

































interpretations presented in the main paper, which were based on categorical comparisons among delineated subsets of the same F<sub>2</sub> hybrids.

Results of linear and logistic regression analyses using all available samples (Extended Data Fig. 2b–g) further reinforce our biological interpretations about niche score and diet deviation score. These supplementary analyses focussed only on the four prey types that, together, accounted for more than 98% of all food items consumed by the F<sub>2</sub> hybrids. Other consumed foods (data provided with source data for Extended Data Fig. 2) were too rare in the digestive tracts of F<sub>2</sub> hybrids to be informative about the dietary ‘meanings’ of stable isotope variation under all of the statistical approaches that we used to investigate feeding patterns. Although we only describe our results from linear and logistic regression, below, no outcomes of any other tests (results not shown) were inconsistent with the following findings.

Linear regression applied to the diet data showed that a more benthic F<sub>2</sub> carbon signature<sup>17</sup> (i.e., higher  $\delta^{13}\text{C}$ ) was positively associated with the consumption of greater numbers of larval chironomids ( $P = 3.70 \times 10^{-6}$ ; Extended Data Fig. 2b; other statistical details given in the figure legend). In addition, a higher value of niche score, which we also interpret as being more benthic-like, was similarly associated with a higher probability of feeding on larval chironomids ( $P = 0.0255$ ; Extended Data Fig. 2c). By contrast, elevated  $\delta^{15}\text{N}$ , which characterised F<sub>2</sub> hybrids in region ‘L’ (Fig. 1a) and was also found in wild Paxton limnetics in a prior study<sup>17</sup>, was positively associated with the amount of *S. oregonensis* consumed by individuals in the experimental population in pond no. 4 ( $P = 0.0139$ ; Extended Data Fig. 2e).

Based on logistic regression, the probability that F<sub>2</sub> hybrids fed on *S. oregonensis* seemed to rise as niche score decreased ( $P = 0.0651$ ; Extended Data Fig. 2f). Though this trend was not strictly significant at the  $P < 0.05$  level, this suggestive result is consistent with an interpretation that F<sub>2</sub> hybrids in region ‘L’ exhibited a more limnetic-like feeding pattern compared to other individuals. Plankton tows made before and during the experiment (with a 0.3-m diameter, 80- $\mu\text{m}$  mesh plankton net) revealed that *S. oregonensis* was the most abundant zooplankton in the pelagic food web of pond no. 4 (M.E. Arnegard, B.W. Matthews, and D. Schluter, *pers. obs.*). This calanoid copepod is also abundant in Paxton Lake<sup>94</sup>. Compared to other planktonic taxa, calanoid copepods are thought to compose a rather nutritious food resource for fish<sup>95</sup>. *Skistodiaptomus oregonensis* has been found to suffer heavier predation from Paxton limnetics than from Paxton benthics, in both a mesocosm setting<sup>55</sup> and the native lake<sup>14,60</sup>. The trophic position of *S. oregonensis* in Paxton Lake is predicted to be elevated relative to other pelagic prey<sup>17</sup>. If *S. oregonensis* indeed had a similarly elevated trophic position in pond no. 4 as well, its  $\delta^{15}\text{N}$  composition and the heavier predation pressure it experienced from ‘L’ individuals during our experiment (Fig. 1c) could have been largely responsible for the elevated  $\delta^{15}\text{N}$  and reduced niche score characterising this subset of F<sub>2</sub> hybrids (Fig 1a).

Using logistic regression, we also found that the second factor axis through stable isotope space (i.e., PC2 or diet deviation score) was negatively associated both with the probability that calanoid copepods were consumed (Extended Data Fig. 2g) and the probability that larval chironomids were consumed (plot not shown). Similarly, linear regression revealed that PC2 was also negatively associated with the amounts of each of these prey types consumed by F<sub>2</sub> hybrids (also not plotted). Results of these four analyses were the following: (logistic regression, calanoid copepod prey) slope coefficient = -0.958,  $z = -2.67$ ,  $P = 0.00766$ ; (logistic regression, larval chironomid prey) slope coefficient = -0.960,  $z = -3.76$ ,  $P = 1.68 \times 10^{-4}$ ; (linear regression, calanoid copepods) slope estimate = -0.270,  $R^2 = 0.0586$ ,  $F_{1,97} = 6.03$ ,  $P = 0.0158$ ; (linear regression, larval chironomids) slope estimate = -0.363,  $R^2 = 0.0882$ ,  $F_{1,97} = 9.38$ ,  $P = 0.00284$ .



The F<sub>2</sub> hybrids from region ‘A’ were characterised by the highest diet deviation scores that we observed, overall, in the mapping population. As described in the main paper, we interpret the feeding pattern of these ‘A’ individuals to be ‘alternative’ (or ‘atypical’) in comparison to the diets of native benthics and limnetics from Paxton Lake. This interpretation is bolstered by the negative relationship between diet deviation score and the extent to which F<sub>2</sub> hybrids fed on either larval chironomids or calanoid copepods, which are typical prey of wild benthics and limnetics, respectively. In fact, chironomid larvae and calanoid copepods are considered to be classic markers<sup>56,96</sup> (or surrogates<sup>97,98</sup>) for opposite sides of the benthic–limnetic resource gradient. Collectively, the findings presented above show that niche score was a measure of where each F<sub>2</sub> hybrid fed along the benthic–limnetic resource gradient, which spans feeding niches occupied by the parental species in the natural setting.

Our conclusion that PC2 indicated the extent of dietary ‘deviation’ away from the classic limnetic–benthic resource axis (as recapitulated in pond no. 4) receives support from the positive relationship between F<sub>2</sub> feeding on symphypleonan collembolans and diet deviation score: (logistic regression) slope coefficient = 1.247,  $z = 4.262$ ,  $P = 2.03 \times 10^{-5}$ , Extended Data Fig. 2d; (linear regression) slope estimate = 1.152,  $R^2 = 0.328$ ,  $F_{1,97} = 47.3$ ,  $P = 6.04 \times 10^{-10}$ , not plotted. Although Hynes<sup>99</sup> demonstrated that some threespine stickleback individuals may feed on collembolans in certain environments, Collembola feeding by either benthic or limnetic sticklebacks has not been observed in Paxton Lake<sup>14,17,84</sup>. Taken together, observed patterns of F<sub>2</sub> hybrid feeding on calanoid copepods, larval chironomids, and symphypleonan collembolans demonstrate that the slowest growing F<sub>2</sub> hybrids in our study, with low  $\delta^{13}\text{C}$ –low  $\delta^{15}\text{N}$  (in region ‘A’), exhibited a divergent pattern of feeding with respect to F<sub>2</sub> hybrids with low (absolute) diet deviation scores and to both of the parental species.

Contrary to data on the consumption of copepods, chironomids, or collembolans by F<sub>2</sub> hybrids, we had no *a priori* grounds for interpreting the pattern of foraging on *Chydorus* sp. with respect to the limnetic–benthic resource spectrum. *Chydorus* sp. (family Chydoridae), a cladoceran microcrustacean taxon, is similar in size to the pelagic zooplankton that are preferred by Paxton limnetics. Yet, this cladoceran is smaller than the average size of prey commonly consumed by sub-adult to adult Paxton benthics<sup>84</sup>. Chydorids tend to be found on aquatic vegetation, leaf litter, and the benthic surfaces of ponds and lakes, rather than among pelagic zooplankton in offshore lentic habitats<sup>84,100</sup>. This distribution pattern applies generally to the Chydoridae, and we also found it to hold true for the *Chydorus* sp. that was common in pond no. 4 during our study (M.E. Arnegard, B. Matthews, and D. Schluter, *pers. obs.*). Paxton benthics and limnetics are both known to feed on cladocerans<sup>14,84</sup>, yet the extent to which either pure species specifically consumes littoral chydorids in the native lake remains unclear. Based on linear and logistic regression, we found no hint of a significant relationship between the consumption of *Chydorus* sp. and either the niche score or diet deviation score of F<sub>2</sub> hybrids (results not shown). Thus, our dietary data on *Chydorus* sp., the only other common food resource found in the digestive tracts of F<sub>2</sub> hybrids, are not inconsistent with our data on ingested copepods, chironomids, or collembolans.

Though there are potential limitations to data obtained from the quantification of ingested food items in the digestive tracts of fish<sup>101</sup>, this approach was the most direct method practical for measuring the feeding activity of F<sub>2</sub> hybrids in our experimental design. When all such data from all available samples were considered together, we found good support for our interpretations of stable isotope variation among the juvenile hybrid stickleback in our mapping population. The overall consistency of findings on F<sub>2</sub> hybrid diet, both between statistical approaches and among commonly consumed prey, justifies our delineation of regions ‘B’, ‘L’, and ‘A’ in isotope space to help explore and illustrate the biological significance of stable isotope variation in a clear and simple manner.

#### 4. Body size as a measure of feeding performance

We used body size of the  $F_2$  hybrid juveniles at capture as a measure of their feeding performance. For all analyses and figures in the main paper we measured body size as body length (i.e., distance between morphometric landmarks 1 and 13; see Methods and Extended Data Fig. 3). We found that our overall results were essentially unchanged when we quantified body size using centroid size instead of body length (results not shown); centroid size is the square root of the sum of squared distances of all morphometric landmarks from the centre of the body form, or centroid<sup>65,102</sup>.

The pre-winter juveniles that we collected for our study had spent their entire lives together in a single closed system (i.e., in pond no. 4), where they had unrestricted access to the same food resource distribution and were able to interact with one another competitively. Given the timeframe and near-natural setting of our experiment, we expected differential foraging success among individuals to be the most important factor responsible for body size (i.e., body length) variation. Although juvenile body size might also reflect differences in fish age, we controlled for this in our analyses by using  $F_2$  family identity (i.e., unique pair of  $F_1$  parents) as a covariate (see Methods). Rather than lifetime reproductive fitness, we were specifically interested in estimating the performance of  $F_2$  hybrids in acquiring food resources during the juvenile growth phase. Moreover, the timing of our sampling was planned to avoid estimating diet and feeding performance after both the onset of the yearly winter crash in zooplankton abundance<sup>103,104</sup> and the increase in northern temperate fish mortality that can occur during severe winters<sup>25,105</sup>. Our rationale for pre-winter sampling was that collecting stable isotope samples after such events could have resulted in a compression of the measured trophic range, or it may have otherwise obscured relationships between diet and performance. Our experimental design also required us to measure feeding performance in a non-invasive way, without handling the  $F_2$  hybrids or disturbing the ecology of pond no. 4, including the competitive interactions among the juvenile stickleback. The overall best approach to achieve these aims was to use body size as our measure of  $F_2$  hybrid feeding performance. High feeding performance has a general positive impact on individual fitness across many groups of fish. Given the general strength of the relationship between feeding performance and fitness in fish, body size and/or growth rate have been used as proxies for fitness in numerous evolutionary studies of threespine stickleback and other teleost fishes<sup>13,14,22,105-107</sup>.

#### 5. Investigating the body size valley in the stable isotope landscape

We statistically evaluated the dip in body size along the niche score axis by means of polynomial regression applied to the 20 largest  $F_2$  families, using regression models that included only linear and quadratic terms as well as the covariate,  $F_2$  family identity. These models provided a reasonable approximation of the shape of the splines shown in Fig. 1f, while avoiding over-fitting with higher order terms. We excluded all but the 20 largest  $F_2$  families because the remaining families (with fewer  $F_2$  full sibs) were too small to allow meaningful within-family analyses (results presented below). The overall polynomial regression model for all individuals in these 20 largest families, across all values of PC2, was significant (model  $R^2 = 33.2\%$ ;  $F_{21,416} = 9.847$ ;  $P < 2.20 \times 10^{-16}$ ). Though the quadratic term in this model was not significant (coefficient estimate =  $0.173 \pm 0.101$  s.e.;  $P = 0.0857$ ), within-family polynomial regression showed that 16 of the 20 largest families individually exhibited positive quadratic coefficients. The probability of obtaining this outcome by chance is  $P = 0.0118$  in a Bernoulli experiment, for which the probability of a positive quadratic term is assumed to be equal to the probability of a non-positive quadratic term (exact binomial test, implemented with R function ‘binom.test’<sup>51</sup>; two-sided).

Using the same model parameterization and family inclusion criterion, we also found statistical support for a body size valley along the niche use axis when only those individuals with  $PC2 < 0$  were considered (model  $R^2 = 34.6\%$ ;  $F_{21,237} = 5.973$ ;  $P = 4.50 \times 10^{-13}$ ). Owing to nuances of stable isotope variation among  $F_2$  families (patterns not shown), we found stronger statistical indications of a positive quadratic term in this analysis (quadratic coefficient estimate =  $0.244 \pm 0.116$  s.e.;  $P = 0.0360$ ) than when all values of  $PC2$  were considered. After restricting the data to  $PC2 < 0$ , we found that 17 of the 20 largest  $F_2$  families individually exhibited positive quadratic coefficients ( $P = 0.00258$ ; exact binomial test; two-sided). Statistical support for a positive quadratic term was also strong when all  $n = 625$   $F_2$  hybrids with complete isotope and body size data were considered (i.e., all sampled individuals across the full range of  $PC2$ ) but the  $F_2$  family covariate was excluded from the model (quadratic term: coefficient estimate =  $0.542 \pm 0.105$  s.e.;  $P = 3.12 \times 10^{-7}$ ). Including the  $F_2$  family covariate in this last model was unjustified because many of the 625  $F_2$  hybrids were from families containing only one sampled individual. No full sibs were detected in the mapping population for a considerable number of these 625 individuals due to the substantially larger total population size of  $F_2$  hybrids (sampled + un-sampled individuals), which were reared together in the experimental pond.

## 6. Selection of phenotypic traits for inclusion in the study

Here, we explain our rationale for selecting the specific phenotypic traits that we opted to include in our study. Focussing on a pair of species undergoing ecological speciation, our primary aim was to test the genetic architecture of niche divergence by genetically mapping relevant phenotypic traits in  $F_2$  hybrids reared under near-natural conditions. Owing to the reasonably well-established ecological and historical context for niche divergence and speciation in the threespine stickleback species pair of Paxton Lake, and due to the availability of suitable genetic tools and genomic resources for *G. aculeatus*, we based our study on an (intercross)  $F_2$  hybrid mapping population derived from wild Paxton benthic and limnetic  $F_0$  adults. After discussing our reasons for choosing the included traits, we briefly report on the finding that the juvenile  $F_2$  hybrids exhibited slight sexual dimorphism, overall, in the chosen traits.

Adaptive divergence in a whole-organism performance phenotype can involve morphological, behavioural, life history, and/or physiological component traits<sup>108-114</sup>. Any of these trait types can contribute to niche divergence, for example. In animals, one of the most generally important factors connecting component trait variation to how well-adapted individuals are to different niches is their performance in acquiring and consuming different food resources<sup>1,2,5,115-119</sup>. This is certainly true of the stickleback species pair in Paxton Lake, where feeding performance is one of the most important factors affecting the adaptation of benthics and limnetics to inshore and offshore areas of the lake (i.e., to littoral and shallow benthic versus pelagic habitats, respectively)<sup>13,14,16,60,83,84,120</sup>. Among all the different types of traits that have diverged between Paxton benthics and limnetics, morphological traits have been the most intensively studied. Many of the strongest known statistical relationships and/or mechanistic connections between any component traits and feeding performance variation in this species pair have been made for morphological traits. Accordingly, we focussed on morphology alone when deciding which candidate component traits of feeding niche divergence to include in our study.

Numerous morphological traits, including several aspects of body shape, differ significantly between wild Paxton benthics and limnetics<sup>16-20,60,84,121-126</sup>. We attempted to include the largest practical number of such traits, for which significant effects on habitat-specific limnetic-benthic feeding performance have been demonstrated or suggested. Many morphological traits have diverged in parallel in multiple benthic-limnetic species pairs<sup>12,17,18,120,123,127</sup>, and the resulting phenotypic differences are

maintained in each species pair in the face of some on-going gene flow<sup>16,20</sup>. The trophic significance of phenotypic variation in some traits is reasonably well understood<sup>16-19</sup>.

Reciprocal transplant experiments, carried out in Paxton Lake, have shown that overall divergence of morphological traits between Paxton benthics and limnetics leads to a twofold difference in feeding efficiency between preferred and non-preferred habitats<sup>14,21,22</sup>. Prior work has also established that general body shape and numerous specific morphological characters breed true for these fish in a common laboratory environment<sup>16,122</sup>. Moreover, heritable variation in this species pair has been demonstrated for some of these traits<sup>122,128,129</sup>. Studies of other threespine stickleback populations, including another benthic-limnetic stickleback species pair from a different lake, have also demonstrated heritable variation in several morphological traits thought to underlie trophic variation<sup>66,130,131</sup>. As described in Methods, we selected three classes of morphological traits on which to make phenotypic measurements and perform quantitative trait locus (QTL) mapping: functional morphological traits playing established roles in feeding; morphometric shape traits; and armour traits. We did not measure colour traits known to differ between Paxton benthics and limnetics, because these traits function primarily in sexual or agonistic signalling between breeding adults<sup>132</sup>, or in crypsis and predator avoidance<sup>133</sup>, rather than in feeding.

Of the three classes of morphological traits that we measured, we only tested the functional morphological traits and the shape traits for significant effects on trophic variation among the juvenile F<sub>2</sub> hybrids. The remaining class of armour traits comprised a small number of defensive characters (pelvic girdle, dorsal spines, and lateral plates), which we were able to rapidly screen alongside our more in-depth measurements of functional morphology and body shape. These armour traits have received a great deal of attention due to their importance in predator avoidance and adaptation of marine stickleback to freshwater environments<sup>24,127,134-136</sup>. Yet, no *a priori* evidence has elucidated specific roles of armour traits in feeding performance variation in the Paxton Lake species pair. Hence, we did not consider armour traits when selecting candidate morphological QTLs for the statistical models that were used to test the genetic basis of feeding niche divergence. We nevertheless performed QTL mapping on these traits (Supplementary Table 3), so that our results could be compared to findings on the genetic architecture of armour trait divergence from other crosses and populations<sup>66,137-140</sup>.

Among all traits investigated to date in the Paxton Lake species pair, functional morphological traits generally exhibit the best-understood effects on trophic divergence between limnetics and benthics. In our study, this class of traits included total counts of two series of gill rakers (Fig. 2e)<sup>141</sup>, stiff processes that project from the branchial arches and serve to limit the loss or escape of small ingested food particles from the buccal cavities of many different kinds of fish<sup>142,143</sup>. Compared to benthic threespine stickleback, limnetic threespine stickleback have longer and more numerous gill rakers, which are more closely spaced on the gill arches, thereby facilitating the retention of captured zooplankton<sup>14,16,17,60</sup>.

Gill rakers were the first functional morphological traits to be identified as having important effects on trophic variation in threespine stickleback<sup>135</sup>, and features of this prey-retention system remained the only well-documented functional morphological traits in the *G. aculeatus* species complex for quite some time. However, using a predictive model based on musculoskeletal morphology, McGee and Wainwright<sup>18</sup> recently quantified how Paxton benthics should be capable of generating greater negative suction pressures in their buccal cavities than Paxton limnetics. The model that was used by these authors describes suction feeding, a mode of prey capture that is important to feeding performance in many different fish groups<sup>144</sup>. An enhanced capacity for suction generation in Paxton benthics allows them to dislodge, and feed on, buried or attached macroinvertebrates in littoral and benthic areas of the

lake, their preferred foraging habitats<sup>13,14,60</sup>. Motivated by these recent findings, we included all five functional morphological traits used as predictor variables in the suction feeding index model<sup>18</sup> (Fig. 2f).

In a subsequent study, McGee *et al.*<sup>19</sup> further investigated the functional basis of feeding performance in the Paxton Lake species pair using biomechanical models for three additional prey capture systems: the lower jaw-opening lever (displacement advantage) system, the (upper) jaw protrusion system, and the opercular four-bar linkage (transmission coefficient) system. These authors used kinematic data from high-speed video recordings of feeding trials to test hypothesized effects of craniofacial morphology on feeding performance, which were predicted from the biomechanical models. Significant functional differences were found between benthics and limnetics for these three systems as well as the prey capture system involving suction feeding<sup>19</sup>. Among component morphological traits of these four mechanical systems, upper jaw protrusion length and lower jaw-opening inlever length were found to be two of the most divergent traits between Paxton benthics and limnetics<sup>19</sup>. Given this finding, as well as the general importance of these two oral jaw traits to feeding performance in a wide variety of ray-finned fishes<sup>145-147</sup>, we included upper jaw protrusion length and lower jaw-opening inlever length in our functional morphological class of measured traits (Fig. 2g).

As shown by McGee *et al.*<sup>19</sup>, upper jaw protrusion is significantly greater in Paxton limnetics than Paxton benthics, resulting in more efficient zooplanktivory by the limnetics. In addition, Paxton limnetics have significantly shorter lower jaw-opening inlevers than benthics, allowing the limnetics to rotate their lower jaws and open their mouths more rapidly than the benthics<sup>19</sup>. The more rapid strike speed of the limnetics, which results from this and other morphological specialisations, facilitates their predation on calanoid copepods (e.g., *S. oregonensis*), a key food resource for limnetics throughout much of the year<sup>14,17,60</sup>. Zooplanktivores like limnetic stickleback require relatively rapid predatory strikes and high jaw protrusion to efficiently capture calanoid copepods, because these microcrustaceans can perform quick escape manoeuvres, called ‘jumps’, in response to water displacements that are detected via their strain sensitivity antennae<sup>148</sup>.

For the shape class of candidate component traits, we employed a geometric morphometric approach<sup>102</sup> to measure several body form features in the juvenile F<sub>2</sub> hybrids. Relative body depth, hydrodynamic streamlining, the sizes and arrangements of fins, and/or mouth position and orientation are thought to generally influence niche occupancy in fish via important habitat-dependent effects on swimming ability, feeding efficiency, and other whole-organism performance traits<sup>149-151</sup>. Parallel evolution of habitat-associated limnetic versus benthic body forms (shallower and more streamlined or deeper and more robust, respectively), in multiple lakes, suggests that these differences in overall body shape have resulted from divergent natural selection for enhanced performance in pelagic versus littoral/benthic habitats<sup>15,84,120,127</sup>. Though some features of stickleback body shape and fin morphology have been related to predator evasion abilities (e.g., fast-start swimming performance) or to swimming abilities in different habitats (e.g., manoeuvrability and sustained swimming performance)<sup>121,152,153</sup>, we had very little *a priori* evidence to guide our choice of specific morphological landmarks for measuring ‘component shape traits’ of trophic divergence in a stickleback species-pair lake. We instead attempted to capture a large number of general body shape features using morphometric landmarks adapted from other threespine stickleback studies<sup>28,61,97,124,125,152,154,155</sup>. We omitted several previously used landmarks and redefined others (Extended Data Fig. 3) to achieve a good balance between: (1) maximal coverage of body regions (especially on the head) that seemed to be most variable among the F<sub>2</sub> hybrids, based on a review of digital images; and (2) use of only those landmarks that could be positioned rather reliably on images of stained juvenile stickleback.

Using the R-package ‘leaps’<sup>70</sup>, we performed exhaustive searches for the ‘best’ multiple linear regression models ( $0 \leq \Delta AIC \leq 2$ ) of the relationships between niche score and subsets of morphological characters from each trait class (i.e., subsets from the nine functional morphological traits or the 38 x- and y- coordinates of 19 morphometric landmarks). Results of this model-selection procedure suggested that the 19 landmarks were rather effective in capturing much of the shape variation that underlies niche divergence in the Paxton Lake species pair. For each model considered, ‘leaps’ returned the standard multiple  $R^2$ , as well as Theil’s<sup>79</sup> adjusted  $R^2$  (also called McNemar’s<sup>156</sup> adjusted  $R^2$ ). Standard  $R^2$  increases monotonically as more parameters (i.e., explanatory variables) are added to a given multiple regression model. To address this general property of the standard  $R^2$ , adjusted  $R^2$  is penalized (i.e., reduced) according to the number of parameters modelled compared to the number of observations used for model fitting<sup>79,156</sup>. Among the best functional morphological trait models predicting niche score, adjusted  $R^2$  ranged from 13.7% to 14.0% (Supplementary Table 1). In contrast, adjusted  $R^2$  ranged from 27.8% to 28.1% among the best shape-trait models predicting niche score (Supplementary Table 2). We cannot conclude from these results that shape variation accounted for approximately twice as much of the total niche score variation as did functional morphology. Despite not being able to quantitatively compare the different trait-class models this way, adjusted  $R^2$  is considered to be a sound goodness-of-fit metric for assessing and contrasting regression equations fitted to different data sets using different numbers of explanatory variables<sup>157</sup>. Thus, whereas the advantage of functional morphological traits was clear from their *a priori* mechanistic connections to niche use, outcomes of the above model comparisons underscore the value of also including body shape traits when investigating the component morphological basis of niche divergence between benthic and limnetic sticklebacks. Though the morphometric landmarks were not as well informed by specific *a priori* hypotheses regarding trophic function, our finding of high adjusted  $R^2$  values for the best shape-trait models (*vis-à-vis* the best functional-trait models) implies that geometric morphometrics also captures a substantial portion of the overall component morphology of benthic-limnetic niche divergence in Paxton Lake.

Although adult threespine stickleback exhibit moderate to strong sexual dimorphism in many morphological traits<sup>61,106,141</sup>, we found that the extent of sexual dimorphism in the juvenile  $F_2$  hybrids of our mapping population was weak or nearly absent in the traits we investigated. Thus, we did not include sex as a covariate in any of our statistical analyses. In an ontogenetic study of morphometric and meristic variation in several stickleback populations, Kitano *et al.*<sup>158</sup> similarly found a lack of sexual dimorphism in juveniles prior to maturation. Even though we did not use sex as a covariate in any of our analyses, we found that none of the measured morphological traits mapped to linkage group (LG) 19 (Extended Data Fig. 3; Supplementary Table 3). Linkage group 19 corresponds to chromosome XIX<sup>29</sup>, the nascent sex chromosome in threespine stickleback, which contains the master sex determination locus<sup>159</sup>. We might have expected to find one or more QTLs on LG 19, rather than none at all (Supplementary Table 3), had there been moderate to strong juvenile sexual dimorphism in some of the measured traits.

## 7. Removing the ‘specimen bending artefact’ during shape analysis

The fixation and preservation of fish typically causes a ‘specimen bending artefact’, represented by a U-shaped displacement of morphometric landmarks that is generally captured by one of the eigenvectors from a PCA of Procrustes-superimposed landmarks<sup>28,61,65,102</sup>. Using the function ‘shapepca’ in the R package ‘shapes’<sup>64</sup>, we plotted vectors beginning at the mean position of each of the 19 superimposed landmarks and ending +3 standard deviations along each PC axis. Plots like these were constructed for the first six PC axes recovered from a principal components analysis of superimposed landmarks for all  $F_2$  hybrids sampled from pond no. 4. The resulting plot for the first PC axis (PC1)

revealed upward pointing vectors for landmarks near the tail and head of each fish (e.g., landmark 1 and landmarks 11–15) and downward pointing vectors near the middle of the body (e.g., landmarks 2–7 and landmark 19; see Extended Data Fig. 3 for landmark positions), reflecting an average U-shaped deformation of the preserved specimens. Similar plots for the other PC axes did not reveal any U-shaped displacements of the landmarks. Instead, the other PC axes appeared to represent different aspects of biologically meaningful shape variation. Rather than being associated with natural shape variation, the first eigenvector therefore appeared to represent the common ‘specimen bending artefact’, which resulted from our fixation of specimens in 7.5% formalin and their subsequent storage in 40% isopropyl alcohol. This eigenvector (PC1) accounted for 23.72% of the total variation in superimposed landmark coordinates. To remove the effect of specimen bending, we transformed the landmark coordinates to their principal components, deleted the first eigenvector and eigenvalue, and then performed an inverse transformation to reconstruct ‘unbent’ landmark coordinates. Following the approach taken in other studies of threespine stickleback shape variation<sup>28,61,152</sup>, we then treated the resulting superimposed and ‘un-bent’ landmark coordinates as individual shape traits in subsequent analyses.

## 8. Patterns of body shape variation among the juvenile F<sub>2</sub> hybrids

We begin this supplementary discussion section by explaining a data visualisation technique that we employed to better understand and characterise juvenile shape variation in the F<sub>2</sub> hybrid mapping population in relation to trophic variation. We then discuss the most noteworthy patterns of shape variation, which were revealed by using this using technique in conjunction with reviewing digital photos of the F<sub>2</sub> hybrids and considering measured functional trait variation in the same individuals. Our morphometric data suggest several key features of F<sub>2</sub> body shape that seemed to show coherent variation across the stable isotope landscape towards benthic-like or limnetic-like forms. Importantly, for each body shape feature in the hybrids that could be related to known patterns of shape variation between parental forms, the corresponding benthic–limnetic shape-shift axis in the hybrids proved to be closely aligned with the niche score axis. For these features of shape, detailed below, the ‘B’ and ‘L’ hybrids tended to somewhat resemble pure Paxton benthics and limnetics, respectively.

A straightforward way to explore, understand, and illustrate patterns of body shape variation across the bivariate isotope landscape was to simultaneously compare F<sub>2</sub> hybrids among regions ‘L’, ‘B’, and ‘A’ (Fig. 1a; Supplementary Discussion Section 3). Our technique for visualising body shape variation among these landscape regions started with the 19 Procrustes-superimposed and ‘un-bent’ morphometric landmarks<sup>63,102</sup> (Methods; Supplementary Discussion Section 7; also see the inset key in Extended Data Fig. 3). The resulting 38 x- and y-coordinates were then used as individual variables in pairwise discriminant function analyses (DFAs) of F<sub>2</sub> hybrids from each of two partitions of the dataset, per DFA. We performed pairwise DFA using MORPHOJ ver. 1.04a<sup>160</sup>. In each analysis, all F<sub>2</sub> hybrids from one of the delineated landscape regions (‘L’, ‘B’, or ‘A’) composed the focal data partition, containing  $n = 91$ – $93$  individuals per region with complete morphometric and isotope data. This focal group was analysed together with a second data partition, consisting of all individuals with complete data ( $n = 335$ ) that fell outside the three focal regions. An overall ‘reference shape’ was derived from this larger data partition in each DFA, allowing the average shape of each focal group to be compared to a standard reference. In turn, this approach allowed us to draw inferences on shape variation among all three focal groups simultaneously. To accomplish this, we again used MORPHOJ to produce and overlay two wireframe diagrams (per pairwise comparison) based on resulting discriminant function (DF) scores for the two groups considered in each DFA. Each resulting pair of wireframes represented the mean shape of individuals in one of the three focal groups in relation to the mean reference shape, after

amplifying the between-group shape difference by a specified factor. An amplification factor of eightfold was used for Extended Data Fig. 4, for example. We only used DF scores to visualise shape variation in the manner described above; DF scores were not used for generating shape traits, identifying component traits of niche use, or QTL mapping.

Based on results of these wireframe renderings, we found that average head shape of the F<sub>2</sub> hybrids in region ‘L’ was noticeably limnetic-like in several respects, albeit only moderately so. The average position of the orbit (i.e., eye socket) in these individuals was ventrally shifted on the head with respect to the ‘B’ individuals (Extended Data Fig. 4). This difference was apparent in the shorter vertical separation (i.e., distance between y-coordinates) of landmark 11 (or 12) and landmark 17 in the ‘L’ individuals, on average. Similarly, the overall size of the orbit, which was captured in the relative positions of landmarks 15–17, was slightly larger in the ‘L’ individuals than the ‘B’ individuals. These differences in eye position and size between ‘L’ and ‘B’ appeared to be in the same direction as those observed between wild limnetics and benthics from Paxton Lake. According to McPhail’s classic illustration of the species pair<sup>16</sup>, for example, the limnetic species exhibits a smaller vertical distance than the benthic species between the ventral margin of the orbit (landmark 17 in our study) and the corner of the mouth (landmark 12 essentially). McPhail’s illustration also depicts a smaller vertical separation in the limnetic species between landmark 17 and the anterior-most extent of the preopercle along the ventral silhouette (landmark 11). In addition, both McPhail<sup>16</sup> and Matthews *et al.*<sup>17</sup> provide data implying that Paxton limnetics have a larger average eye width (relative to head length) than Paxton benthics. Our results suggest that such a pattern was recapitulated among the F<sub>2</sub> hybrids in pond no. 4, to some extent, along the niche score axis. Interestingly, however, threespine stickleback populations of the Cook Inlet Basin (Alaska)<sup>155</sup> showed a pattern of orbit size variation that differs from the findings reported for benthic-limnetic species-pair lakes in British Columbia<sup>16,17,123,161</sup>. Solitary-lake populations in the Cook Inlet Basin with more benthic-like head shapes exhibited larger residual mean eye sizes (from linear regression of vertical eye diameter against within-population mean centroid size of the skull) than solitary limnetic-like populations in the same region of Alaska<sup>155</sup>.

In the present study, F<sub>2</sub> hybrids in region ‘L’ also exhibited a larger average distance between the x-coordinates of landmarks 13 and 15 than hybrids from other regions of trophic space. A review of digital images of the F<sub>2</sub> hybrids hinted that, overall, ‘L’ individuals also tended to have a more elongated ventral head profile rostrally (i.e., along their lower ‘jaw lines’) than other individuals in the F<sub>2</sub> mapping population. Upon investigating this suggested difference more formally, we found that, on average, the ventral silhouette of the head in lateral view was elongated in ‘L’ individuals between the rotation point of the lower jaw — i.e., the point of articulation between the quadrate and articular bones<sup>19,127</sup> (near landmark 11) — and the rostral tip of the closed lower jaw (near landmark 13; Extended Data Fig. 4). This extended ‘jaw line’ seen in many ‘L’ individuals was associated with a large average shift (both dorsally and anteriorly) in the relative position of landmark 13 in this F<sub>2</sub> hybrid group, compared to other individuals in the mapping population. In fact, landmark 13 exhibited a greater average shift in relative position on the head, with respect to variation across isotope space, than any other landmark included in our study. This was evident in the large oblique shift of landmark 13 away from the centre of the body form (i.e., centroid) of ‘L’ individuals, on average, compared both to other landmarks and to other F<sub>2</sub> hybrids from different regions of trophic space (Extended Data Fig. 4).

Many morphological trait differences between Paxton benthic and limnetic sticklebacks breed true in a common laboratory environment<sup>16,122</sup>, yet phenotypic plasticity also contributes to variation in a number of traits in this species pair<sup>128</sup>. Phenotypic plasticity has been further documented in several other threespine stickleback populations. When subjected to experimental diet manipulations, for example,



individuals from Alaskan populations exhibited developmental plasticity in several aspects of body shape, including position of the upper lip's anterior tip<sup>97,154</sup>, which is located extremely close to our landmark 13. A diet consisting of limnetic prey caused the Alaskan sticklebacks to shift the anterior tip of their upper lip in a direction that was similar to the average shift seen in landmark 13 in 'L' individuals relative to the rest of the mapping population (Extended Data Fig. 4). Intriguingly, over all morphometric landmarks, we found that the x- and y-coordinates of landmark 13 exhibited among the best-supported statistical associations with niche score. Both coordinates (x13 and y13) were present in the overall-best model (i.e., minimum-AIC model) predicting niche score from body shape coordinates. Both of the landmark 13 coordinates were also included as explanatory variables in all other, essentially equally well-supported models ( $\Delta\text{AIC} \leq 2$ ; Supplementary Table 2). Accordingly, landmark 13 — anterodorsal extent of the maxilla — appears to be a strong candidate for having acted as one of the many important component traits contributing to niche use and whole-organism feeding performance in the Paxton Lake species pair.

In spite of the strong statistical association between landmark 13 and niche score, we found no QTLs for either x13 or y13 (Extended Data Fig. 3; Supplementary Table 3). This lack of QTLs for landmark 13 is consistent, both with an underlying genetic architecture of divergence in this trait possibly involving multiple genes with effects too small to be detected<sup>162</sup>, and with the potential for diet-induced plasticity to produce phenotypic variation in this trait. Given that developmental plasticity has been observed for several morphological characters in a number of threespine stickleback populations<sup>97,128,154</sup>, and that opportunity for diet-induced plasticity certainly occurred in pond no. 4 (Supplementary Discussion Section 3; Extended Data Fig. 1), phenotypic plasticity should be considered an important candidate cause of phenotypic variation among the F<sub>2</sub> hybrids in our study. Phenotypic plasticity is especially important to consider for traits that did not map to any QTLs but were nevertheless strongly associated with niche score, like the Cartesian coordinates of landmark 13. As with other whole-organism performance phenotypes, divergence in the component morphology of niche use in threespine stickleback almost certainly reflects the joint action of heritable genetic effects and phenotypic plasticity. In this regard, it may prove quite interesting to carry out additional investigations that help to: (1) quantify the potential for diet-induced plasticity in landmark 13, specifically in Paxton limnetics, benthics, and their hybrids; and (2) determine the extent to which phenotypic plasticity of landmark 13 affects feeding performance and diet in this species pair.

In the Paxton Lake stickleback system, the genetic architecture of trophic performance (described at length in the main paper) and diet-associated phenotypic variation in the relative position of landmark 13 (Extended Data Fig. 4) suggest a hypothesized process by which the interaction of genotypes underlying certain component traits and phenotypic plasticity in other component traits could accelerate niche divergence. Our main findings illustrate how phenotypic variation in component traits due to underlying genetic factors influence an emergent whole-organism performance phenotype, such as niche use. The specific environmental conditions that individuals in a population find most profitable should often depend on phenotypic variation in whole-organism niche use, which in turn depends on genotypes underlying the associated component traits. In animals, individuals are often able to seek out profitable microenvironments or specific resources within a habitat<sup>115,117</sup> (e.g., preferred foods), producing a potential effect of underlying genotypes on the environmental conditions that are most often experienced during an individual's own development and growth. More broadly, genotypes underlying whole-organism performance in parents will affect which environmental conditions prove most favourable for offspring growth, survival, and/or reproduction. As individuals and their offspring begin exploiting a novel habitat, the new environmental conditions of that habitat can induce new phenotypic variation in plastic traits that may not be directly affected by the genetic architecture of divergence in whole-organism

performance. If divergence in a plastic trait of this kind effectively has no underlying genetic basis, yet the trait is also an important component trait and the induced phenotypically-plastic variation is also directed toward the performance optimum in the new habitat, such a trait may ‘boost’ performance (and likely fitness) beyond that predicted from the genotypes of all other component traits. As a consequence, adaptation to new habitats and/or niche divergence may proceed more rapidly when such plastic ‘booster traits’ contribute to an individual’s whole-organism performance phenotype in a coherent direction with respect to selection on the genetically-based component trait variation. Owing to the lack of QTLs detected for landmark 13 in the present study, and the finding from other stickleback studies of coherent diet-induced plasticity in the anterior tip of the upper lip<sup>97,154</sup>, we hypothesize that ecological divergence between Paxton benthics and limnetics potentially could have been facilitated by this sort of interaction between the genetic architecture of niche use and one or more ‘booster traits’. We further speculate that the relative position of landmark 13 with respect to other landmarks on the head could potentially be an example of such a phenotypically plastic booster trait. Additional research is needed to test these hypotheses.

Among the most distinctive hallmarks of body shape divergence in the Paxton Lake species pair are the large overall body depth and prominent dorsal hump (immediately posterior to the neurocranium) that characterise the adult benthic form<sup>16,84</sup>. The dorsal hump contributes directly to the elevated capacity that benthics have for suction generation in comparison to limnetics<sup>18,19</sup>. In turn, greater suction forces during feeding, which Paxton benthic stickleback are predicted to be capable of generating, are thought to enhance foraging efficiency in littoral and shallow benthic habitats<sup>14,21,22</sup>. Mean body depth was greatest in the F<sub>2</sub> hybrids of region ‘B’, as illustrated by the large distance between y5 and y18 in the wireframe diagram for this group of hybrids compared to hybrids from other regions of trophic space (Extended Data Fig. 4). Upon examining digital images of fish specimens collected from pond no. 4, we identified a number of F<sub>2</sub> hybrids that appeared to have a distinctive dorsal hump, and most of these individuals belonged to landscape region ‘B’. Unfortunately, the relative height of the dorsal hump was not particularly well captured by our chosen morphometric landmarks, which were based, wherever possible, on homologous bony structures that could be identified reliably in images of stained juveniles. Landmarks like these are generally not well suited to detecting variation in soft, fleshy morphological characters<sup>102</sup>. However, height of the dorsal hump is directly related to anterior epaxial muscle height, which we did measure (Fig. 2f). Indeed, height of the anterior epaxial muscle was greater in ‘B’ individuals, on average, than any other group of F<sub>2</sub> hybrids (Extended Data Fig. 5b). Moreover, anterior epaxial muscle height was included as an explanatory variable in three of nine well-supported models predicting niche score from F<sub>2</sub> hybrid functional morphology ( $0 \leq \Delta\text{AIC} \leq 2$ ; Supplementary Table 1). In addition, we mapped anterior epaxial muscle height to a QTL on LG 14 (Supplementary Table 3).

Use of the data visualisation technique also suggested that the average snout shape of F<sub>2</sub> hybrids in landscape region ‘A’ was somewhat more similar to individuals in region ‘B’ than individuals in region ‘L’ (Extended Data Fig. 4). Conversely, relative eye position of the ‘A’ individuals appeared to be somewhat more similar to that of ‘L’ individuals than ‘B’ individuals (and slightly more similar to that of wild limnetics than wild benthics<sup>16</sup>). Based on body length (i.e., the Euclidean distance between landmarks 1 and 13), the individuals in region ‘A’ of trophic space were the smallest F<sub>2</sub> hybrids in the mapping population (Fig. 1a). The small body sizes of these juvenile F<sub>2</sub> hybrids, overall, were also apparent in the small values that the ‘A’ individuals exhibited for functional morphological traits measured as linear distances in and around the head (Fig. 2f). Examples of these traits include neurocranium outlever length (Extended Data Fig. 5d), buccal cavity length (Extended Data Fig. 5e), and gape (Extended Data Fig. 5f). The wireframe diagram for ‘A’ further suggested that the ‘A’ individuals

tended to be characterised, in particular, by slightly smaller relative head sizes (as scaled by centroid size for all landmarks) than other  $F_2$  hybrids in the mapping population. In the  $F_2$  hybrids of landscape region 'A', the average positions of many morphometric landmarks on the anterior region of the head generally appeared to be contracted toward the centre of the body form (i.e., centroid) to a slightly greater extent, perhaps, than landmarks located elsewhere on the body (Extended Data Fig. 4).

Lastly, we used the data visualisation technique described above to examine average body shape of the 15% of  $F_2$  hybrids that were located closest to the bivariate mode of the stable isotope distribution. Relative to the bivariate mean of the distribution, the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  mode was slightly skewed toward landscape region 'B' (Fig. 1a). The geometry of the experimental pond (Extended Data Fig. 1) potentially contributed to this slight skew in the stable isotope data, as the ratio of littoral/benthic to pelagic habitat in pond no. 4 likely supported a higher abundance of benthos and littoral food resources for threespine stickleback, compared to zooplankton and other pelagic food resources for threespine stickleback. Despite the slight skew in isotopes, the modal  $F_2$  hybrids nevertheless exhibited fairly intermediate trophic signatures of past diet (e.g., niche score and diet deviation score; Fig. 1, Extended Data Fig. 2) in relation to the overall mapping population in pond no. 4. In addition, we found that  $F_2$  hybrids near the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  mode exhibited an intermediate average body shape (results not shown) relative to  $F_2$  hybrids from each of the three isotopically extreme regions ('B', 'L', or 'A'). Not surprisingly, the modal  $F_2$  hybrids also tended to have intermediate phenotypic values for functional morphological traits (Extended Data Fig. 6). In contrast to this centrally located landscape region around the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  mode, regions 'B', 'L', and 'A' represented extreme 'corners' of a roughly triangle-shaped isotope distribution. As we have shown in detail above,  $F_2$  hybrids from regions 'B', 'L', and 'A' tended to exhibit more extreme features of body shape compared to all other  $F_2$  hybrids in the mapping population.

## 9. Testing for QTL clustering in the *Gasterosteus aculeatus* genome

We were interested in testing whether QTLs were non-randomly distributed either (1) among or (2) within linkage LGs, the numbering of which matches that used for the 21 chromosomes in the threespine stickleback's nuclear genome<sup>29,163</sup> (Supplementary Table 4). To accomplish the first aim, we conducted goodness-of-fit tests on the observed numbers of QTLs per LG (either all QTLs or just those for the morphological 'component traits' of niche use). These tests were performed under a null hypothesis of randomly distributed QTLs, according to a simple proportional model for the expected number of QTLs on each LG. Expectations for the tests were based on either the relative physical sizes of the corresponding chromosomes or the number of genes reported for each chromosome (Extended Data Table 1). Goodness of fit was evaluated using Pearson's chi-squared test, performed by means of the R function 'chisq.test'<sup>51</sup>. Given the small expected cell values in the contingency tables, we computed the  $P$ -value for each test by Monte-Carlo simulation (10,000 replicates per test). No matter how the test was constructed, the outcome favoured the alternate hypothesis that QTLs were non-randomly distributed in the genome:  $\chi^2_{20} = 45.17$ ,  $P = 0.0016$  (for all traits, with an expectation based on chromosome size);  $\chi^2_{20} = 55.76$ ,  $P = 0.0002$  (all traits, based on gene number);  $\chi^2_{20} = 34.87$ ,  $P = 0.0219$  ('component traits', based on chromosome size); and  $\chi^2_{20} = 39.12$ ,  $P = 0.0083$  ('component traits', based on gene number). Under each of these four test constructions, we also computed standardised residuals<sup>164</sup> ('stdres') as follows for each LG:

$$\text{stdres} = (\text{observed count} - \text{expected count}) / (\text{resvar})^{-1/2},$$

where 'resvar' is the residual cell variance<sup>164</sup>. Based on stdres, we identified which LGs contained more or fewer QTLs than expected by chance. Across groups, stdres values roughly sum up to the  $\chi^2$  value of

the corresponding goodness-of-fit test. Thus, *stdres* for each LG can be thought of as an approximate z-score from a standard normal distribution for that linkage group. Following Agresti<sup>164</sup>, we considered extreme values of *stdres* (i.e.,  $\text{stdres} \geq +2$  or  $\text{stdres} \leq -2$ ) to be significant at  $\alpha = 0.05$ . It was thereby revealed that significantly more QTLs than expected for component traits occurred on LGs 16 and 20, and that significantly more QTLs than expected for all traits occurred on LGs 4 and 16 (Extended Data Table 1).

To accomplish the second aim (testing whether QTLs were non-randomly distributed within LGs), we compared the vector of all QTL peak positions within each linkage group (i.e., 'Position' in cM in Supplementary Table 3) to the uniform probability distribution function, using a one-sample Kolmogorov-Smirnov test. Such an approach tests the null hypothesis that each detected QTL on a LG occurred with equal probability at any recombination distance along that LG. Kolmogorov-Smirnov tests for the different LGs were performed using the R function 'ks.test'<sup>51</sup>. Outcomes of these tests for LGs 4 and 16 favoured the alternate hypothesis of a deviation from randomly distributed QTLs along the respective LGs, indicating either clustering or overdispersion of QTLs. For LG 4,  $D = 0.4446$  and two-sided  $P = 0.00734$ ; for LG 16,  $D = 0.5418$  and two-sided  $P = 0.00531$ . In both cases, it was readily apparent that QTLs were clustered on the LGs rather than overdispersed (Extended Data Fig. 3; Supplementary Table 3).

## Supplementary Notes

### References cited only in Supplementary Tables and Supplementary Discussion

- 83 Larson, G. L. Social behavior and feeding ability of two phenotypes of *Gasterosteus aculeatus* in relation to their spatial and trophic segregation in a temperate lake. *Can. J. Zool.* **54**, 107-121 (1976).
- 84 Schluter, D. & McPhail, J. D. Ecological character displacement and speciation in sticklebacks. *Am. Nat.* **140**, 85-108 (1992).
- 85 Tieszen, L. L., Boutton, T. W., Tesdahl, K. G. & Slade, N. A. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for  $\delta^{13}\text{C}$  analysis of diet. *Oecologia* **57**, 32-37 (1983).
- 86 Hesslein, R. H., Hallard, K. A. & Ramlal, P. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by  $\delta^{34}\text{S}$ ,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$ . *Can. J. Fish. Aquat. Sci.* **50**, 2071-2076 (1993).
- 87 O'Reilly, C. M., Hecky, R. E., Cohen, A. S. & Plisnier, P.-D. Interpreting stable isotopes in food webs: recognizing the role of time averaging at different trophic levels. *Limnol. Oceanogr.* **47**, 306-309 (2002).
- 88 Post, D. M. Individual variation in the timing of ontogenetic niche shifts in largemouth bass. *Ecology* **84**, 1298-1310 (2003).
- 89 Matthews, B. & Mazumder, A. Consequences of large temporal variability of zooplankton  $\delta^{15}\text{N}$  for modeling fish trophic position and variation. *Limnol. Oceanogr.* **50**, 1404-1414 (2005).
- 90 Araújo, M. S., Bolnick, D. I., Machado, G., Giaretta, A. A. & dos Reis, S. F. Using  $\delta^{13}\text{C}$  stable isotopes to quantify individual-level diet variation. *Oecologia* **152**, 643-654 (2007).
- 91 Vander Zanden, H. B., Bjorndal, K. A., Reich, K. J. & Bolten, A. B. Individual specialists in a generalist population: results from a long-term stable isotope series. *Biol. Lett.* **6**, 711-714 (2010).
- 92 Vander Zanden, M. J. & Rasmussen, J. B. Primary consumer  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and the trophic position of aquatic consumers. *Ecology* **80**, 1395-1404 (1999).
- 93 Quevedo, M., Svanbäck, R. & Eklöv, P. Intrapopulation niche partitioning in a generalist predator limits food web connectivity. *Ecology* **90**, 2263-2274 (2009).
- 94 Ghan, D., Hyatt, K. D. & McPhail, J. D. Benefits and costs of vertical migration by the freshwater copepod *Skistodiaptomus oregonensis*: testing hypotheses through population comparison. *Can. J. Fish. Aquat. Sci.* **55**, 1338-1349 (1998).
- 95 Persson, J. & Vrede, T. Polyunsaturated fatty acids in zooplankton: variation due to taxonomy and trophic position. *Freshwater Biol.* **51**, 887-900 (2006).
- 96 Knudsen, R., Klemetsen, A., Amundsen, P.-A. & Hermansen, B. Incipient speciation through niche expansion: an example from the Arctic charr in a subarctic lake. *Proc. R. Soc. B* **273**, 2291-2298 (2006).
- 97 Wund, M. A., Valena, S., Wood, S. & Baker, J. A. Ancestral plasticity and allometry in threespine stickleback reveal phenotypes associated with derived, freshwater ecotypes. *Biol. J. Linn. Soc.* **105**, 573-583 (2012).
- 98 Lucek, K., Sivasundar, A. & Seehausen, O. Evidence of adaptive evolutionary divergence during biological invasion. *PLoS ONE* **7** (2012).
- 99 Hynes, H. B. N. The food of fresh-water sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. *J. Anim. Ecol.* **19**, 36-58 (1950).
- 100 Whiteside, M. C. & Swindoll, M. R. Guidelines and limitations to cladoceran paleoecological interpretations. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **62**, 405-412 (1988).

- 101 Hyslop, E. J. Stomach contents analysis — a review of methods and their application. *J. Fish Biol.* **17**, 411-429 (1980).
- 102 Zelditch, M. L., Swiderski, D. L. & Sheets, H. D. *Geometric Morphometrics for Biologists*. 2nd edn, (Academic Press, 2012).
- 103 Kalff, J. *Limnology: Inland Water Ecosystems*. (Prentice Hall, 2002).
- 104 Steinhart, G. B. & Wurtsbaugh, W. A. Winter ecology of kokanee: implications for salmon management. *T. Am. Fish. Soc.* **132**, 1076-1088 (2003).
- 105 Pangle, K. L., Sutton, T. M., Kinnunen, R. E. & Hoff, M. H. Overwinter survival of juvenile lake herring in relation to body size, physiological condition, energy stores, and food ration. *Trans. Am. Fish. Soc.* **133**, 1235-1246 (2004).
- 106 Bolnick, D. I. & Lau, O. L. Predictable patterns of disruptive selection in stickleback in postglacial lakes. *Am. Nat.* **172**, 1-11 (2008).
- 107 Martin, C. H. & Wainwright, P. C. Multiple fitness peaks on the adaptive landscape drive adaptive radiation in the wild. *Science* **339**, 208-211 (2013).
- 108 Roff, D. A. *Life History Evolution*. (Sinauer Associates, Inc., 2002).
- 109 Storz, J. F. Hemoglobin function and physiological adaptation to hypoxia in high-altitude mammals. *J. Mammal.* **88**, 24-31 (2007).
- 110 Losos, J. B. *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*. (University of California Press, 2009).
- 111 Székely, T., Moore, A. J. & Komdeur, J. *Social Behaviour: Genes, Ecology and Evolution*. (Cambridge University Press, 2010).
- 112 Wu, X. *et al.* Comparative genomics and functional analysis of niche-specific adaptation in *Pseudomonas putida*. *FEMS Microbiol. Rev.* **35**, 299-323 (2011).
- 113 Feder, J. L., Egan, S. P. & Forbes, A. A. Ecological adaptation and speciation: the evolutionary significance of habitat avoidance as a postzygotic reproductive barrier to gene flow. *Int. J. Ecol.* **2012**, Article ID 456374 (2012).
- 114 Keller, I. & Seehausen, O. Thermal adaptation and ecological speciation. *Mol. Ecol.* **21**, 782-799 (2012).
- 115 MacArthur, R. H. & Pianka, E. R. On optimal use of a patchy environment. *Am. Nat.* **100**, 603-609 (1966).
- 116 Wainwright, P. C. & Reilly, S. M. *Ecological Morphology: Integrative Organismal Biology*. (The University of Chicago Press, 1994).
- 117 Stephens, D. W., Brown, J. S. & Ydenberg, R. C. *Foraging: Behavior and Ecology*. (The University of Chicago Press, 2007).
- 118 Svanbäck, R. & Bolnick, D. I. Intraspecific competition drives increased resource use diversity within a natural population. *Proc. R. Soc. B* **274**, 839-844 (2007).
- 119 Grant, P. R. & Grant, B. R. *How and Why Species Multiply: The Radiation of Darwin's Finches*. (Princeton Univ. Press, 2008).
- 120 McPhail, J. D. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): origin of the species pairs. *Can. J. Zool.* **71**, 515-523 (1993).
- 121 Law, T. C. & Blake, R. W. Comparison of the fast-start performances of closely related, morphologically distinct threespine sticklebacks (*Gasterosteus* spp.). *J. Exp. Biol.* **199**, 2595-2604 (1996).
- 122 Ahn, D.-g. & Gibson, G. Axial variation in the threespine stickleback: genetic and environmental factors. *Evol. Dev.* **1**, 100-112 (1999).

- 123 Gow, J. L., Rogers, S. M., Jackson, M. & Schluter, D. Ecological predictions lead to the discovery of a benthic-limnetic sympatric species pair of threespine stickleback in Little Quarry Lake, British Columbia. *Can. J. Zool.* **86**, 564-571 (2008).
- 124 Cooper, I. A., Gilman, R. T. & Boughman, J. W. Sexual dimorphism and speciation on two ecological coins: patterns from nature and theoretical predictions. *Evolution* **65**, 2553-2571 (2011).
- 125 Kimmel, C. B. *et al.* Independent axes of genetic variation and parallel evolutionary divergence of opercle bone shape in threespine stickleback. *Evolution* **66**, 419-434 (2012).
- 126 Head, M. L., Kozak, G. M. & Boughman, J. W. Female mate preferences for male body size and shape promote sexual isolation in threespine sticklebacks. *Ecol. Evol.* **3**, 2183-2196 (2013).
- 127 Bell, M. A. & Foster, S. A. *The Evolutionary Biology of the Threespine Stickleback*. (Oxford University Press, 1994).
- 128 Day, T., Pritchard, J. & Schluter, D. Ecology and genetics of phenotypic plasticity: a comparison of two sticklebacks. *Evolution* **48**, 1723-1734 (1994).
- 129 Hatfield, T. Genetic divergence in adaptive characters between sympatric species of stickleback. *Am. Nat.* **149**, 1009-1029 (1997).
- 130 Hermida, M., Fernández, C., Amaro, R. & San Miguel, E. Heritability and “evolvability” of meristic characters in a natural population of *Gasterosteus aculeatus*. *Can. J. Zool.* **80**, 532-541 (2002).
- 131 Berner, D. *et al.* Quantitative genetic inheritance of morphological divergence in a lake–stream stickleback ecotype pair: implications for reproductive isolation. *J. Evol. Biol.* **24**, 1975-1983 (2011).
- 132 Albert, A. Y. K. & Schluter, D. Reproductive character displacement of male stickleback mate preference: reinforcement or direct selection? *Evolution* **58**, 1099-1107 (2004).
- 133 Clarke, J. M. & Schluter, D. Colour plasticity and background matching in a threespine stickleback species pair. *Biol. J. Linn. Soc.* **102**, 902-914 (2011).
- 134 Hoogland, R., Morris, D. & Tinbergen, N. The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as means of defence against predators (*Perca* and *Esox*). *Behaviour* **10**, 205-236 (1956).
- 135 Hagen, D. W. & Gilbertson, L. G. Geographic variation and environmental selection in *Gasterosteus aculeatus* L. in the Pacific Northwest, America. *Evolution* **26**, 32-51 (1972).
- 136 Reimchen, T. E. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators? *Can. J. Zool.* **58**, 1232-1244 (1980).
- 137 Cresko, W. A. *et al.* Parallel genetic basis for repeated evolution of armor loss in Alaskan threespine stickleback populations. *Proc. Natl. Acad. Sci. USA* **101**, 6050-6055 (2004).
- 138 Shapiro, M. D. *et al.* Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature* **428**, 717-723 (2004).
- 139 Colosimo, P. F. *et al.* Widespread parallel evolution in sticklebacks by repeated fixation of *Ectodysplasin* alleles. *Science* **307**, 1928-1933 (2005).
- 140 Chan, Y. F. *et al.* Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx1* enhancer. *Science* **327**, 302-305 (2010).
- 141 Miller, C. T. *et al.* Modular skeletal evolution in sticklebacks is controlled by additive and clustered quantitative trait loci. *Genetics*, early online publication, doi:10.1534/genetics.1114.162420 (Mar 19, 2014).
- 142 Sanderson, S. L., Cech, J. J. J. & Patterson, M. R. Fluid dynamics in suspension-feeding blackfish. *Science* **251**, 1346-1348 (1991).
- 143 Kahilainen, K. K. *et al.* The role of gill raker number variability in adaptive radiation of coregonid fish. *Evol. Ecol.* **25**, 573-588 (2011).

- 144 Carroll, A. M., Wainwright, P. C., Huskey, S. H., Collar, D. C. & Turingan, R. G. Morphology predicts suction feeding performance in centrarchid fishes. *J. Exp. Biol.* **207**, 3873-3881 (2004).
- 145 Coughlin, D. J. & Strickler, J. R. Zooplankton capture by a coral reef fish: an adaptive response to evasive prey. *Env. Biol. Fish.* **29**, 35-42 (1990).
- 146 Westneat, M. W. Evolution of levers and linkages in the feeding mechanisms of fishes. *Integr. Comp. Biol.* **44**, 378-389 (2004).
- 147 Holzman, R., Day, S. W., Mehta, R. S. & Wainwright, P. C. Jaw protrusion enhances forces exerted on prey by suction feeding fishes. *J. R. Soc. Interface* **5**, 1445-1457 (2008).
- 148 Jiang, H. & Kjørboe, T. Propulsion efficiency and imposed flow fields of a copepod jump. *J. Exp. Biol.* **214**, 476-486 (2011).
- 149 Winemiller, K. O. Ecomorphological diversification in lowland freshwater fish assemblages from five biotic regions. *Ecol. Monogr.* **61**, 343-365 (1991).
- 150 Douglas, M. E. & Matthews, W. J. Does morphology predict ecology? Hypothesis testing within a freshwater stream fish assemblage. *Oikos* **65**, 213-224 (1992).
- 151 Langerhans, R. B. Predictability of phenotypic differentiation across flow regimes in fishes. *Integr. Comp. Biol.* **48**, 750-768 (2008).
- 152 Walker, J. A. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc.* **61**, 3-50 (1997).
- 153 Hendry, A. P., Hudson, K., Walker, J. A., Räsänen, K. & Chapman, L. J. Genetic divergence in morphology-performance mapping between Misty Lake and inlet stickleback. *J. Evol. Biol.* **24**, 23-35 (2011).
- 154 Wund, M. A., Baker, J. A., Clancy, B., Golub, J. L. & Foster, S. A. A test of the “flexible stem” model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. *Am. Nat.* **172**, 449-462 (2008).
- 155 Willacker, J. J., von Hippel, F. A., Wilton, P. R. & Walton, K. M. Classification of threespine stickleback along the benthic–limnetic axis. *Biol. J. Linn. Soc.* **101**, 595-608 (2010).
- 156 Armstrong, J. S. *Principles of Forecasting: A Handbook for Researchers and Practitioners*. (Springer Science+Business Media, Inc., 2001).
- 157 Legendre, P. Studying beta diversity: ecological variation partitioning by multiple regression and canonical analysis. *J. Plant Ecol.* **1**, 3-8 (2008).
- 158 Kitano, J., Mori, S. & Peichel, C. L. Sexual dimorphism in the external morphology of the threespine stickleback (*Gasterosteus aculeatus*). *Copeia* **2007**, 336-349 (2007).
- 159 Peichel, C. L. *et al.* The master sex-determination locus in threespine sticklebacks is on a nascent Y chromosome. *Curr. Biol.* **14**, 1416-1424 (2004).
- 160 Klingenberg, C. P. MORPHOJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* **11**, 353-357 (2011).
- 161 McPhail, J. D. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. *Can. J. Zool.* **62**, 1402-1408 (1984).
- 162 Lynch, M. & Walsh, B. *Genetics and Analysis of Quantitative Traits*. (Sinauer Associates, Inc., 1998).
- 163 Chen, T.-R. & Reisman, H. M. A comparative chromosome study of the North American species of sticklebacks (Teleostei: Gasterosteidae). *Cytogenetics* **9**, 321-332 (1970).
- 164 Agresti, A. *An Introduction to Categorical Data Analysis*. 2nd edn, (John Wiley & Sons, Inc., 2007).