Transgenerational selection driven by divergent ecological impacts of hybridizing lineages

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Dynamic interactions between ecological conditions and the phenotypic composition of populations likely play an important role in evolution, but the direction and strength of these feedbacks remain difficult to characterize. We investigated these dynamics across two generations of threespine sticklebacks from two evolutionary lineages undergoing secondary contact and hybridization. Independently manipulating the density and lineage of adults in experimental mesocosms led to contrasting ecosystem conditions with strong effects on total survival in a subsequent generation of juveniles. Ecosystem modifications by adults also varied the strength of selection on competing hybrid and non-hybrid juveniles. This variation in selection indicated (1) a negative eco-evolutionary feedback driven by lineage-specific resource depletion and dependence and (2) a large performance advantage of hybrid juveniles in depleted environments. This work illustrates the importance of interactions between phenotype, population density and the environment in shaping selection and evolutionary trajectories, especially in the context of range expansion with secondary contact and hybridization.

There is mounting evidence that phenotypic differences can both shape and be shaped by dynamic interactions between organisms and their environment[1–5]. Previous studies suggest that recent evolutionary divergence can impact the same ecological conditions (for example, resource abundance and composition[6–9]) that affect selection on heritable phenotypes[10–12]. Work across a broad range of systems shows resulting feedbacks between ecological and evolutionary processes when they occur on similar timescales[13,14]. However, moving from documenting the existence of such dynamics to understanding what controls their magnitude and direction across a variety of complex systems has only just begun and remains a major challenge at the intersection of ecology and evolution.

Interest in quantifying the nature and impact of eco-evolutionary dynamics has fuelled the development of theory linking ecological processes to trait evolution[13,15], as well as the exploration of increasing complexity in those dynamics observed in nature. Theoretical investigations of eco-evolutionary dynamics have demonstrated that they can either promote or constrain phenotypic divergence and that this outcome depends on the nature of resource use and supply[16], the complexity of species interactions[13], the temporal overlap in rates of ecological and evolutionary processes[13] and the way that changing trait frequencies impact population density[15]. Despite this expanding body of theory, it remains challenging to formulate general predictions about the way eco-evolutionary dynamics alter evolutionary trajectories[17].

Empirical work in individual study systems mirrors the complexity of theoretical predictions, with eco-evolutionary feedbacks in nature depending on abiotic variation in time and space[18], community complexity[19], standing genetic variation[20] and density-dependent influence on resource availability[10,21]. The focus on population density in particular has served as an important bridge between

theoretical and empirical work, with both viewing population density as the ecological link in a chain of evolutionary events[21,22]. This is the basis for a recent re-emphasis on soft selection[1], whereby selection results from the interaction between population density and phenotype frequencies, rather than from fixed links between phenotype and fitness. Work in the Trinidadian guppy system has demonstrated the importance of population density in eco-evolutionary dynamics, with predation intensity and density-mediated per-capita resource availability driving repeated life history divergence[1,10]. However, both theory and experiments have demonstrated that variation in predator density can impact not only total prey availability, but also prey composition[16,23]. While some increases in density may promote the simultaneous use of alternate resources and facilitate dietary differentiation among interacting competitors[24,25], high population densities may also reduce the availability of alternative resources to a point where no dietary segregation can occur[23,26]. Thus, the ecological component of eco-evolutionary feedbacks is not simply the population ecology of the focal species (that is, its density), but also the community and ecosystem ecology of the whole system, which defines the composition and rate of supply of multiple available resources.

One approach to translating the potential complexity of factors contributing to eco-evolutionary dynamics into general predictions is to use experiments that isolate the impacts of both phenotype and population density on the dynamics of multiple resources and test how this affects selection in subsequent generations. This approach can help pull apart mechanisms by which eco-evolutionary interactions might control the rate of evolutionary divergence, convergence or directional change. Here, we used a two-phase mesocosm experiment with adult and juvenile sticklebacks to investigate their ecosystem impacts and subsequent effects on selection dynamics. First, we independently manipulated the density and multivariate

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phenotype of adult sticklebacks (using two distinct evolutionary lineages) and measured the effects on replicate aquatic environments (1,000 l mesocosms seeded with equal quantities of diverse benthic and planktonic algae and invertebrates). Second, we evaluated the consequences of these ecosystem modifications for the direction and strength of selection on juveniles from both parental lineages and their hybrids. Combining both elements of an eco-evolutionary feedback in a single experiment is essential for determining how the particular ecological effects induced by phenotypic differences (as opposed to externally imposed ecological contrasts) translate into further selection on phenotypic variation.

The threespine stickleback (*Gasterosteus aculeatus*) is a species complex widely distributed throughout northern temperate oceans and in lakes and streams, many of which were colonized in the late Pleistocene or early Holocene. In Switzerland, documented introductions in the 1870s seeded a southwestern (Rhône) lineage into Lake Geneva and a northeastern (Baltic) lineage into Lake Constance—two watersheds at opposite ends of the country[27] (Fig. 1a). Sticklebacks of the Lake Geneva lineage differ from the Lake Constance lineage in traits related to defense (fewer lateral plates) and feeding morphology (deeper bodies, shorter gill rakers and smaller eyes) (Fig. 1b–d and refs [28–30]), with some of these traits

linked to quantitative trait loci[31]. The combination of traits in Lake Geneva fish predicts higher feeding efficiency on benthic prey[31–33] and is probably responsible for their lower feeding efficiency on pelagic prey (Fig. 1e). These differences are consistent with divergent habitat selection in the period before their introduction to the large Swiss lakes; while the Geneva lineage occupied smaller streams in western Europe, the Constance lineage probably evolved in marine and large-lake habitats in northern Europe (Lucek, K., et al., manuscript in preparation). In addition, recent hybridization between these two lineages has resulted in a wide range of populations with variable phenotypes colonizing new waterbodies across western Switzerland[27,34].

Hybridization events involving the spread of populations with variable densities and variable compositions and frequencies of genotypes and phenotypes provide an excellent opportunity for investigating the interplay between ecological and evolutionary dynamics[35]. In this context, first, we predicted that the contrasting phenotypes that have evolved in these two lineages would, along with density, lead to differences in ecosystem properties such as resource availability, habitat structure and productivity. Second, we predicted that these ecosystem modifications would impact the total and relative success of a subsequent generation of juveniles representing the two
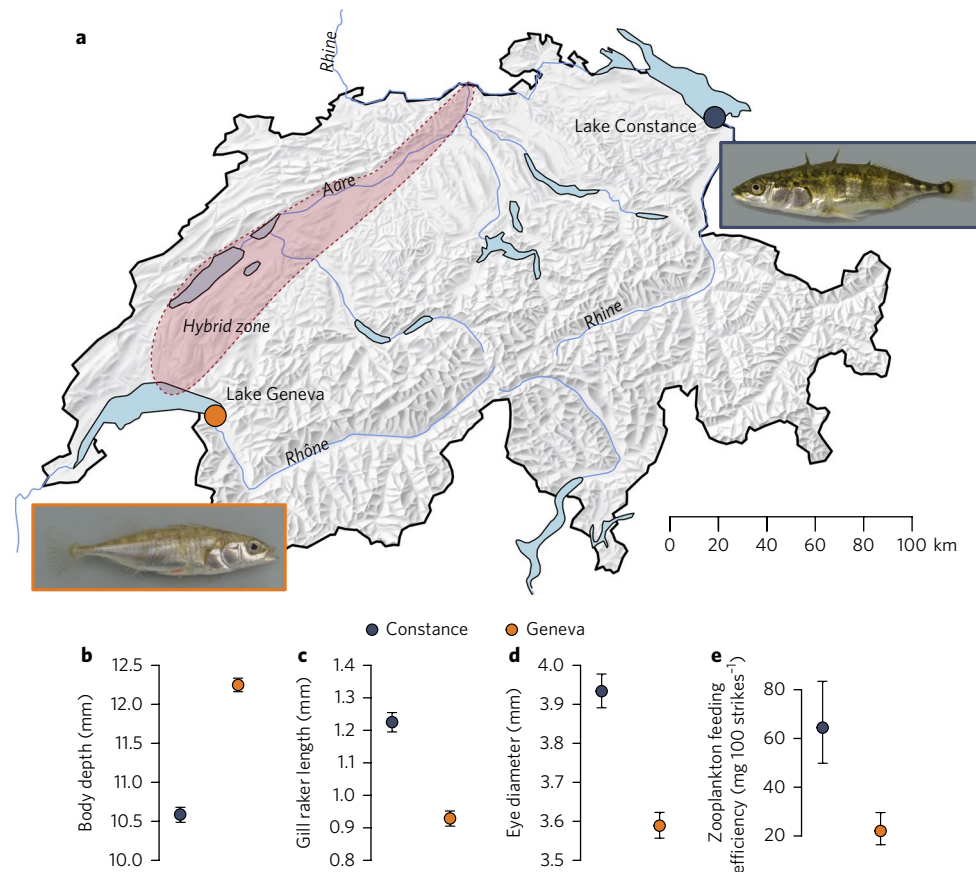

Fig. 1 | Location and phenotypic divergence of two major evolutionary lineages of threespine sticklebacks in Switzerland. a, Map showing the locations of the study populations from the Lake Constance and Lake Geneva lineages, as well as the known extent of the hybrid zone (shown in pink). This zone is based on ten sampled populations falling into three genetic clusters, each of which has been identified as comprising individuals representing multiple generations of inter-lineage hybrids (NEU, Neuchatel; WOH, Wohlensee; GIP, Gippingen in ref. [34]). Analysis of population genetic structure and connectivity based on 17 microsatellite markers has shown that these populations are all substantially connected by migration to each other and/or one of the nonhybrid Geneva or Constance populations [34]. Insets, images showing the typical morphology of each lineage. **b–d**, Data for morphological traits related to feeding efficiency, including body depth (**b**), gill raker length (**c**) and eye diameter (**d**) were obtained from ref. [30] and standardized for average fish length across all individuals ($n = 28$ for the Constance lineage and $n = 58$ for the Geneva lineage). **e**, The efficiency of feeding on macrozooplankton was measured in the laboratory as described in ref. [58] ($n = 19$ for the Constance lineage and $n = 16$ for the Geneva lineage). The data represent population means ± 1 s.e. $P < 0.007$ for all population differences assessed using a 2-sample *t*-test. Fig. 1a base map: Federal Office of Topography swisstopo.

lineages and their F1 hybrids. Specifically, we aimed to determine whether eco-evolutionary feedbacks help to maintain existing phenotypic diversity (if adults modify ecosystems in ways that favour juveniles of the other lineage), facilitate further phenotypic divergence (if ecosystem modifications favour juveniles of the same lineage) or reduce phenotypic divergence (if ecosystem modifications favour hybrids). Our approach expands on previous research on hybrid zones by explicitly investigating the impact of phenotype–environment interactions on the relative performance of hybrids, as opposed to external environmental drivers. Using detailed data on prey community composition and fish dietary signatures, we integrated the results from both phases of the experiment to examine the mechanisms of broad-sense eco-evolutionary dynamics, where one type of evolutionary change dynamically alters ecology in a way that impacts further evolution[4]. This approach links the effects of adult fish on resource availability to the form and intensity of selection on juveniles representing the subsequent generation.

Results

Phase I: effects of adult fish on aquatic ecosystems. After five weeks with adult sticklebacks, ecosystem parameters in the mesocosms were modified by fish density, lineage or their interaction (Fig. 2 and Supplementary Table 1). Whereas higher fish density increased the abundance of benthic invertebrates (Fig. 2a), lineage marginally impacted the average benthic prey size (Fig. 2b), with Geneva fish leaving smaller individual prey (mostly mayflies and chironomids; Supplementary Table 2). For zooplankton, the abundance and mean prey mass were interactively affected by fish density and lineage (Supplementary Table 1). At low density, Geneva fish left more but somewhat smaller zooplankton, while at high density the same lineage left fewer, larger zooplankton (Fig. 2c,d).

We also found strong effects of fish lineage and density on macrophyte structure. The cover of filamentous algae, which can produce thick mats covering the bottom and impacting fish foraging, was strongly diminished by increasing fish density (Fig. 2e). In addition, the three-dimensional macroalgae (*Chara* species) produced higher numbers of upright shoots in mesocosms with Geneva fish, even at low fish density (Fig. 2f). Finally, both the presence and the lineage of adult fish affected the overall ecosystem gross primary productivity (GPP; Fig. 2g).

Phase II: effects of ecosystem variability on juvenile survival and condition. After the adults were removed, the juveniles were added and allowed to feed and grow for six weeks. These juveniles had been bred in the laboratory from fish collected at the same time and place as the mesocosm adults, but not from the mesocosm adults themselves (see Methods). Thus, all mesocosm impacts on juveniles were mediated by the ecological effects of the adults rather than direct maternal effects. Total juvenile survival was strongly influenced by the evolutionary lineage (and not the density) of the preceding adult generation, with the ecosystem effects of the Geneva adults leading to lower total survival (Fig. 3a). There were also extremely strong genetic effects on the relative survival and condition (length-standardized wet weight) of the juveniles: hybrids had higher survival and condition than Geneva juveniles, which did better than Constance juveniles (Fig. 3b,c). We also found an effect of maternal lineage within the hybrids in the same direction as the contrast between parental types: hybrids with Geneva mothers had higher average body condition than those with Constance mothers ($P < 0.0001$; Fig. 3c).

Despite strong effects of adult lineage on ecosystem conditions and total juvenile survival, and strong differences in performance among the three types of juveniles, we did not detect any interactions between adult treatment and relative juvenile survival or condition. However, particular ecological effects of adult lineage did mediate
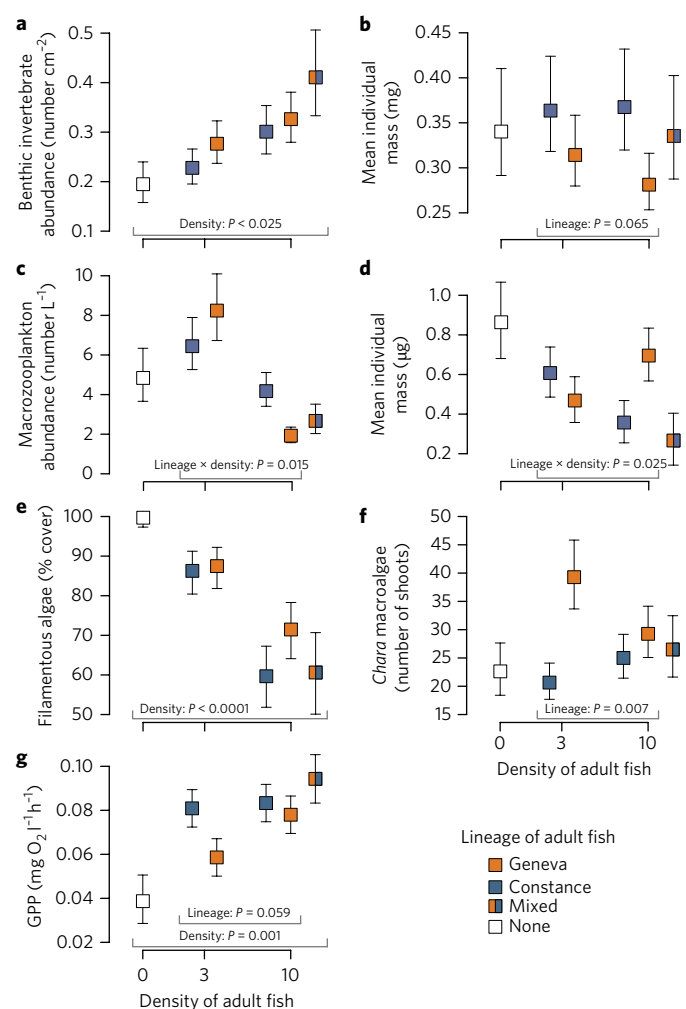

Fig. 2 | Effects of adult fish on experimental ecosystems, including prey availability, habitat structure and GPP. a, Abundance of benthic invertebrates remaining in the environment. **b**, Mean individual mass of benthic invertebrates remaining in the environment. **c**, Abundance of macrozooplankton remaining in the environment. **d**, Mean individual mass of macrozooplankton remaining in the environment. **e**, Cover of filamentous algae. **f**, Number of upright shoots of *Chara* macroalgae. **g**, GPP. Treatments included fish-free controls ($n = 5$), low-density mesocosms with three Geneva or three Constance adults ($n = 10$), high-density mesocosms with ten Geneva adults or ten Constance adults ($n = 10$) and a mixed-lineage treatment with five fish from each lineage ($n = 5$). The data represent treatment means ± 1 s.e. and the effects are highlighted for the core factorial density x lineage contrast (the full statistical results are shown in Supplementary Table 1) and additionally for a density contrast across all treatments. The statistical tests performed were linear mixed models.

both the total and relative juvenile survival. First, larger benthic prey and fewer upright macrophytes increased the total juvenile survival (Fig. 4a,b and Table 1). These ecological variables also had strong effects on the relative juvenile survival, with the combination of juvenile type and ecological parameters explaining 64% of the variation in survival (Table 1). The number of survivors of each type was differentially dependent on variation in benthic prey size, productivity and algal cover. In particular, a larger mean benthic prey mass increased the survival of Geneva juveniles but not that of Constance or hybrid juveniles (Fig. 4c and Table 1). Furthermore, the proportion of survivors that were hybrids increased from 40 to 80% as the total juvenile survival decreased (Fig. 4d).
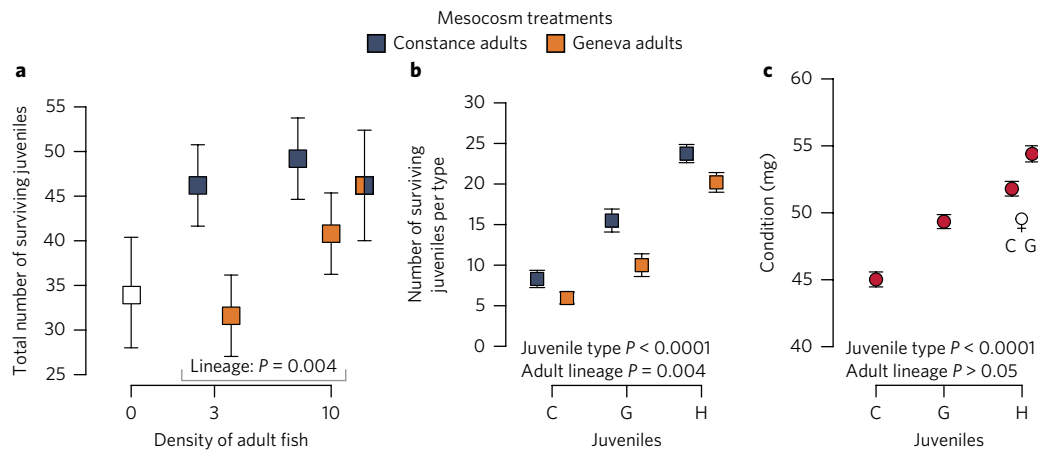
Fig. 3 | Juvenile survival and condition. a, Total juvenile survival was affected by the preceding adult lineage (not density) and was lower in mesocosms modified by Geneva adults (the initial number of juveniles was 99 per mesocosm; 33 of each type). **b,c,** The relative survival (**b**) and body condition, measured as the residual wet weight for a fish of average standard length (**c**), both differed strongly and consistently between Constance (C), Geneva (G) and hybrid (H) juveniles. The body condition also differed within the hybrids depending on the lineage of the mother. The data points represent means ± 1 s.e. and the statistical tests used were linear mixed models.

Variation in the performance and survival of juveniles might reflect differences in nutritional status due to differences in diet, which we quantified using stable isotopes at the end of the experiment. All three types of juvenile differed in their $\delta^{15}N$ signatures (Fig. 5a) and hybrids and Geneva juveniles differed in their $\delta^{13}C$ signatures. The range of invertebrate taxa sampled at the end of phase I also had distinct C and N signatures (Fig. 5b). In general, the higher $\delta^{15}N$ values in the Geneva juveniles could have been associated with consumption of more easily accessible, soft-bodied prey such as mayfly larvae rather than more difficult-to-digest prey such as snails. Consuming prey with higher $\delta^{15}N$ signatures had a positive impact on the condition of both Constance and Geneva juveniles (Supplementary Fig. 1; $P < 0.0001$). In contrast, the higher average condition of the hybrids was not correlated with dietary signature, either within the hybrids (Supplementary Fig. 1; $P = 0.31$) or among the juvenile types (the hybrids had the highest condition (Fig. 3c) but only intermediate $\delta^{15}N$ (Fig. 5a)).

Parallel laboratory experiment: baseline differences in condition. As the relative condition of the hybrid and parental-type juveniles may be due to either intrinsic morphological and physiological differences or ecological factors[36,37], we assessed condition in a parallel laboratory study. In the laboratory experiment, with low mortality, hybrids and Geneva juveniles reached the same final condition (Supplementary Fig. 2). However, in the mesocosm experiment hybrids maintained substantially higher condition than both Geneva and Constance juveniles (Fig. 3c). Thus, relative fitness among juveniles of different types was ecologically dependent: hybrids had a larger performance advantage in low-resource, high-mortality conditions.

Discussion

Previous theoretical and empirical work on eco-evolutionary dynamics has highlighted the importance of interactions between the density and phenotypic composition of populations for determining their ecological impacts and evolutionary trajectories[1,4]. However, these interactions are rarely investigated within single experiments, where the ecological change created in the first part of an eco-evolutionary interaction can be directly translated to selection (evolution) in the second part. This is especially true for eco-evolutionary dynamics involving diverse resources. To the best of our knowledge, this approach to quantifying eco-evolutionary

interactions has also never been applied in the context of hybridization between lineages in secondary contact, even though range expansions leading to secondary contact are predicted to increase with global change.

We found that adult density and lineage affected different ecosystem components in different ways, but that the predominant effect on the total survival of juvenile fish was due to adult lineage (Fig. 3a). This result was further substantiated by the finding that the ecosystem parameters that were quantitative predictors of total juvenile survival were also those driven by lineage rather than density: the size composition of benthic invertebrates and the density of upright macrophytes (Figs. 2 and 4a,b and Table 1). The lineage effect on benthic prey size (which Geneva adults decreased; Fig. 2b) is consistent with morphological differences (Fig. 1), which predict greater success capturing benthic invertebrates for Geneva fish[28,31,33]. The subsequent effects on total juvenile survival (Fig. 4a,b) were also consistent with research showing that larger prey (up to a limit determined by fish gape) provide a higher net energy return[38], and that the complex habitat structure provided by *Chara* species macroalgae can decrease predation efficiency on benthic invertebrates[39]. Both of these mechanisms could influence survival, creating links between the phenotype-specific ecological effects of one generation and selection in the next.

The structure of the benthic prey community impacted not only on total survival but also the magnitude of the selection differential (Fig. 4e), inducing negative feedback in the connection between phenotype and fitness across generations. Geneva adults reduced the availability of larger benthic prey, which were particularly beneficial to Geneva juveniles. Given that larger prey require longer handling times and there is therefore a greater probability that they will be intercepted by a superior consumer, morphological adaptations in the Geneva lineage may have been particularly beneficial in competitive environments. The ability of Geneva juveniles to profit from this prey type is further supported by stable isotope signatures. High $\delta^{15}N$ signatures in Geneva juveniles (Fig. 5a), which they may have obtained from chironomids and mayfly larvae (Fig. 5b), were associated with higher body condition (Supplementary Fig. 1). This type of negative feedback should be an important feature of eco-evolutionary dynamics across generations whenever multiple life stages share strategies for resource use, and can directly impact coexistence potential via negative frequency-dependent selection[40]. Thus, this process deserves explicit
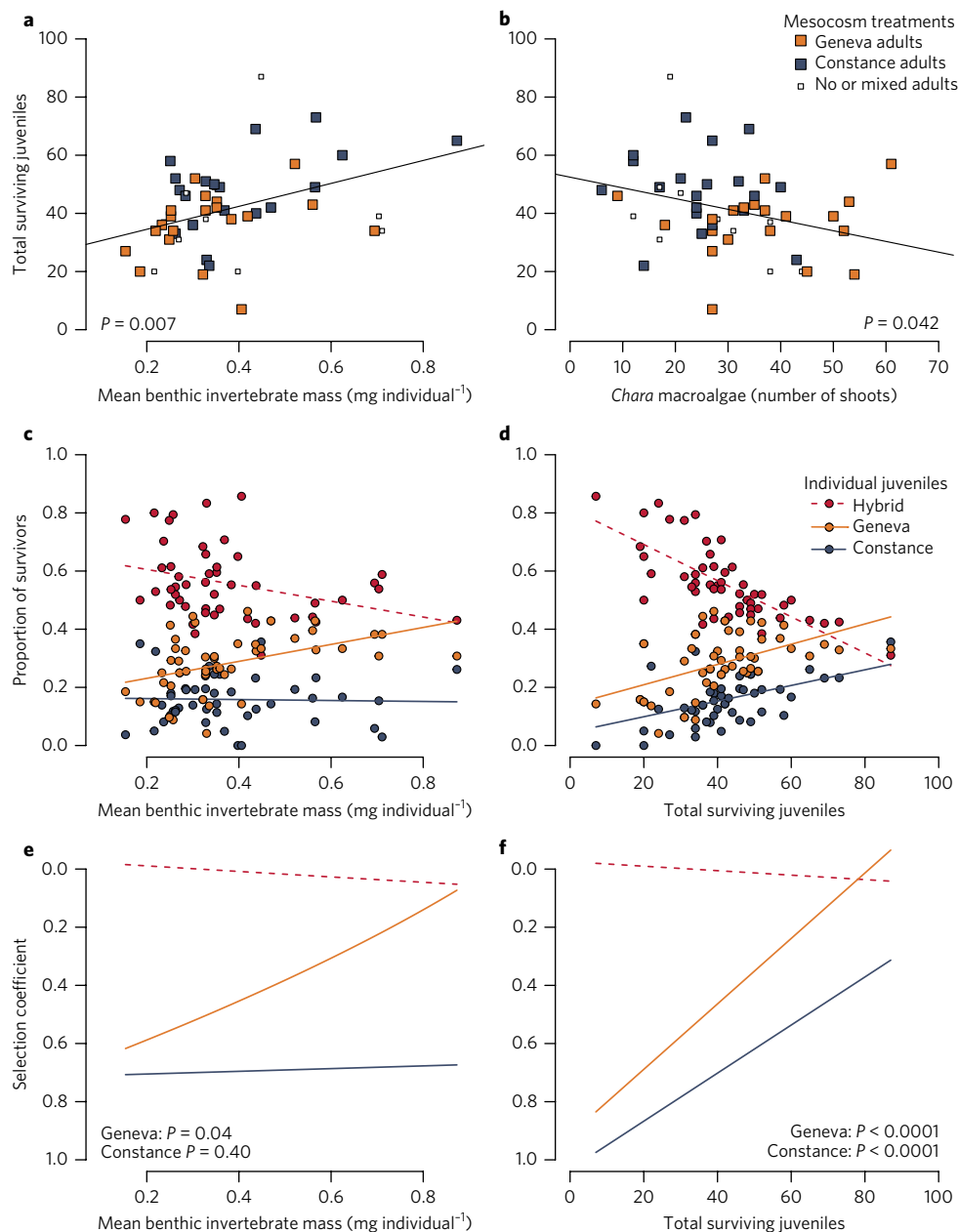
Fig. 4 | Determinants of selection on juveniles. a–f, The total survival of juveniles was related to the benthic prey mass (**a**) and abundance of upright algae (**b**), which were both impacted by the lineage of the preceding adults. The relative survival of juveniles was also differentially dependent on the benthic prey mass (**c**) and varied with total juvenile survival (**d**). This translated into variable selection against Geneva juveniles (**e**), which experienced increasingly negative selection with decreasing benthic prey size, and against both parental types (**f**), which experienced strong negative selection under challenging conditions when juvenile survival was limited. The statistical tests used were linear mixed models, and the full results are shown in Table 1.

attention in the theoretical and empirical development of our understanding of eco-evolutionary processes.

An additional key insight from this feedback based on the size structure of prey is that eco-evolutionary dynamics can be mediated by changes to the multivariate trait structure of prey communities. This is a more complex kind of ecological change than the abundance of a single homogeneous resource, which is the implicit variable underlying population density effects in many studies. Thus, the nature of selection in real ecosystems is determined not only by environment-independent fitness consequences (hard selection), and not only by the population ecology (density and phenotype frequency) of the focal species (soft selection). In reality, the population ecology of a focal species also interacts with complex ecosystems in

ways that are central to community and ecosystem ecology—via the composition and structure of multiple alternate resources with their own population dynamics. The importance of resource composition is also underscored in our experiment by the different effects of benthic and planktonic prey. Despite the fact that Constance fish are more efficient at capturing zooplankton (Fig. 1e), we detected neither consistent lineage effects on zooplankton abundance nor consequences of zooplankton availability for the survival of Constance juveniles. This is probably because the profitability of this alternate niche was limited by low zooplankton density at the end of the adult phase. This could curtail the potential for divergence[23,24,26] and cause selection to depend on the composition of the primary available resource (benthic invertebrates) rather than alternative resources.

Table 1 | Statistical results for juvenile survival and condition

Response	Fixed effect	F	Degrees of freedom	P^a	$R^2_{marginal}{}^b$	$R^2_{conditional}{}^b$
Total survivors[c]	Mean benthic invertebrate mass	8.44	1, 32.8	0.007	0.324	0.324
	Mean zooplankton mass	0.00	1, 38.9	0.954		
	Gross primary productivity	1.77	1, 29.1	0.194		
	Filamentous algae cover	3.36	1, 30.1	0.077		
	Number of *Chara* macroalgae shoots	4.43	1, 37.9	0.042		
Survivors[d]	Juvenile type	194.83	2, 86	<0.0001	0.638	0.814
	Mean benthic invertebrate mass	8.46	1, 32.8	0.006		
	Mean zooplankton mass	0.01	1, 38.9	0.929		
	Gross primary productivity	1.72	1, 29.1	0.200		
	Filamentous algae cover	3.32	1, 30.1	0.079		
	Number of *Chara* macroalgae shoots	4.37	1, 37.9	0.043		
	Juvenile type × mean benthic invertebrate mass	3.65	2, 86	0.030		
	Juvenile type × mean zooplankton mass	0.88	2, 86	0.419		
	Juvenile type × gross primary productivity	3.62	2, 86	0.031		
	Juvenile type × filamentous algae cover	4.22	2, 86	0.018		
	Juvenile type × number of *Chara* macroalgae shoots	0.30	2, 86	0.742		
Condition[e]	Juvenile type	39.819	2, 474.5	<0.0001	0.218	0.222
	$\delta^{13}C$	2.245	1, 469.5	0.135		
	$\delta^{15}N$	20.801	1, 477.7	<0.0001		
	Juvenile type × $\delta^{13}C$	0.870	2, 435.5	0.420		
	Juvenile type × $\delta^{15}N$	3.656	2, 496.6	0.027		

[a]Variables with $P < 0.05$ were also identified as the best combination of predictor variables using a model selection approach. Excluding any of these variables caused the Akaike Information Criterion (small sample-size corrected, AICc) to increase by more than 2, indicating less support[57]. [b]$R^2_{marginal}$ and $R^2_{conditional}$ give the percent variance explained by all fixed effects, and by both fixed and random effects (mesocosm and block), respectively. [c]One data point per mesocosm ($n = 50$); random effect = block. [d]One data point per type of juvenile per mesocosm ($n = 150$); random effects = block + mesocosm. [e]One data point per individual fish assayed for stable isotopes, with one individual with an extreme δC value removed ($n = 515$); random effects = block + mesocosm: response was log-transformed for analysis.

Variation in the success of non-hybrid juveniles also translated into dramatic consequences for selection on hybrids. In several previous studies, hybrids between ecologically divergent sticklebacks showed intermediate feeding strategies, which may be disadvantageous in natural resource environments[36]. For example, F1, F2 and multi-generation hybrids between sympatric benthic and limnetic species of threespine stickleback consistently have lower feeding efficiency in both parental habitats[32,37,41,42]. Given a shift in environmental conditions, the same hybrid strategies can be successful and ecological isolation can break down[43]. In our experiment, however, the survival and condition of F1 hybrids was uniformly high and independent of prey size and juvenile stable isotope signatures, whereas non-hybrid condition depended on $\delta^{15}N$. Because of their lower sensitivity to environmental contexts, the relative advantage of the hybrids was dramatically higher under conditions that were challenging for non-hybrid juveniles (Fig. 4d,f). This contrast among mesocosms was also consistent with the contrast between mesocosms and the laboratory environment. Whereas hybrids had superior condition in the mesocosms (where total survival ranged from around 10 to 90%), under the more benign conditions in the laboratory (very close to 100% survival), hybrid and Geneva juveniles had equal condition at the end of the experimental period (Supplementary Fig. 2).

Without additional information on ecologically relevant traits at the individual level, it was not possible to determine whether consistent hybrid success across the full range of ecological conditions in this experiment came from transgressive[44] or intermediate trait values. The high performance in F1 hybrids could have resulted from increased heterozygosity overcoming the effects of mildly deleterious alleles[45] or from novel combinations of dominant alleles at

different loci[46]. Previous work testing for context dependency in these types of advantages has shown mixed results. For example, heterozygosity can have a strong positive impact on fitness regardless of competition[47], provide an advantage via superior immune condition only in stressful environments[48] or be strongest in benign or productive conditions[49]. Heterosis can also lead to an increased range of tolerated stress conditions[50] or more consistent fitness within a particular set of conditions[49].

Although the higher fitness in F1 hybrids may have been a temporary feature of a first-generation cross, it was certainly consistent with the rapid and widespread mixing of Constance and Geneva lineages in western Swiss waterbodies previously unoccupied by sticklebacks[27,34]. Whether the hybridization of these lineages leads to homogenization or a wider range of phenotypes coexisting across or within habitats will depend on the interactions between ecological conditions, population density and the range of phenotypes generated by continued mixing. Hybrid zones between species or distinct lineages are characterized by unusually large variation in phenotypic traits and potentially rapid changes in trait frequencies. In Switzerland, hybridization between the Constance and Geneva lineages has created these conditions across western and northwestern Switzerland, while hybridization with an additional northern (Rhine) lineage has created additional hybrid zone dynamics in the northern part of the country. Density- and phenotype-driven feedbacks between ecology and evolution may thus play an important role in shaping both ecological conditions and evolutionary processes throughout this range. Our results suggest that populations might be dominated by hybrids in habitats with low resource availability that decrease non-hybrid survival. In addition, alleles controlling the morphological adaptations of Geneva fish for
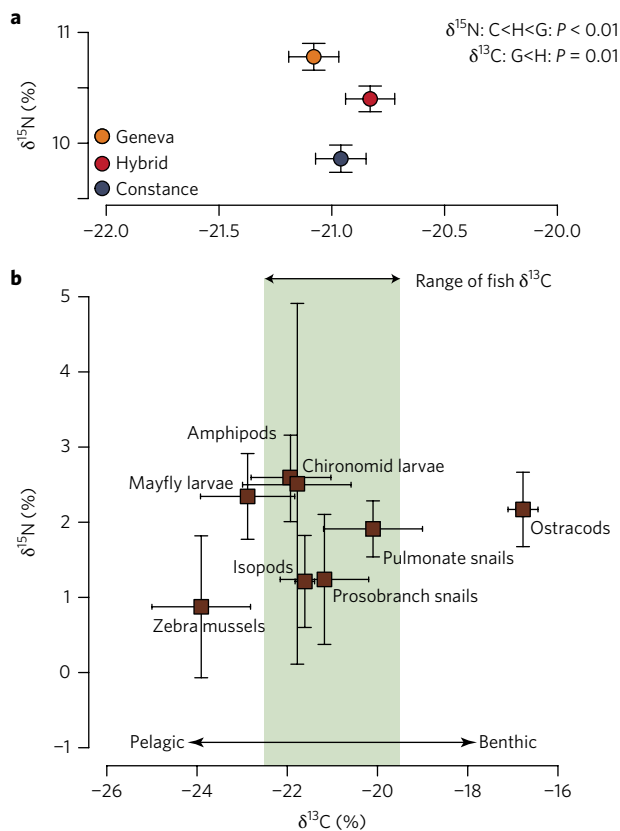
Fig. 5 | Stable isotope signatures of juvenile fish and potential prey.
a, C and N stable isotope signatures differed among juveniles (mean ± 1 s.e.; linear mixed models). **b**, They also varied across a range of mesocosm invertebrates (mean ± 1 s.d. to show the full range of potential dietary inputs). No differences in either $\delta^{13}C$ or $\delta^{15}N$ were detected in ten juveniles per type sampled from the rearing aquaria at the start of the mesocosm experiment, confirming that the observed difference developed during feeding and growth within the mesocosms. In **b**, the invertebrate values are based on at least five individuals taken from at least two mesocosms for each taxon, with the exception of ostracods (two individuals from the same mesocosm) and mussels and prosobranch snails (one sample of three pooled individuals for each of 35 of the 50 total mesocosms).

consuming larger benthic prey should increase in habitats where this resource is consistently available, whether this is controlled by abiotic factors, the density or phenotype frequency of colonizing sticklebacks, or (most likely) their interaction.

Foundational examples of eco-evolutionary dynamics have illustrated the role of population density in shaping the direction of phenotypic evolution[1]. Here, we show that phenotypic differences among lineages—and between these and their hybrids—can also impact eco-evolutionary dynamics by altering resource composition, carrying capacity and juvenile survival rates in colonized environments. Depending on the colonization history and environmental conditions, this may have substantial implications for the evolutionary trajectories of individual populations and the spread of hybrid phenotypes. Given the logistical and ethical constraints associated with manipulating population composition across natural environments, the use of contained experiments representing a wide range of ecological conditions can be a valuable approach, especially when it produces specific hypotheses that can then be tested with targeted surveys of resource conditions and phenotypic frequencies across natural habitats. This is true both for testing the existence of positive or negative feedbacks in the course of phenotypic divergence and for forecasting the effects of rapid evolution following

secondary contact. It is particularly important to understand such dynamics in hybrid zones in the context of anthropogenic climate change, habitat modification and species introductions, all of which may induce rapid range shifts and expansions.

Methods

Mesocosm experiment phase I: effects of phenotypic evolution on aquatic ecosystems. In April 2014, we established 50 replicate mesocosms (1,000 l in volume and 1.3 m deep) with a base of sand and fine sediment and water pumped from Lake Lucerne. To stock the prey and plant community, we added zooplankton, benthic invertebrates, soft sediment and macroalgae (*Chara* species) collected from Lake Constance, Lake Geneva and Lake Lucerne. For each resource, all mesocosms received the same initial quantity. Next, we assigned the mesocosms to six different treatments using a randomized block design with five blocks. These treatments were no fish added, four factorial combinations of fish density (low = three fish or high = ten fish) crossed with evolutionary lineage (Geneva or Constance) and one high-density mixture treatment with five fish from each of the two lineages. Within the core factorial contrast of density versus a single lineage we established two replicates per treatment per block and treated the fish for one of these replicates with medication to reduce the parasite load before the experiment[51]. However, since this treatment had no impact on the variables considered in this paper, we have presented results as an average across the fish medication treatment. Thus, for each of the core treatments $n = 10$.

The adult fish for this phase of the experiment were taken from a single population in each of Lake Geneva and Lake Constance, which were chosen to represent evolutionary lineage characteristics with minimal effects of recent introgression[34]. Fish were caught in early April at Lake Geneva (Le Grand Canal; 46° 23′ 53″ N, 6° 53′ 13″ E) and Lake Constance (Marina Rheinhof; 47° 29′ 55″ N, 9° 33′ 25″ E) (Fig. 1a) and kept in the laboratory for one month before the experiment. To prevent breeding and breeding-related behavioral alterations we used only males. We introduced the fish to the mesocosms on 12 May and removed them after five weeks on 14 June.

At the time of adult removal, we quantified the macrophyte cover, GPP, macrozooplankton and benthic invertebrates. The abundance of macrophytes (both prostrate filamentous and upright *Chara* macroalgae) was quantified using visual surveys. GPP was measured as the whole-ecosystem rate of net production plus the consumption of dissolved oxygen—the sum of the daytime rate of increase in dissolved oxygen concentration and the nighttime rate of decrease due to respiration. Zooplankton communities were sampled by filtering a total of 10 l of water collected from multiple locations and depths within each mesocosm through a 90 μm mesh and preserving and identifying the retained zooplankton. Benthic invertebrate communities were sampled by preserving and identifying the community present in a 10.5 cm × 10.5 cm box pre-installed in the sediment at the outset of the experiment. The taxa that were identified in the zooplankton and benthic surveys are listed in Supplementary Table 2, along with the average linear measurements for each taxon. For our analysis of benthic prey availability, we focused on probable prey items (crustaceans and insects but not Zebra mussels or snails). For both zooplankton and benthic invertebrates, we obtained average length measurements per species per mesocosm and used published length–weight regressions (Supplementary Table 3) to estimate biomass. The overall average linear prey size for these taxa was 5 mm, which is accessible to both juveniles and adults.

We tested for the effects of adult stickleback density and lineage and their interaction on these ecosystem components using linear mixed models. We ran separate models for each of the following response variables: abundance of benthic invertebrates, abundance of macrozooplankton, average per-individual biomass of benthic and planktonic invertebrates as a measure of the energy available per captured prey item, cover of filamentous algae, number of shoots of upright *Chara* algae and GPP. In each case, we first ran a model on only the factorial subset of four treatments (low or high density × Geneva or Constance lineage) to test for the main and interactive effects. Following this, we tested the effect of adult density across all treatments using three levels: zero, three or ten fish, regardless of lineage. We coded block as a single random effect.

Mesocosm experiment phase II: effects of ecological variation on juvenile selection. From 7 to 12 July (one month after removing the adults), we added 99 juvenile sticklebacks (of six to eight weeks of age) to each mesocosm in an equal mixture of Constance lineage (33), Geneva lineage (33) and F1 hybrids (33). No additional resources were added to the mesocosms with the addition of the juveniles. To produce the juveniles, we used artificial mating of reproductively mature parents collected from the same populations given above and raised six families of each type for each experimental block, with half of the hybrid families having mothers from each lineage. While in the laboratory, all fish were fed daily with artemia and live zooplankton (collected from Lake Lucerne). All fish collections and husbandry for adults and juveniles was according to fishing permits obtained from the cantons of St. Gallen and Vaud and animal care permits obtained from the Swiss federal veterinary office and the canton of Lucerne.

To detect variation in survival both within and among the mesocosms, we ended the experiment when visual surveys indicated 50% mortality in at least some mesocosms. This occurred after six weeks, at which point we removed all surviving juveniles using dipnets, euthanized them with MS222 and froze them individually. We recovered 2,082 total survivors from the original 4,950 juveniles, with 98% of all the surviving juveniles found in the first ten days of removal. Later, surveying and emptying of the mesocosms recovered an additional 49 fish (fewer than two per mesocosm on average). These later fish were included in the calculated survival rates but not in the analyses of fish condition.

We identified all the surviving juveniles as Constance, Geneva or hybrid by genotyping each fish and all of the parents used for breeding at nine microsatellite markers[52] and inferring parentage using the COLONY software[53]. This method assigned over 99% of fish with an average of 99% certainty. Microsatellite data were obtained in ref. [52] by running a single multiplexed polymerase chain reaction product on an ABI 3130XL and scoring the alleles with GENEMAPPER 4.0 (Applied Biosystems). We ran COLONY in a separate run for each block to infer parentage and thus lineage. Of the total 2,082 recovered juveniles, 98% were assigned to a real combination of parents with an average certainty of 99% and 2% were assigned to an impossible combination of parents but always from the same parental lineage, resulting in zero uncertainty about type assignment. Only four fish could not be assigned due to incomplete genotype information.

We measured the standard length and wet weight for each juvenile and calculated body condition by regressing log-transformed weight on log-transformed length and extracting the residuals. To characterize diet variation, we selected one to six juveniles of each type (Constance, Geneva and hybrid) from each mesocosm (mean = three of each type per mesocosm) and processed a section of the caudal peduncle for stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$). We also collected a range of invertebrates thought to represent benthic versus pelagic food webs, with individuals of each taxon taken from multiple mesocosms. All fish and invertebrate samples were freeze dried, ground to a fine powder and analysed for $\delta^{13}C$ and $\delta^{15}N$. All samples were run using a Thermo Fisher Delta V Advantage Isotope Ratio mass spectrometer coupled to a Thermo Flash 2000 Organic Elemental Analyzer. Isotopic reference materials (acetanilide; Stable Isotope Research Facility) were assayed at the beginning of each run and after every ten samples. The average precision of standards for $\delta^{13}C$ and $\delta^{15}N$ was 0.04 and 0.12, respectively. To allow for a comparison of the diet signatures across the mesocosms varying in their baseline $\delta^{13}C$ and $\delta^{15}N$ values, we obtained residual values of $\delta^{13}C$ and $\delta^{15}N$ after removing random effects of mesocosm (while accounting for lineage). We also assayed $\delta^{13}C$ and $\delta^{15}N$ for ten juveniles of each type sampled in the laboratory just before the mesocosm experiment to determine any initial differences in stable isotope signatures.

To quantify any possible effects of ecosystem modification by adult fish on the juveniles, we tested for both discrete treatment effects and continuous ecological effects on juvenile survival and condition. First, we tested for treatment effects on total juvenile survival using the model structure described for the ecosystem variables described in phase I. Second, we tested for continuous effects of the core ecosystem variables (described in phase I) on total and type-specific juvenile survival and type-specific juvenile condition. To do this we included the mean individual biomass of benthic and zooplankton prey items, GPP and abundance of filamentous and upright algae in a single model with all the main and interactive effects involving these variables and juvenile stickleback type. We also calculated the selection coefficient for each type of juvenile in each mesocosm as S = 1 − (surviving juveniles of that type/number surviving of the best performing type in that mesocosm). Finally, we tested for differences in stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) among the types of juveniles and for joint effects of $\delta^{13}C$ and $\delta^{15}N$ on individual fish condition. We included a random effect of block in all the models, as well as a random effect of mesocosm for all the models with multiple data points per mesocosm (either one per type of juvenile or one per individual fish). For models with count data (for example, number of survivors) as the response variable, we tested both general linear mixed models and generalized linear models (see the footer in Supplementary Table 1 for details).

Parallel laboratory experiment. To test for inherent differences in body condition among juveniles in a controlled environment, we established a parallel laboratory growth experiment, which ran at the same time and for the same duration as the mesocosm experiment. For this, we established one laboratory aquarium of fish per block at each of two densities: 15 fish (5 of each lineage) or 30 fish (10 of each lineage). Regardless of density, each aquarium of fish was fed three times per week with the same volume of chironomid larvae per aquarium. Following this experiment all juveniles were genotyped, weighed and measured as above.

Statistical analyses. All analyses were carried out in R (ref. [54]). The linear mixed models described in the sections above were implemented using the *lme4* package[55]. After checking residuals for normality and equal variance (and transforming response variables when necessary), we obtained P values using type II Wald F tests with Kenward–Roger approximations for the denominator degrees of freedom using the *car* package[56]. As an alternative form of inference, which was not subject to multiple comparison issues but provided relative rather

than absolute support, we used model selection to identify important predictor variables. To do this, we ran separate models (fit with maximum likelihood rather than restricted maximum likelihood) with all possible combinations of variables (except the models with interactions had to also include the corresponding main effects). We then compared these models based on their AICc (small sample-size corrected Akaike Information Criterion) values, which indicated relative support for all the tested models[57]. The full statistical results are reported in Supplementary Table 1 and Table 1.

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Author contributions
B.M., J.M.A.-R., O.S. and R.J.B. designed the core experiment. R.J.B. and J.M.A.-R. carried out the experiment. M.C.L. designed and carried out the isotopic analysis, and D.W.S. provided supplementary data on feeding efficiency. R.J.B. analysed the data and wrote the manuscript with substantial contributions and revisions from all authors.

Competing interests
The authors declare no competing finacial interests.

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