNA concentration in muscle tissue. The RNA/DNA ratio is highly correlated with growth rate in stickleback [$r = 0.92$ (Ali and Wootton, 2003)] and other fishes (Caldarone *et al.*, 2001; Dahlhoff, 2004), because fish that grow faster synthesize more protein and have higher RNA titre as a result (particularly ribosomal RNA), whereas the amount of DNA remains constant in the cell. In a field experiment, increased intraspecific competition depressed growth rate as measured by the RNA/DNA ratio because of reduced prey availability (Svanbäck and Bolnick, 2007). This reduction in the RNA/DNA ratio occurred over 2 weeks, whereas brief periods of starvation (e.g. 24 h) have no effect on the ratio. Thus, it appears that the RNA/DNA ratio reveals growth variation among individuals arising from differences in energy income over the preceding several days to weeks. The RNA/DNA ratio has previously successfully revealed disruptive selection on gill raker length and number in stickleback in the lakes studied here (Bolnick and Lau, 2008). We followed Bolnick and Lau's (2008) RNA/DNA quantitation protocol.

Statistical analyses

For each of the two lakes, we used the following analyses to test our hypothesis that selection acts most directly on diet but is indirectly translated into selection on trophic morphology. We estimated the fitness landscape within each lake using linear and quadratic regression of our fitness proxy (ln-transformed RNA/DNA) against morphology (quantitative traits and geometric shape variables) and diets ($\delta^{13}C$ and $\delta^{15}N$) (Lande and Arnold, 1983). For the quantitative traits, we focused on size-corrected gill raker length and gill raker number, which are most strongly correlated with diet here and in previous studies (Schluter, 1995; Robinson, 2000; Bolnick, 2004a; Bolnick *et al.*, 2008; Snowberg and Bolnick, 2008) and which have previously been implicated as a target of selection (Bolnick, 2004a; Bolnick and Lau, 2008; Bolnick *et al.*, 2008). For body shape, we focused on RW_1 and RW_2. For diet, we examined the separate effects of $\delta^{13}C$ and

$\delta^{15}N$, which are only weakly correlated (First Lake: $r = -0.14$, $P = 0.003$; McNair: $r = -0.27$, $P < 0.001$).

We regressed our fitness proxy (ln-transformed RNA/DNA) on three sets of variables: (1) size-adjusted gill raker length and number; (2) body shape (RW_1 and RW_2); and (3) diet (measured as $\delta^{13}C$ and $\delta^{15}N$). We size-corrected morphological traits by calculating the residuals from regressions of the ln-transformed traits on an independent metric of overall body size (centroid size computed from the 23 digitized landmarks). All independent and dependent variables were transformed to have standard normal distributions (unit standard deviation, mean of zero) to allow for comparison of selection gradients with other studies (Lande and Arnold, 1983; Kingsolver *et al.*, 2001).

Inspection of residual plots indicated linearity of the models and normal distribution of errors in all cases. Applying a PCA-based size standardization yielded equivalent results to our size-standardized measures. We therefore focus on the analyses of univariate size-standardized traits. Several studies of selection on trophic morphology used the PCA size standardization (Bolnick, 2004a; Bolnick and Lau, 2008; Bolnick *et al.*, 2008), so for comparison with those previous studies we also provide the results of selection gradient estimates for PC axes, presented in the Appendix (Tables A1–A4; evolutionary-ecology.com/data/2657Appendix.pdf). Finally, we undertook cubic spline analysis for all quadratic regressions to evaluate whether a quadratic model was appropriate (Schluter, 1988). In all cases where quadratic regression terms were significant, cubic spline confirmed that a quadratic model was an appropriate fit to the data.

We ran separate quadratic multiple regressions for each of the three sets of traits (gill raker traits, body shape, and diet). For each multiple regression, we included sex as a factor but excluded sex × trait interactions, which were never supported by *F*-tests or AIC. Sex was removed from the models when non-significant (McNair Lake), but were retained when it had a significant effect on growth (First Lake). Also, we found no significant selection on correlations among morphological traits (consistent with Bolnick and Lau, 2008) or $\delta^{13}C$ and $\delta^{15}N$, and so do not discuss those analyses here. To test for possible multicollinearity between independent variables, we calculated variance inflation factors (VIFs) and found no evidence of multicollinearity in any of the models (all VIFs < 2.5).

Whenever we found simultaneous significant selection gradients on diet and morphology within a population, we used quadratic path analysis (Scheiner *et al.*, 2000) to partition the linear and quadratic effects of morphology (gill raker length) and diet on growth. The path model is presented in the Results section. A more complex path model with gill raker number and/or body shape yielded no additional significant path terms and did not change the qualitative results, so we focus on gill raker length alone. A multiple regression model-fitting approach using Akaike's Information Criterion yielded equivalent inferences as the path analysis, so we focus our presentation on the conclusions arising from the path analysis.

Effects of diet or trait disparity on growth

Quadratic regression works well when disruptive selection acts along a single univariate trait axis. Alternatively, frequency-dependent competition may favour any individual who departs from the population mean regardless of the trait(s). To evaluate this possibility, we took an alternative approach, in which we tested whether individuals' distance ('disparity') from the population centroid (in any direction in multivariate trait space) conferred higher fitness (RNA/DNA). First, we calculated the morphological (quantitative traits and relative

warps) and isotopic Mahalanobis distance between each individual and the population centroid. With this metric, two individuals could differ from the population mean by the same amount but along very different phenotypic axes. We then regressed standardized ln(RNA/DNA) onto trait disparity (normalized to unit standard deviation), with sex included where significant. We ran three separate disparity regressions, one for all quantitative traits, one for the relative warps, and one for isotopes. A positive slope would indicate that phenotypically peripheral individuals have higher growth rate, consistent with disruptive selection, while a negative slope would indicate stabilizing selection. This approach requires no *a priori* assumption as to which trait(s) or trait combinations are subject to selection. As with the quadratic regressions described above, whenever both morphology and isotopes showed significant fitness gradients, we used a path analysis to partition the relative effects of morphological and dietary disparity on growth rates (see Results for the model). All analyses were carried out in the R environment (R Development Core Team, 2007.)

RESULTS

McNair Lake

There was no significant linear or quadratic relationship between growth rate and $\delta^{13}C$ in McNair Lake (Fig. 1A). In contrast, we found a negative linear relationship between growth and $\delta^{15}N$ (Table 1; Fig. 1B), suggesting there may be a directional component to selection favouring individuals who feed on lower trophic position prey. There was no quadratic relationship between growth and $\delta^{15}N$ (Table 1) or correlation between growth and diet disparity (Table 2).

We found evidence of directional selection on trophic morphology, paralleling the selection on diet. Individuals with shorter gill rakers exhibited higher growth rates (Table 1; Fig. 2A). Growth rates were also positively correlated with RW_1 (Fig. 2C), although the relationship was only marginally significant (Table 1). Gill raker number and RW2 were not correlated with growth rate (Fig. 2B, D). None of the morphological axes exhibited quadratic relationships with growth. Consistent with the absence of quadratic selection gradients, there was no relationship between growth rate and multivariate disparity in body shape, morphology or stable isotopes (Fig. 3A–C).

Gill raker length was positively correlated with trophic position ($\delta^{15}N$; see Table A5 at evolutionary-ecology.com/data/2657Appendix.pdf). Consequently, the inferred directional selection for lower trophic position is consistent with the directional selection for shorter gill rakers. To isolate the relative contributions of morphology and diet to variation in growth rates, we constructed a path analysis including the effect of gill raker length (linear and quadratic) on diet (measured by linear and quadratic $\delta^{15}N$ and $\delta^{13}C$), and the effects of gill rakers and diet on growth (Fig. 4). The path analysis supports a model in which gill raker length affects diet (both $\delta^{15}N$ and $\delta^{13}C$, which are correlated with each other). However, only relative trophic position ($\delta^{15}N$) influences growth rate. Thus, the directional selection on gill raker length, noted above, is entirely explained by indirect selection in which gill raker length affects trophic position, which affects growth rates. In the terminology of Arnold (1983), there is a performance function relating morphology to diet, and a fitness function relating diet to growth.

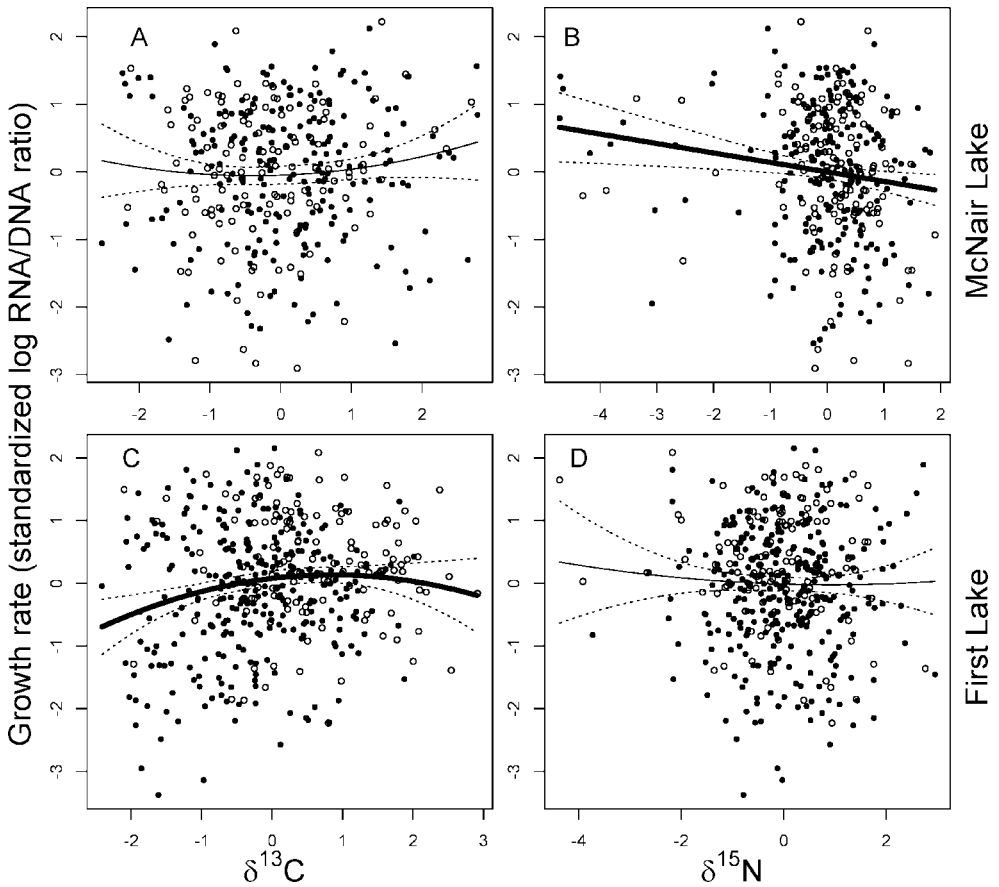

Fig. 1. Relationships between growth rate (RNA/DNA ratio) and two weakly inter-correlated measures of diet ($\delta^{13}C$ in panels A and C; $\delta^{15}N$ in panels B and D) for McNair Lake (A, B) and First Lake (C, D). Isotope ratios and the RNA/DNA ratio were transformed to a standard normal distribution. Quadratic (or linear) regression results are plotted as a solid line with dashed lines representing 95% confidence intervals for the slope. Significant relationships are plotted as thick trend lines, non-significant relationships as thin lines. Sexes are plotted with separate symbols (solid = male, open = female). A single trend line is provided for both sexes because trends are the same with or without sex.

First Lake

In First Lake, there was a negative quadratic relationship between growth and $\delta^{13}C$ (Table 1; Fig. 1C), implying a stabilizing component to selection. In this lake, growth rate was maximized for individuals with average values of $\delta^{13}C$, representing a roughly intermediate mixture of benthic and limnetic resources. Note that both quadratic regression (Fig. 1C) and cubic spline indicate that the fitness maximum is within the observed range of trait values. There was no significant association between growth and $\delta^{15}N$ in First Lake (Fig. 1D), unlike McNair. Finally, regression of growth on isotopic disparity revealed a significant negative association, implying that individuals far from the isotopic centroid

Table 1. Linear and quadratic relationships between growth and morphology or isotopes, by lake. Estimates of linear and quadratic selection gradients (β and 2γ) for size-standardized log gill raker length (GRL), size-standardized gill raker number (GRN), geometric shape variables (relative warps; RW_1 and RW_2), and $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. All traits were transformed into standard normal distributions to provide gradient estimates comparable to other studies of selection

Lake and trait	β	s.e. (β)	*t*	*P*	2γ	2 s.e. (γ)	*t*	*P*
McNair Lake								
GRL	**−0.079**	**0.036**	**−2.224**	**0.027**	−0.009	0.055	−0.160	0.873
GRN	−0.002	0.036	−0.065	0.948	−0.054	0.055	−0.982	0.327
RW_1	0.057	0.030	1.912	0.057	−0.027	0.042	−0.640	0.522
RW_2	0.036	0.030	1.220	0.223	0.044	0.042	1.043	0.298
$\delta^{13}C$	0.008	0.031	0.266	0.790	0.032	0.043	0.734	0.463
$\delta^{15}N$	**−0.074**	**0.031**	**−2.423**	**0.016**	−0.029	0.029	−0.997	0.320
First Lake								
GRL	**0.095**	**0.029**	**3.240**	**0.001**	−0.011	0.041	−0.278	0.781
GRN	0.001	0.025	0.045	0.964	0.037	0.031	1.180	0.239
RW_1	−0.027	0.025	−1.089	0.277	0.560	0.037	−1.516	0.130
RW_2	0.024	0.025	0.931	0.352	−0.041	0.037	−1.128	0.260
$\delta^{13}C$	0.029	0.028	1.021	0.308	**−0.099**	**0.038**	**−2.589**	**0.010**
$\delta^{15}N$	−0.006	0.025	−0.255	0.799	0.011	0.027	0.392	0.695

Note: GRL is the standardized log transformed mean length of the two longest gill rakers. For each trait in each lake we provide the least squares slope estimate, its standard error, and statistical significance. Values in **bold** indicate regression terms that are significantly different from 0 at $\alpha = 0.05$. Linear and quadratic gradients were estimated from different linear models. A sex effect was included in each model initially, but was dropped when not supported by AIC. Sex had no significant effect on growth rate in McNair Lake, so was not included in the models presented here. In First Lake, sex had a significant effect on growth rate ($P < 0.001$) in all the regression models and so were retained, but we only present the regression slopes for the traits putatively under directional selection.

Table 2. Linear correlations between growth rates and isotopic and morphological disparity, by lake. Morphological disparity (Mahalanobis distance from population centroid) was based on quantitative traits (Morphological disparity_{qt}) and geometric shape variables (Morphological disparity_{geo}). Isotopic disparity was based on $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. The *P*-value for the sex effect on growth is listed in the final column

Lake and trait	β	s.e. (β)	*t*	*P*	Sex
McNair Lake					
Morphological disparity_{qt}	−0.056	0.038	−1.479	0.140	**0.027**
Morphological disparity_{geo}	0.015	0.033	0.485	0.628	0.244
Isotopic disparity	−0.010	0.032	−0.313	0.754	0.210
First Lake					
Morphological disparity_{qt}	−0.048	0.026	−1.877	0.061	**< 0.001**
Morphological disparity_{geo}	**−0.079**	**0.025**	**−4.389**	**0.002**	**< 0.001**
Isotopic disparity	**−0.076**	**0.025**	**−3.024**	**0.003**	**< 0.001**

Note: Quantitative traits were body mass, standard length, body width, lower jaw length, gill raker length, and gill raker number. For each trait in each lake we provide the least squares slope estimate, its standard error, and statistical significance. Values in **bold** indicate regression terms that are significantly different from 0 at $\alpha = 0.05$.

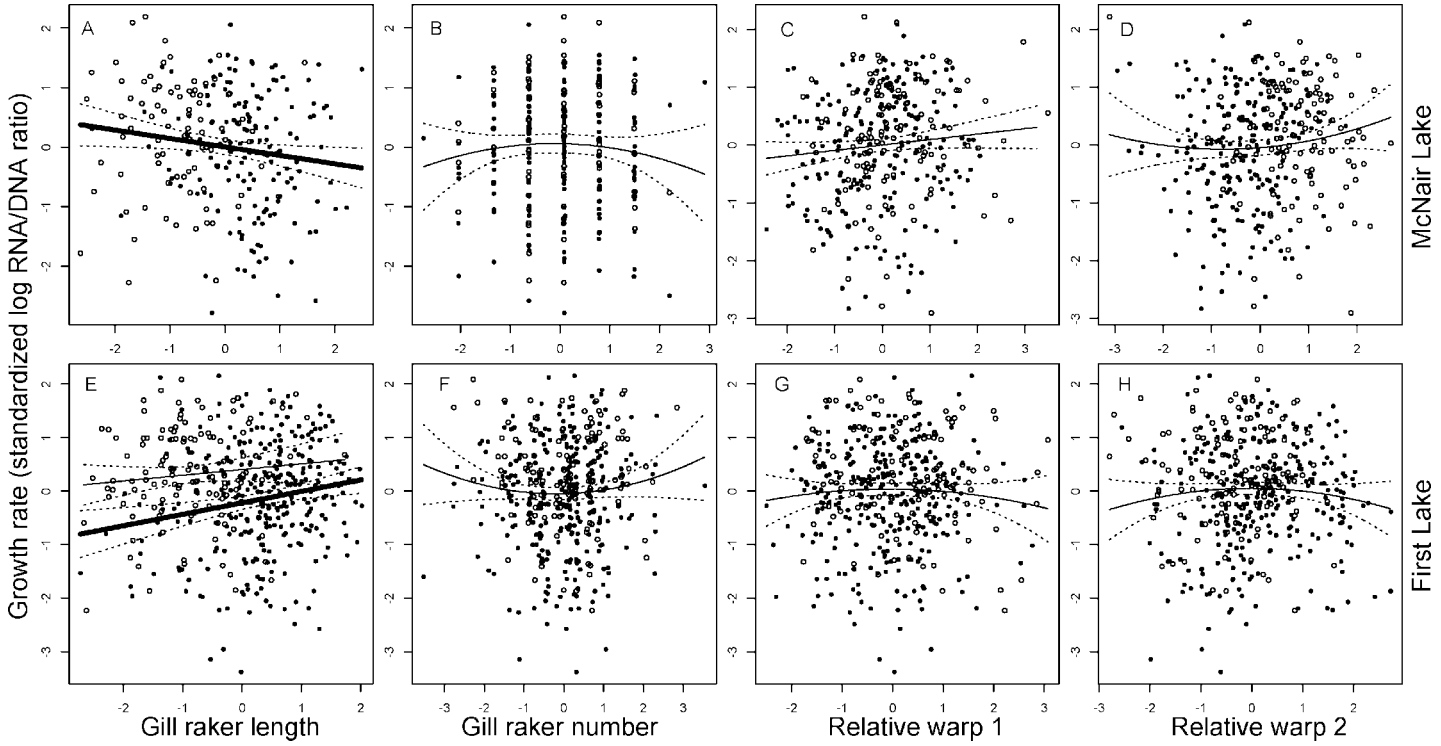

Fig. 2. Relationships between growth rate and four morphological traits (relative gill raker length in panels A and E; gill raker number in panels B and F; and body shape relative warp 1 in panels C and G, relative warp 2 in panels D and H) for McNair Lake (A–D) and First Lake (E–H). Gill raker length was log-transformed and standardized by body size. All traits and growth rate were transformed to standard normal distributions. Quadratic (or linear) regression results are plotted as a solid line with dashed lines representing 95% confidence intervals for the slope. Significant linear and/or quadratic relationships are plotted as thick trend lines, non-significant relationships as thin lines. Sexes are plotted with separate symbols (solid = male, open = female). With one exception, a single trend line is provided for both sexes because quadratic and linear terms are similar whether sex is included or excluded. The exception is panel E, where a linear effect is only detected when sex is included in the model. For panel E we therefore plot the trends for the sexes separately; in females there is a positive but non-significant effect of gill raker length on growth (thin line), while in males there is a positive significant effect of gill raker length on growth (bold line). The linear gradient does not differ significantly between the sexes (no sex × raker length interaction).

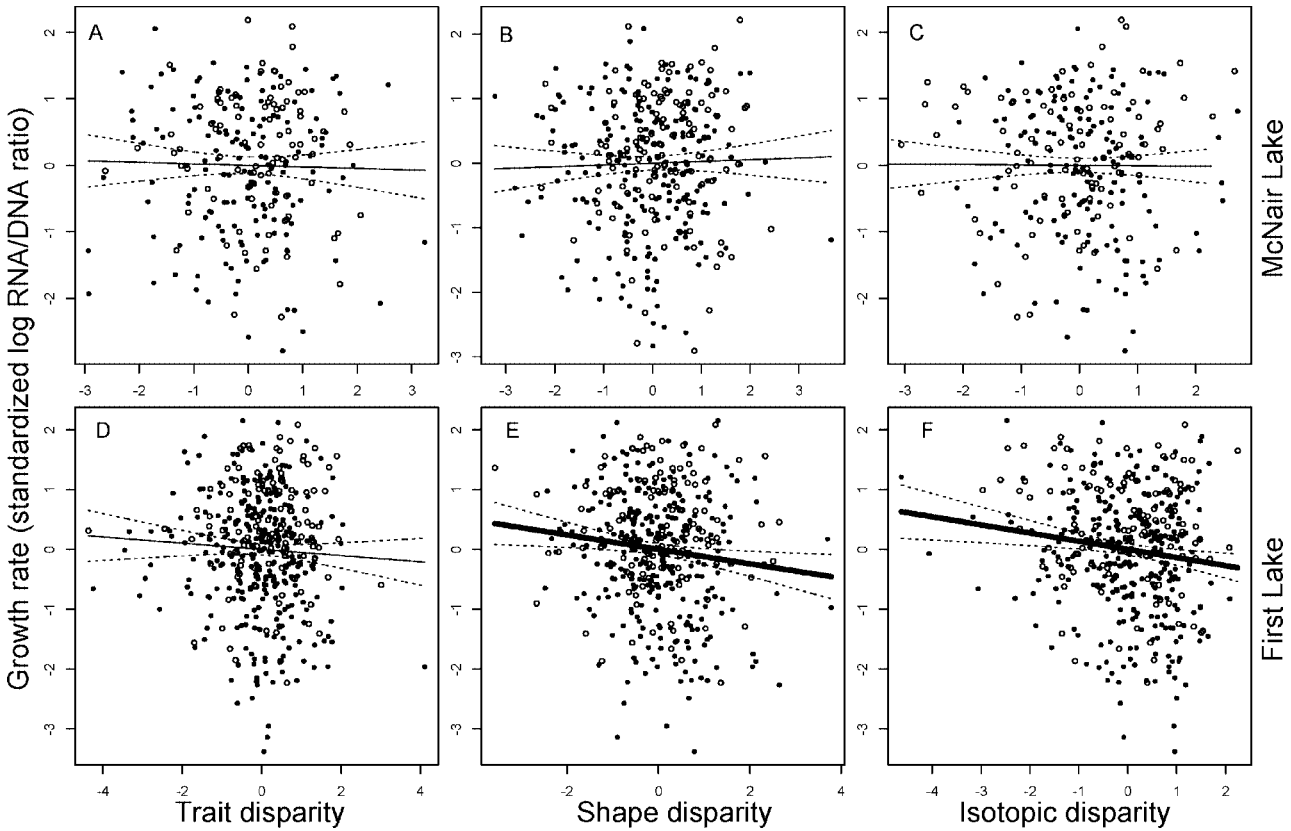

Fig. 3. Correlations between growth rate and three measures of trait disparity (quantitative trait morphology in panels A and D; geometric shape in panels B and E; isotope ratios in panels C and F) in McNair Lake (A–C) and First Lake (D–F). Linear regression trends are plotted as a solid line with dashed lines representing 95% confidence intervals for the slope. Significant linear relationships are plotted as thick trend lines, non-significant relationships as thin lines. Sexes are plotted with separate symbols (solid = male, open = female). A single trend line is provided for both sexes because trends are the same with or without sex.

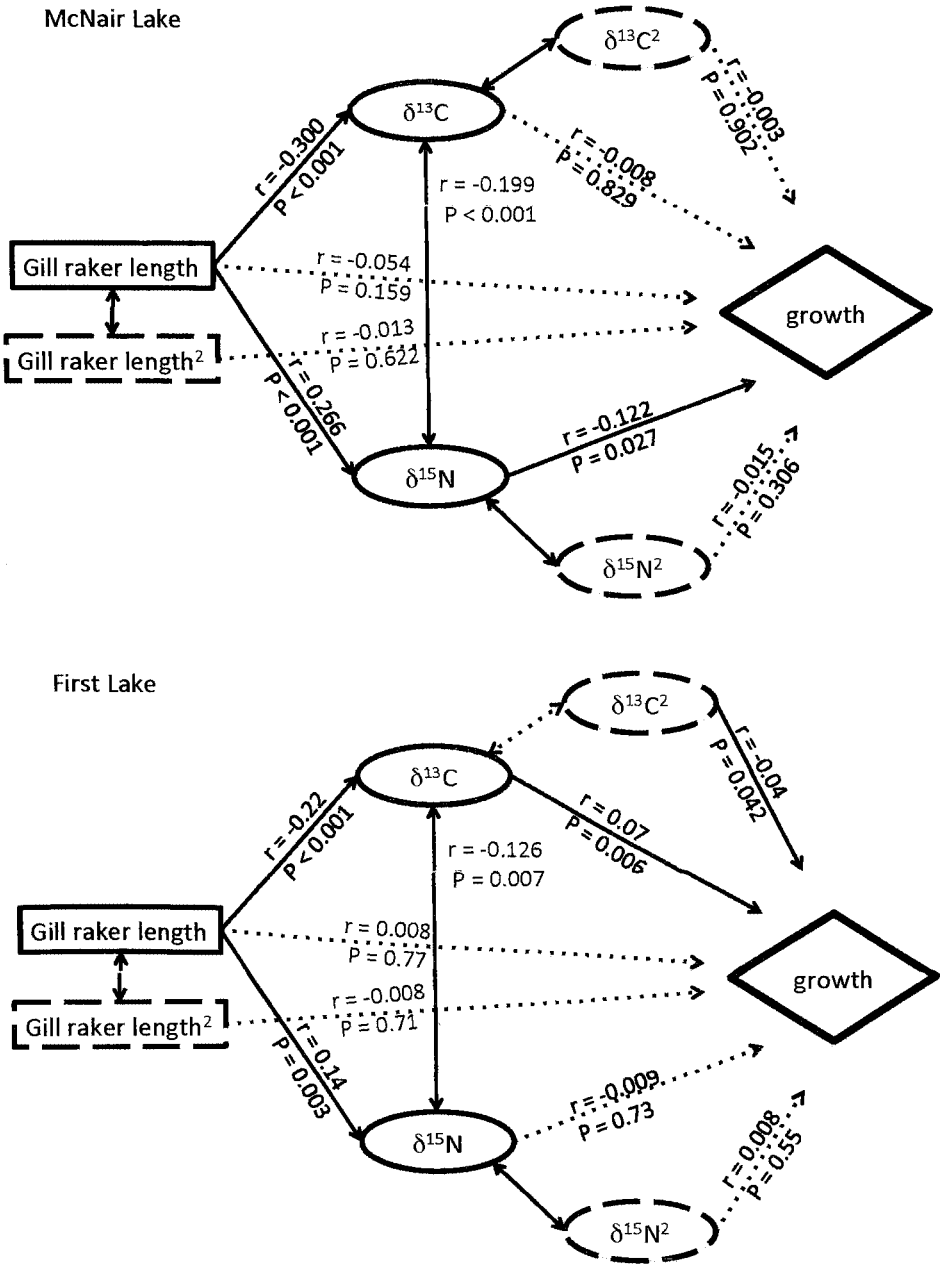

Fig. 4. The results of quadratic path analysis, for each of the two lakes, estimating the relationships between trophic morphology (gill raker length), diet ($\delta^{13}C$ and $\delta^{15}N$), and a measure of growth rate (RNA/DNA ratio; a fitness proxy). Morphology (rectangles) is assumed to determine individuals' diets (a performance trait, indicated by ovals), which affect fitness (diamond). Quadratic terms are symbolized by shapes with dashed-line margins. Significant partial regressions are indicated by solid arrows, non-significant regressions by dashed arrows. Correlations (no direction of causation assumed) are indicated by double-headed arrows. Correlation coefficients and *P*-values are presented for each element of the path (except the trait variances, and correlations between linear and quadratic terms for a given trait). Path models with gill raker number yield identical patterns, but with no significant effects of gill raker number on diet or growth; consequently, we do not present those components of the path analysis.

grew poorly (Table 2; Fig. 3F). Both the quadratic and disparity approaches suggest that ecologically generalist intermediate individuals had highest growth.

We found no significant quadratic selection gradients on gill raker traits or geometric shape variables (Table 1; Fig. 2E–H). However, in a model omitting the quadratic effect we did find a significant linear relationship between gill raker length and growth rate (Table 1; Fig. 2E). Thus, the stabilizing selection acting on $\delta^{13}C$ is not translated into stabilizing selection on gill raker length, in spite of the correlation between gill raker length and $\delta^{13}C$.

Path analysis clarified the relationship between the stabilizing selection on diet and the concurrent directional selection on gill raker length (Fig. 4B). Much like McNair Lake, in First Lake there was a correlation between gill raker length and both $\delta^{13}C$ and $\delta^{15}N$. However, in First Lake growth rate exhibited both a linear and quadratic relationship with $\delta^{13}C$, but had no independent relationship with gill raker length. The path analysis thus reveals that the inferred directional selection on gill raker length is an artefact of the linear association between gill raker length and diet, together with the linear component of the relationship between diet and growth. As with McNair Lake, we therefore conclude that gill raker morphology only indirectly affects growth via their shared relationship with diet.

Consistent with our inference of stabilizing selection on diet, multivariate disparity in body shape was negatively related with growth rate (Table 2; Fig. 3E). Thus, there appears to be a component of selection acting to reduce isotopic and body shape disparity in First Lake. We observed no such effect on quantitative trophic morphology traits (Fig. 3D). A multiple regression model including both isotopic and shape disparity suggested that both terms are independently and significantly related to growth rate (isotopic disparity: $t = -2.825$, $P = 0.005$; morphological disparity: $t = -2.967$, $P = 0.003$). A path analysis further clarifies this simultaneous trait–growth and diet–growth association (Fig. 5). The path analysis confirms that body shape disparity and isotopic disparity both have a significant direct negative effect on growth rate. However, the path analysis also reveals that shape and isotopic disparity are not significantly correlated with each other. Thus, in First Lake body shape must be linked to growth rate via some other, unmeasured performance function. In contrast, quantitative trait disparity is positively correlated with isotopic disparity, and thereby indirectly associated with growth rate. However, that indirect correlation between quantitative trait disparity and growth is too weak to reach significance when measured in a simple regression ($P = 0.061$; Table 2).

DISCUSSION

Several models of quantitative genetics and adaptive dynamics assume that selection on individuals' resource use (a performance trait) leads to correlated selection on trophic morphology (Roughgarden, 1972; Rosenzweig, 1978; Taper and Case, 1985; Abrams *et al.*, 1993; Dieckmann and Doebeli, 1999; Bolnick and Doebeli, 2003; Ackermann and Doebeli, 2004). This indirect effect of morphology on fitness can be tested by partitioning the effects of morphology and resource use on growth (Arnold, 1983). In the present study, individuals with different diets exhibited different current growth rates. In McNair Lake, individuals feeding at lower trophic position exhibited higher growth rates; in First Lake, growth was higher for ecological generalists with an intermediate carbon signature representing a mixture of benthic and limnetic prey. To the extent that current growth is a proxy for fitness, we conclude that selection does act on sticklebacks' resource use.

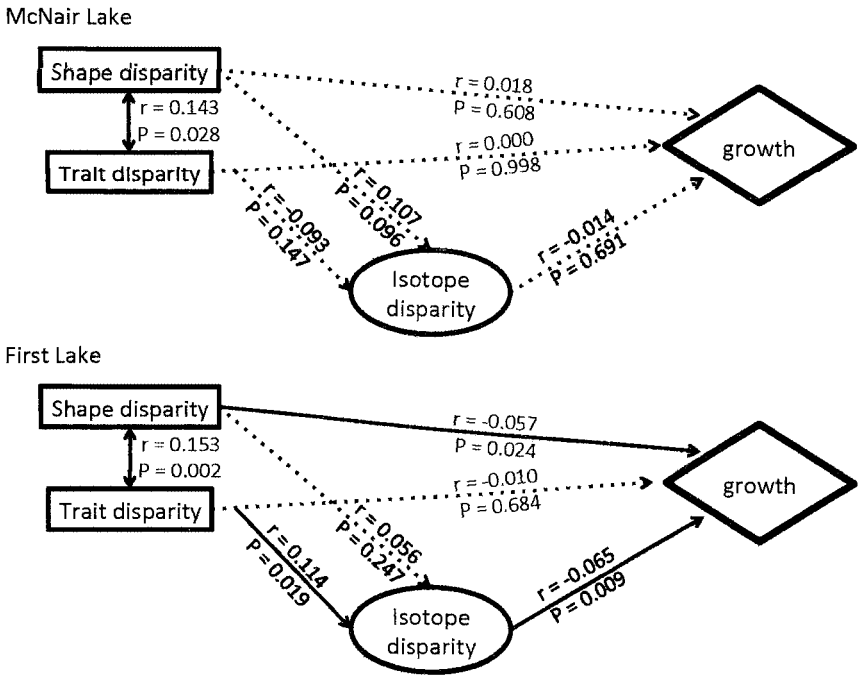

Fig. 5. The results of path analysis, for each of the two lakes, estimating the relationships between morphological disparity (in trophic morphology or body shape), isotopic disparity, and a measure of growth rate (RNA/DNA ratio; a fitness proxy). The meanings of various line and box shapes are as described for Fig. 4.

The selection on diet indirectly generates correlations between growth and morphology. In McNair Lake, growth was highest for individuals who both feed at lower trophic levels and have shorter gill rakers. In First Lake, both gill raker length and benthic/limnetic carbon use were correlated with growth and with each other. In both cases, path analysis revealed no independent morphology–growth correlation. Instead, morphology was correlated with diet, which was correlated with growth. Thus, selection on gill raker morphology in these systems conforms to a model of trait–performance–fitness mapping (Arnold, 1983), in which diet is an emergent (performance) trait that is most relevant for selection.

The exception to this pattern of indirect selection on morphology was seen for body shape and isotopic disparity. Positive or negative relationships between growth and disparity should generally conform to patterns of disruptive or stabilizing selection, respectively. The difference in these approaches is that disparity measures multivariate differences between individuals, and thus is not restricted to a pre-defined (or even a single) trait axis. Consistent with our inference of stabilizing selection on $\delta^{13}C$, we found a negative relationship between isotopic disparity and growth. Notably, we found a similar negative trend for body shape disparity, even though the first two body shape axes (RW1–2) were not under stabilizing selection. Given the simultaneous selection against shape and diet disparity, we might have expected another case of indirect selection on morphology via diet. However, in this

instance diet and shape disparity have independent effects on growth, and are not noticeably correlated with each other.

It is not immediately clear why body shape and diet have separate effects on growth. We speculate that body shape might affect growth primarily via locomotion (and associated energetic costs), or prey-capture success rates, rather than diet composition. Another possible explanation could be that body shape affects aspects of diet that are not effectively measured using carbon and nitrogen stable isotopes. Carbon and nitrogen isotopes have been very effectively used to identify basic benthic/pelagic differences within stickleback (Snowberg and Bolnick, 2008; Bolnick and Paull, 2009; Matthews *et al.*, 2010), but additional axes of diet variation do occur and are correlated with morphology (Araújo *et al.*, 2008). If these other axes of diet variation entail isotopically similar prey, our measures would fail to detect that diet variation. In this case, selection acting on undetected diet variation would appear to directly affect morphology. Finally, there is a potential mismatch between the time-scale of our fitness and diet metrics. The RNA/DNA ratios reveal growth rate variation that can respond to adult feeding success within ~2 weeks (Svanbäck and Bolnick, 2007). In contrast, isotopes are retained in muscle tissue for months. Consequently, any recent diet switch could generate variation in growth rate (our fitness metric) that has not yet had time to affect muscle isotopes.

One important caveat regarding our results is our use of growth rate as a proxy for fitness. The justification for this proxy is described in more detail in Bolnick and Lau (2008), but comparisons of growth versus diet raise a few novel challenges. An obvious concern is that different prey may inherently differ in energy content or capture rate, and thus confer different growth rates. However, inasmuch as these differences in growth rate lead to differences in survival or reproductive success, it is still appropriate to view growth rate variation as indicative of selection. That is, selection favours individuals that specialize on higher-value resources and thus achieve higher growth rates. This scenario might explain the directional selection in McNair Lake favouring individuals with lower trophic position, although we cannot be sure without detailed analyses of prey energy content and availability. Similarly, the stabilizing selection on $\delta^{13}C$ observed in First Lake might reflect a higher overall prey encounter rate for generalized individuals, because they consume both benthic and limnetic invertebrates. It is not clear why the lakes differ in their selection regimes, especially because in a 2005 sample they exhibited similar fitness landscapes (see below).

We emphasize that our selection estimates only reflect variation in foraging success – additional selective sources (predators, parasites, mate choice) might generate additional selection gradients that counterbalance or even overwhelm the partial selection observed here. However, we were specifically interested in testing the often-modelled association between morphology, diet, and the component of selection arising from variation in foraging success. As a final caveat, we acknowledge that in certain environments there may be selection against fast growth (Barber *et al.*, 2001), contrary to the general trend that larger stickleback have larger clutch sizes (Wootton, 1973, 1977; Ali and Wootton, 1999). For instance, fast growth may coincide with a weakened immune system or greater visibility to predators.

Evolutionary implications

Direct selection on diet preferences may have important evolutionary implications for correlated phenotypic traits. In our study, gill raker length and number explained between 6%

and 34% of variation in stable isotopes (see Table A4: evolutionary-ecology.com/data/2657Appendix.pdf). While the unexplained variation may be noise, it is possible that diet variation also depends on variation in unmeasured morphological, behavioural or physiological traits. For example, among-individual variation in microhabitat use is not only correlated with gill raker length but also with jaw lever ratios that are not typically measured in stickleback (D.I. Bolnick, unpublished results). Direct selection on diet could thus drive evolution not just of individual morphological structures such as gill rakers, but suites of traits that might even include important but unmeasured behavioural or physiological traits for which there is substantial genetic variation (Gibbons *et al.*, 2005; Latshaw and Smith, 2005). Conversely, weak correlations between diet and these various phenotypic traits may mean that strong frequency-dependent selection is nevertheless ineffective at driving adaptive diversification on commonly measured trophic phenotypes.

A second implication of our results is more specific to stickleback. Namely, a previous study found that diet variation, as measured by isotopes, is subject to positive assortative mating (Snowberg and Bolnick, 2008). We here show that the same diet metric is subject to natural selection. Several models of sympatric speciation predict that, in sexual populations, evolutionary divergence depends on a combination of ecological disruptive selection and assortative mating (Dieckmann and Doebeli, 1999; Bolnick, 2004b; Doebeli *et al.*, 2007). In these models, speciation is greatly facilitated when selection and assortative mating act on the same trait ['magic traits' (Gavrilets, 2004; Servedio *et al.*, 2011)]. Our results, combined with those of Bolnick and Lau (2008) and Snowberg and Bolnick (2008), suggest that in stickleback, diet may represent an instance of a magic trait, as isotopic measures of diet are correlated with fitness proxies (implying they are a target of natural selection), and are subject to assortative mating. Of course, this does not guarantee that speciation will ensue, especially because (1) both assortative mating and selection on diet are very weak in stickleback (Bolnick, 2011), and (2) selection is not consistently disruptive.

The temporal dynamics of selection in stickleback

In a previous study on these two populations based on samples collected in 2005 (First Lake) and 2006 (McNair), Bolnick and Lau (2008) found support for significant disruptive selection on gill raker number in both lakes. Using the same phenotypes and fitness proxies, the 2008 samples analysed here revealed no significant disruptive selection on this trait in either lake [the tables in the Appendix present quadratic selection estimates on principal component measures of diet and morphology that are directly comparable to Bolnick and Lau (2008); see evolutionary-ecology.com/data/2657Appendix.pdf]. In the more recent sample, we found significant directional selection on $\delta^{15}N$ and gill raker length (McNair Lake), and stabilizing selection on $\delta^{13}C$ (First Lake). The selection gradients observed in 2008 differ significantly from those of 2006. For example, quadratic regression coefficients of growth on gill raker number in McNair Lake differ significantly between years ($t = 2.25$, $P < 0.03$; 2008: $\gamma = -0.026 \pm 0.0275$; 2006: $\gamma = 0.074 \pm 0.035$). We therefore conclude that the fitness landscape in lake stickleback varies across years. Our results add to the literature showing that fitness landscapes can be rather dynamic and change over short time-scales in natural populations (Siepielski *et al.*, 2009, 2011; Kingsolver and Diamond, 2011; Morrissey and Hadfield, 2011). Some of the temporal heterogeneity in selection may simply be due to sampling error, although here we find statistically significant differences in selection between years.

Changes in the form and direction of selection can arise from a variety of sources, which we cannot distinguish among at present. First, inter-annual or seasonal variation in resource availability may give rise to fluctuating selection gradients (Reimchen and Nosil, 2004). Second, selection landscapes may change as a function of population density and the degree of intraspecific competition (Svanbäck and Bolnick, 2005; Abrams *et al.*, 2008), a prediction that has recently received observational support in Eurasian perch, *Perca fluviatilis* (Svanbäck and Persson, 2009), and experimental support in stickleback (Bolnick, 2004a). Under low competition, selection is either directional or stabilizing, favouring phenotypes functionally associated with benthic resources; as population density increases and competition for these preferred resources becomes stronger, the use of pelagic resources becomes increasingly advantageous, and selection turns disruptive (Svanbäck and Persson, 2009). Although we do not have rigorous population density estimates, natural stickleback populations do cycle in a density-dependent fashion (Wootton *et al.*, 2005; Wootton, 2008). We therefore speculate that fluctuations in either stickleback and/or resource densities may lead to fluctuations in the sticklebacks' fitness landscape, varying between stabilizing and disruptive selection. Long-term monitoring of fitness landscapes and lake ecology is required to test this prediction. Such fluctuating selection would likely undermine any progress towards speciation arising from the joint action of disruptive selection and assortative mating on a magic trait.

CONCLUSIONS

In the present study, we provide evidence for an implicit but largely untested assumption of models of frequency-dependent competition, by showing that resource use may be a primary target for natural selection that is indirectly transferred to trophic morphology. For most (but not all) selection gradients found here, morphology had no independent effect on growth rates except via a performance function relating morphology to diet. This finding suggests that natural selection acting on diets may be an important evolutionary force on traits correlated with foraging in natural populations. On the other hand, the same finding suggests that weak correlations between diet and phenotypic traits may mean that strong frequency-dependent selection is nevertheless ineffective at driving adaptive diversification on commonly measured trophic phenotypes. Theoretical models, therefore, should be re-evaluated in light of the general weak morphology–diet correlations in empirical systems and relax the assumption of one-to-one mapping between morphology and diet. Models of competition-induced disruptive selection would benefit from recognizing that trait–performance–fitness mapping may be complex and noisy.

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