PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Lürig MD, Matthews B. 2021 Dietary-based developmental plasticity affects juvenile survival in an aquatic detritivore. *Proc. R. Soc. B* **288**: 20203136. https://doi.org/10.1098/rspb.2020.3136

Received: 21 December 2020
Accepted: 19 January 2021

Subject Category:
Ecology

Subject Areas:
ecology

Keywords:
development, stoichiometry, fitness, life history, phenotypic plasticity, diet quality

Author for correspondence:
Moritz D. Lürig
e-mail: moritz.lurig@biol.lu.se

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5291646.

THE ROYAL SOCIETY PUBLISHING

Dietary-based developmental plasticity affects juvenile survival in an aquatic detritivore

Moritz D. Lürig[1,2,3] and Blake Matthews[2]

[1]Department of Biology, Lund University, 22362 Lund, Sweden
[2]Department of Fish Ecology and Evolution, and [3]Department of Aquatic Ecology, Eawag, Seestrasse 79, 6047 Kastanienbaum, Switzerland

MDL, 0000-0002-8175-6234

Developmental plasticity is ubiquitous in natural populations, but the underlying causes and fitness consequences are poorly understood. For consumers, nutritional variation of juvenile diets is probably associated with plasticity in developmental rates, but little is known about how diet quality can affect phenotypic trajectories in ways that might influence survival to maturity and lifetime reproductive output. Here, we tested how the diet quality of a freshwater detritivorous isopod (*Asellus aquaticus*), in terms of elemental ratios of diet (i.e. carbon : nitrogen : phosphorus; C : N : P), can affect (i) developmental rates of body size and pigmentation and (ii) variation in juvenile survival. We reared 1047 individuals, in a full-sib split-family design (29 families), on either a high- (low C : P, C : N) or low-quality (high C : P, C : N) diet, and quantified developmental trajectories of body size and pigmentation for every individual over 12 weeks. Our diet contrast caused strong divergence in the developmental rates of pigmentation but not growth, culminating in a distribution of adult pigmentation spanning the broad range of phenotypes observed both within and among natural populations. Under low-quality diet, we found highest survival at intermediate growth and pigmentation rates. By contrast, survival under high-quality diet survival increased continuously with pigmentation rate, with longest lifespans at intermediate growth rates and high pigmentation rates. Building on previous work which suggests that visual predation mediates the evolution of cryptic pigmentation in *A. aquaticus*, our study shows how diet quality and composition can generate substantial phenotypic variation by affecting rates of growth and pigmentation during development in the absence of predation.

1. Introduction

Developmental plasticity, when the phenotypic expression of genotypes depends on the environmental conditions during development, is ubiquitous in animals [1–3]. There are several mechanisms by which environmental conditions can affect the phenotypic trajectories of individual juveniles [4,5], and several ways in which such developmental plasticity can affect fitness variation: for example, juveniles can experience physiological trade-offs that manifest in lowered performance, such as reduced locomotion [6,7] or maintenance of basic body functions [8], that might ultimately increase mortality prior to adulthood [1,9]. Over an individual's lifetime, the environmental dependence of phenotypic expression can weaken (e.g. irreversible developmental plasticity), and, in some cases, can culminate in adult phenotypes that are maladaptive. Cryptic coloration, for example, is often determined during early developmental environments in response to potentially imperfect environmental cues about predation risk in adult environments [10,11]. Despite the ubiquity of developmental plasticity, surprisingly little is known about the ecological factors

affecting divergence in developmental trajectories and the consequences of these trajectories for fitness variation.

The dietary quality of resources throughout juvenile development is probably an important cause of developmental plasticity, because of its potentially large effects on the expression of morphological, physiological and behavioural traits of adults [12,13]. Across their lifetimes, organisms need to balance the allocation of acquired resources for growth, maintenance and reproduction [1,2,14]. Especially during early life, when investments in somatic growth are high [15,16], developmental trajectories might be more susceptible to variation in both resource quantity and quality [17,18]. The stoichiometric composition of essential elements (carbon, nitrogen and phosphorus) varies broadly among primary producers within and across ecosystems [19], and is a useful proxy of variation in diet quality of consumers [20]. Substantial mismatches between consumers and their diets are common [21–23], and if they occur early in development, they might be an important ecological cause of plasticity [6,10,24] and of fitness variation [25].

The effects of diet variation on developmental trajectories are likely to have important fitness consequences for consumers in general [3,26], and for detritivores in particular [27]. Dietary-based developmental plasticity can vary from maladaptive to adaptive depending on the specific ecological context [3,28]. For example, high-quality diets that are available during juvenile development may allow organisms to reduce predation risk (e.g. by outgrowing vulnerable stages or sizes [6], maturing earlier [29] or expressing adult phenotypes that increase mating success [30]). For detritivores, who have adapted in various ways to low-quality food throughout their lifetime [31], we might expect nutrition to be an important source of individual variation in both developmental trajectories and fitness in natural populations [32]. However, few studies (either of detritivores or other consumers) have quantified how the link between fitness variation and developmental trajectories of individuals depends on the nutritional quality of diets.

The detritivorous freshwater isopod *Asellus aquaticus* is a useful model to explore how dietary variation can affect phenotypic variation throughout juvenile development. Previous work in Swedish lakes has shown habitat-specificity of adult isopod pigmentation and body size [33,34]. The matching of body pigmentation with habitat backgrounds has been primarily interpreted in the context of the evolution of crypsis in response to visual predation [33–35]. However, *A. aquaticus* also exhibits diet-based plasticity in terms of both growth rate [36] and accumulation rates of pigmentation through development [27]. At birth, isopods completely lack pigmentation and become increasingly pigmented as they grow [27]. The development of pigmentation of *A. aquaticus* is cumulative and irreversible through development [37], and may be linked to environmental sources of tryptophan, an amino acid that is a metabolic precursor for the pigment xanthommatin [38,39]. Tryptophan varies strongly among detrital resources of *A. aquaticus* [40], but neither the effects of tryptophan nor the dietary quality of resources has been investigated in the context of survival variation of *A. aquaticus* through development.

Here, building on our previous work [27], we perform a large laboratory experiment to test how varying dietary environments affect developmental trajectories of juveniles, and investigate the joint effects of diet and divergent developmental trajectories for juvenile fitness. Using the freshwater isopod *A. aquaticus*, we manipulated stoichiometric ratios and availability of pigmentation precursors (i.e. tryptophan) and tracked individual growth and pigmentation rates, as well as survival, of over 1000 individuals from 29 families. Specifically, our rearing experiment allowed us to investigate (i) the extent of developmental plasticity in growth and pigmentation caused by our diet manipulations, and (ii) how such variation in developmental rates of growth and pigmentation can jointly affect the survival of juveniles, in the absence of predators or their cues [27,33]. Based on previous work regarding the physiological mechanisms of isopod development [27,36,38], we expected to find higher pigmentation rates under a high-quality (= high-protein) diet. Moreover, we anticipated associations between developmental rates of growth and pigmentation, partly because high-quality diets often covary with pigmentation precursors—a covariation that we attempted to disentangle with our manipulation of tryptophan. Our results confirm pronounced developmental plasticity in pigmentation, and, to a lesser degree, in growth rates, and underscore the need to consider diet- or resource-based developmental plasticity as an important source of phenotypic variation, which may affect fitness before reproduction or selection from predation later in life.

2. Material and methods

(a) *Asellus aquaticus*

The freshwater isopod *A. aquaticus* is common in benthic communities across Europe and parts of Asia [41]. The small crustaceans (mature animals are 4–15 mm; figure 1) are found in many different microhabitats, like beds of *Chara tomentosa*, *Phragmites australis* (reed) or bare sand [33,34,36], and are considered to play a significant role in freshwater food webs [33,36,41]. While *A. aquaticus* can feed on fresh plant material, they often prefer substrates colonized with microbiota (i.e. bacteria and fungi; figure 1*d*) on leaf litter or decaying macrophytes [36,42–44]. Feeding on fungal and microbial biofilms may help alleviate stoichiometric mismatches between *A. aquaticus* and their nutritionally poor detritial diets [36,43]. Moreover, the amino acid tryptophan, which is essential for the main pigment in *A. aquaticus*, is known to vary strongly across various detrital resources [40], but neither the effects of tryptophan or nutrition have been investigated in the context of isopod life history and development. Here, we manipulate both diet quality and tryptophan availability to explore the link between variation in developmental trajectories and juvenile survival.

(b) Common garden experiment

(i) Contrasts and food preparation

Using a common garden experiment, we quantified the extent of variation in developmental rates of growth and pigmentation, and their effects on survival in *A. aquaticus* in response to diet composition (stoichiometric quality and tryptophan availability). To do so, we exposed 1047 juvenile isopods from 29 families shortly after their birth (1–3 days) to four different dietary contrasts: high elemental ratios (C : P and C : N, hereafter low-quality (LQ) diet) and low elemental ratios (hereafter high-quality diet (HQ)), as well as each of these diet combinations crossed with a supplement (or not) of tryptophan. We measured growth, pigmentation and survival of each individual over the course of 12 weeks. For each family, half of the juveniles were randomly assigned to either low or high diet quality (full-sib/split-family design). For the eight families with the highest number of offspring (50–60 juveniles), we crossed the diet quality treatment with a

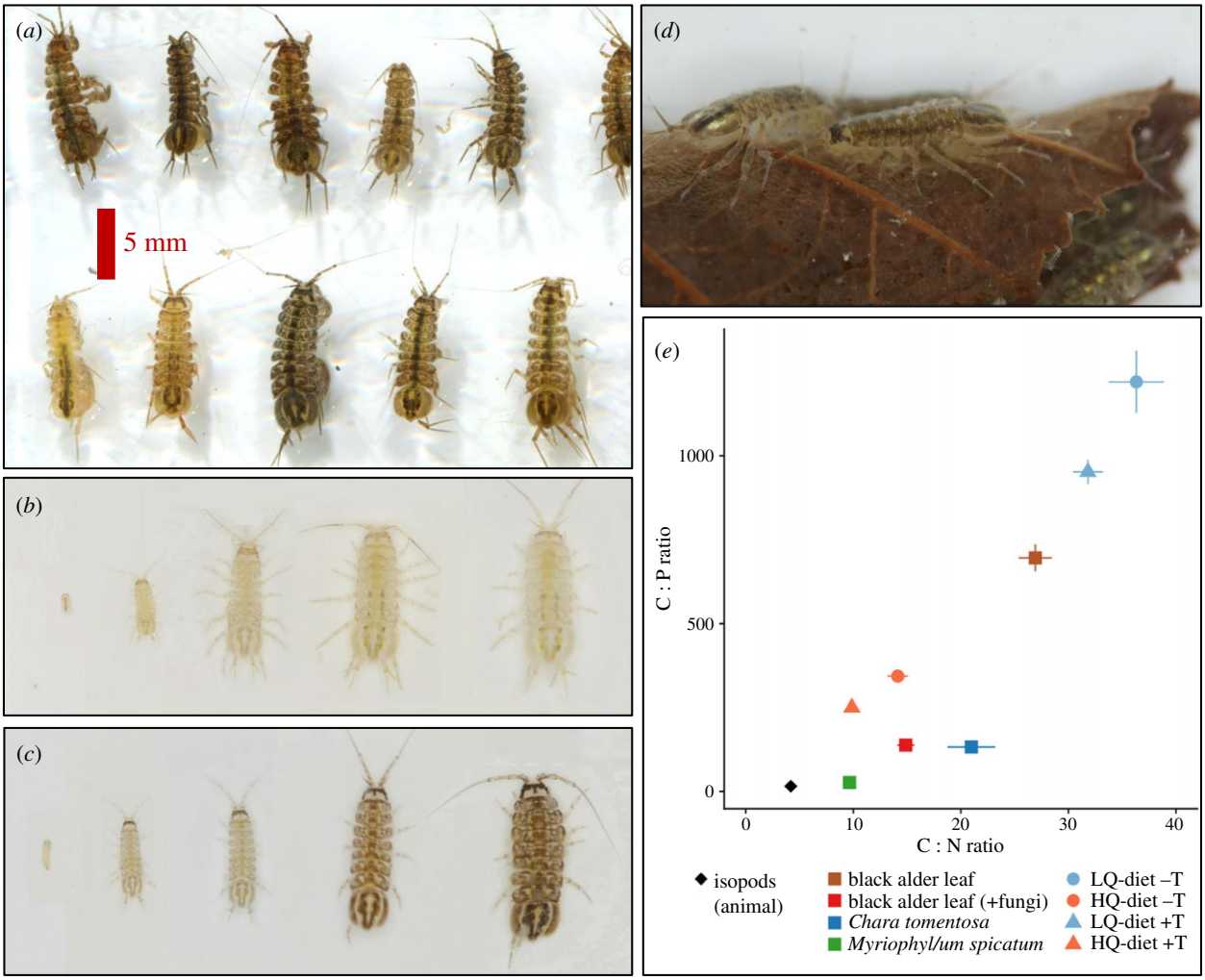

Figure 1. Phenotypic variation in pigmentation in the freshwater isopod *A. aquaticus* can be determined by diet. (*a*) Random sample of isopods taken from beds of *Chara tomentosa* in Lake Lucerne at Kastanienbaum (measured with a flatbed scanner, brightness adjusted to match images from camera stand; size scale is for *a*–*c*). (*b*,*c*) Example of an isopod reared under (*b*) low-quality and (*c*) high-quality diet (both no tryptophan, photographed with a camera stand). The levels of adult isopod pigmentation measured throughout the diet manipulation fall well within the range of isopod pigmentation found in nature (figure 2*d*) [27]. (*d*) Isopods feeding on fungi that form on the surface of alder leaves in standing water. (*e*) Elemental composition of various natural food items that isopods encounter in Lake Lucerne, as well as the artificial diets used in this experiment (LQ, low quality/high elemental ratio; HQ, high quality/low elemental ratio; −T, without tryptophan supplement; +T, with tryptophan supplement). This panel also shows the elemental composition of isopods collected from Lake Lucerne (black diamond). Elemental ratios are scaled by the molar mass of the respective elements. The data for the figure can be found in electronic supplementary material, table S1. (Online version in colour.)

supplemental tryptophan treatment: in these eight families, 40 juveniles were randomly distributed among high- and low-quality treatments, and the remaining 10–20 individuals among the two treatments with tryptophan supplement. For the high-quality diet, we used 80% dry yeast (*Saccharomyces cerevisiae*) and 20% potato starch that was autoclaved together with agar and filtered lake water into a paste that was dried and cut into pellets (dry weight 1.2 ± 0.1 g). The low-quality diet was prepared in the same way, but with 20% yeast and 80% starch. For the tryptophan supplement, we added 0.1 g of tryptophan per 1 g of food substrate. We constructed these diets so as to capture some of the broad range of stoichiometric variation that isopods encounter in nature, from high-quality macrophyte detritus to low-quality terrestrial detritus (figure 1). Our tryptophan manipulation unintentionally lowered the C : P of this diet treatment (figure 1*e*), but this effect was small relative to the overall diet contrast.

(ii) Experimental set-up and procedure

We used juvenile isopods from a total of 29 successful matings (for details on isopod collection and breeding, see electronic supplementary material) and started the common garden experiment in three temporal blocks. From each family, juvenile isopods were randomly distributed across jars (50 ml, PE), which contained filtered lake water and a pellet of either of the diet types. We placed the jars inside racks that were arranged randomly inside a flow-through water trough to buffer against fluctuations in temperature. The set-up was maintained at 20°C with a 16 : 8 h light dark cycle, and temperature was controlled every day. We took pictures of all live isopods from each block every three weeks. Using small pipettes (for isopods bigger than approx. 5 mm, we used soft steel forceps), we transferred an individual from its tube into a small container with lake water, and from there onto a flat tray containing lake water underneath a camera mounted on a camera stand. After taking the picture, we transferred each isopod into a new (autoclaved) tube with fresh lake water and a new food pellet. We repeated this procedure with every individual, yielding up to five phenotypic measurements for each developmental trajectory.

(iii) Isopod pictures and phenotyping

We took pictures of isopods using a camera stand with a digital single lens reflex camera (Canon) and a 100 mm macro lens (Tamron). The tray was uniformly illuminated with an LED spot ring (Leica). We ensured that each isopod specimen was flat on

the tray, without movement or curling up. To quantify pigmentation and body size of isopods from the digital images, we applied computer vision techniques. For this purpose, we used the python package *phenopype* [45]. It uses thresholding algorithms to segment isopods from the image background, to then extract the phenotypic information from the pixels marking the animal (dorsal region of isopod torso = carapace, excluding legs and antennae). The greyscale values from these pixels were averaged and converted to a pigmentation scale from 0 (greyscale value of 255) to 1 (greyscale value of 0). Body size was measured as carapace length, excluding legs and antennae. Previous work has confirmed that *phenopype* results are highly correlated with measurements of the same images using ImageJ (linear correlation between methods: slope = 0.98, $R^2 = 0.97$ [27]).

(c) Statistical analyses

(i) Common garden experiment

We tested for effects of diet composition and tryptophan supplement on developmental rates of body size and pigmentation, as well as survival over the course of the experiment using a series of generalized additive mixed models (GAM), using the 'gamm' function in *mgcv* [46]. We fitted separate models each for body size (GAM1; table 1) and pigmentation (log transformed, GAM2), with time separated by diet contrast as the fixed effect and a thin plate spline term with time in weeks. Furthermore, we fit a GAM with a binomial distribution family to test for differences in survival as a binary dependent variable, and fixed effect and spline terms identical to the developmental rate models (GAM3; table 1). All three models contained nested random terms for family and individual, and used diet as a parametric component in the spline terms.

In a further step, we tested for effects of diet composition and of juvenile phenotypes right after birth on growth and pigmentation rates and survival by performing a path analysis using Bayesian multilevel modelling [47]. In a single model, we implemented three hierarchical levels, and included family as the grouping term, allowing us to estimate relative effect sizes of developmental rates and starting conditions on lifespan under all diet treatment contrasts (see electronic supplementary material, table S2 for details). We applied both types of analysis in a complementary fashion: with separate additive models, we accounted for the nonlinearity in developmental rates, and with the path analysis, we were able to disentangle complex interactions linking rearing conditions and juvenile traits through development with survival variation.

To test for interactions between growth and pigmentation on survival, we also applied a more complex multivariate GAM. To do so, we first converted measurements of body size and pigmentation up until week 6 (dashed line in figure 2) to a single linear slope per individual isopod (hereafter growth and pigmentation rate, respectively). We chose to calculate slopes from this time frame, because pigmentation and growth increased linearly to this point, and isopod survival up to this point was high. We then implemented an additive model (GAM4) with the 'gam' function from *mgcv*, using lifespan (in weeks) as the dependent variable, single thin plate spline terms for growth and pigmentation rate, and a tensor smooth product term to test for the interaction (table 1). The model included family as a random effect, and the spline and tensor term included diet as a parametric component (see electronic supplementary material, for details).

3. Results

We found that growth rates were only weakly affected by diet quality and tryptophan supplement (GAM 1; table 1 and figures 2 and 3), whereas rates of pigmentation were strongly affected by diet quality. Tryptophan only resulted in significantly higher pigmentation rates under low-quality diet (significant interaction diet quality × tryptophan; table 1 and figures 2 and 3). As indicated by the path analysis (figure 3) and GAM2 (table 1 and figure 2), pigmentation rates were lowest when juveniles were reared under low-quality diet and in the absence of the tryptophan supplement. On the other hand, the tryptophan supplement resulted in slightly higher pigmentation rates under low-quality diet, but not under high-quality diet. This was indicated by a significant interactive effect of diet and tryptophan in GAM2 (table 1 and figure 2) and in the path analysis (figure 3). Overall, and despite the presence of significant variation at the family level for growth and pigmentation rates (see random effect of family in table 1; electronic supplementary material, figure S2), the diet contrast resulted in clear divergence in the build-up of pigmentation through development (figure 2*b*). For a given body size, these diet-induced differences in pigmentation are comparable in magnitude to the observed habitat-specific variation in nature (figure 2*d*).

Multiple lines of analysis indicate that there were complex interactions between diet quality and developmental rates that affected survival of isopods. We found that survival of juvenile isopods during the experiment depended strongly on both diet and tryptophan supplement: survival was much higher on low-quality diets, and further increased by the tryptophan supplement. However, under a high-quality diet, the tryptophan supplement did not affect survival (GAM3; table 1 and figure 3). Using the path analysis, we found that higher concurrent rates of growth and pigmentation also had a negative impact on survival independent of diet, as indicated by the interaction term (figure 3*d*). For a more in-depth analysis of the full three-way interaction of diet, growth rate and pigmentation rate, we used a multivariate additive framework, where we tested diet-specific relationships between both developmental rates (GAM4; figure 4 and table 1). This analysis revealed two distinct 'survival surfaces': under low-quality diet, a single, high survival peak existed at intermediate growth and pigmentation rates. Survival under high quality was overall lower and varied nonlinearly across a wide range of both developmental rates (figure 4), as indicated by a significant nonlinear interaction of diet and rates (table 1). Specifically, survival on high-quality diet peaked at intermediate growth rates and high rates of pigmentation (figure 4).

4. Discussion

Our experiment confirms and expands the results of a previous study [27] that found diet-based developmental plasticity in pigmentation, and weak diet-based plasticity in growth in *A. aquaticus*. In the current paper, we found that growth of juvenile isopods was only weakly affected by our manipulation of diet stoichiometry and the tryptophan supplement (figures 2*a* and 3*a* and table 1). The growth rates we measured are comparable to previous rearing experiments that used naturally occurring food items [36], confirming that the caloric content and nutritional balance of the pellets that we provided ad libitum were an appropriate rearing environment. Maintaining high growth rates on low-quality food might be an important mechanism in natural habitats to escape (outgrow) gape limited predators (e.g. juvenile fish) or have a higher chance of escaping slow

Table 1. Statistical results of generalized additive models. Models GAM1–GAM3 tested for an effect of diet quality content on growth, pigmentation and survival (figure 2), GAM4 tested for interactive effects of diet quality, growth rates, pigmentation rates on survival of isopods (figure 4). Reported are results for linear (*fixed effect)* and nonlinear (*smooth term*) part of the model (tprs, thin plate regression spline; tp, tensor product). For each model, the degrees of freedom for the fixed effect term are 1, and the number of knots for each smooth function is 3. Significance of random effects was tested with a likelihood ratio test. Significant (less than 0.05) and marginally significant (less than 0.1) results are in bold.

model	response variable	fixed effect	*F*	*p*- value	smooth term	smooth function	edf	*F*	*p*-value	random effect	d.f.	Chisq	*p*-value
GAM1	log(Length)	diet	4.644	**0.031**	high-quality − T	Tprs	2	4739.25	**>0.001**	individual	1	89.921	**>0.001**
		tryptophan	3.434	**0.064**	high-quality +T	tprs	1.99	603.079	**>0.001**	family	1	495.419	**>0.001**
		diet × tryptophan	2.202	0.138	low-quality − T	tprs	2	7036.52	**>0.001**	block	1	199.2	**>0.001**
					low-quality +T	tprs	2	1196.43	**>0.001**				
GAM2	log(Pigmentation)	diet	221.96	**>0.001**	high-quality − T	tprs	1.96	1426.96	**>0.001**	individual	1	61.161	**>0.001**
		tryptophan	2.735	0.098	high-quality +T	tprs	1	271.881	**>0.001**	family	1	541.715	**>0.001**
		diet × tryptophan	7.003	**0.008**	low-quality −T	tprs	1.87	1179.35	**>0.001**	block	1	111.844	**>0.001**
					low-quality +T	tprs	1.9	267.761	**>0.001**				
GAM3	survival	diet	37.109	**>0.001**	high-quality T	tprs	1.97	342.591	**>0.001**	individual	1	3318.86	**>0.001**
		tryptophan	2.721	0.099	high-quality +T	tprs	1.51	51.396	**>0.001**	family	1	384.212	**>0.001**
		diet × tryptophan	7.71	**0.006**	low-quality −T	tprs	1.95	324.69	**>0.001**	block	1	644.953	**>0.001**
					low-quality +T	tprs	1	58.669	**>0.001**				
GAM4	survival	diet	107.56	**<0.001**	high-quality × growth rate	tprs	1.96	14.856	**>0.001**	family	1	23.466	0.217
		growth rate	652.88	**<0.002**	low-quality × growth rate	tprs	1.94	4.39	**0.014**	block	1	60.419	**>0.001**
		pigmentation rate	246.89	**<0.003**	high-quality × pigmentation rate	tprs	1	23.212	**>0.001**				
		diet × growth rate	108.07	**<0.004**	low-quality × pigmentation rate	tprs	1.98	6.501	**0.002**				
		diet × pigmentation rate	66.537	**<0.005**	high-quality × growth rate × pigmentation rate	tp	3.21	7.755	**>0.001**				
		growth rate × pigmentation rate	2.709	0.1	low-quality+growth rate × pigmentation rate	tp	1	1.187	0.276				
		diet × growth rate × pigmentation rate	2.778	**0.09**									

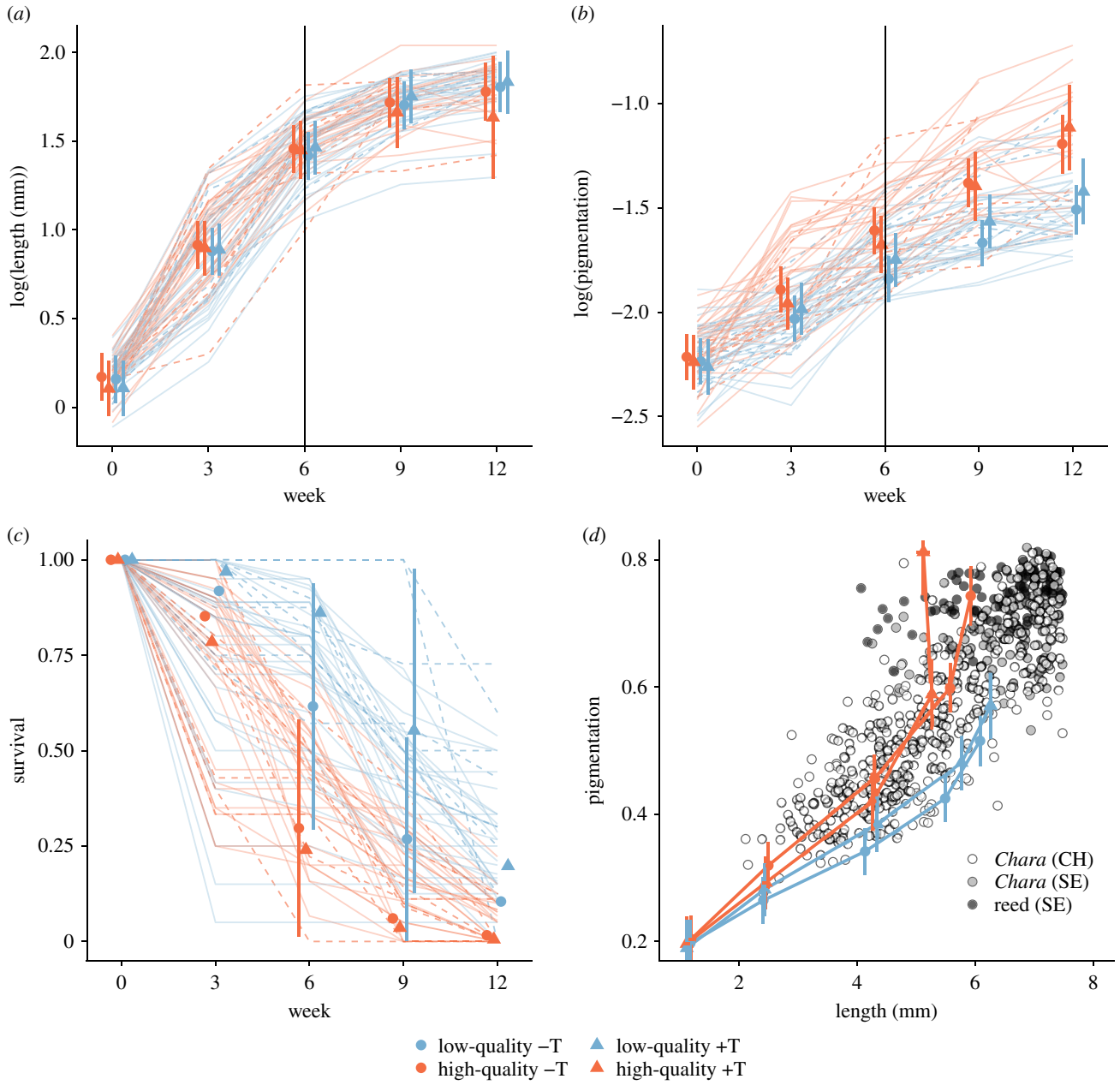

Figure 2. Treatment-level model estimates (symbols) and family-level developmental trajectories (lines). The symbols with error bars show model estimates for log-transformed length (*a*, GAM1), log-transformed pigmentation (*b*, GAM2) and survival (*c*, GAM3) for both diet contrasts (diet quality = circles, tryptophan = triangles) at a given time point (details on the model statistics are given in table 1). Each line shows the family-level average of body size, pigmentation or survival at a given time point. Solid lines indicate only protein manipulation; dashed lines indicate averages for the part of the families that were reared under tryptophan supplement. The vertical line in *a* and *b* indicates the cut-off of values used for the multivariate additive model (*t*1–*t*3, GAM4). (*d*) The untransformed treatment-level averages for length and pigmentation at each time point (same symbol and colour coding as in *a*–*c*), and length and pigmentation of wild caught isopods from different habitats. Differences in length and pigmentation due to the diet manipulation at the end of this experiment resemble phenotypic variation in isopods from two different habitats in southern Sweden (SE, reed, black points; *Chara tomentosa*, dark grey points). Moreover, developmental trajectories we measured in this experiment fall within the range of phenotypes of isopods collected from Lake Lucerne in Switzerland (CH, *Chara tomentosa*, light grey points). (Online version in colour.)

moving invertebrate predators (e.g. odonate larvae) when they are larger [35]. Although our diet contrast spanned beyond the range of natural food items that we measured in our study population (figure 1), our treatments with high stoichiometric mismatch (i.e. high C : P/C : N) was sufficient near natural growth [36] and pigmentation rates [27].

Pigmentation rates were strongly affected by our manipulation of diet stoichiometry (figures 2*b* and 3*b* and table 1): when reared under high-quality diet (low C : P, C : N) juvenile isopods from a majority of families (22 out of 29, electronic supplementary material, figure S2) showed greatly increased rates of pigmentation, and also higher final levels pigmentation at the end of the experiment. This is in agreement with a previous study [27] and provides additional support for plasticity of pigmentation during juvenile development, which is irreversible for adult isopods [33]. Indeed, our dietary manipulations recapitulated the entire phenotypic range of pigmentation for a given body size in the Lake Lucerne population (see figures 1*a*–*c* and 2*d*) [27]. While variation among families in the extent of phenotypic divergence probably results from a mixture of genetic and environmental factors, our experimental design can neither quantify additive genetic

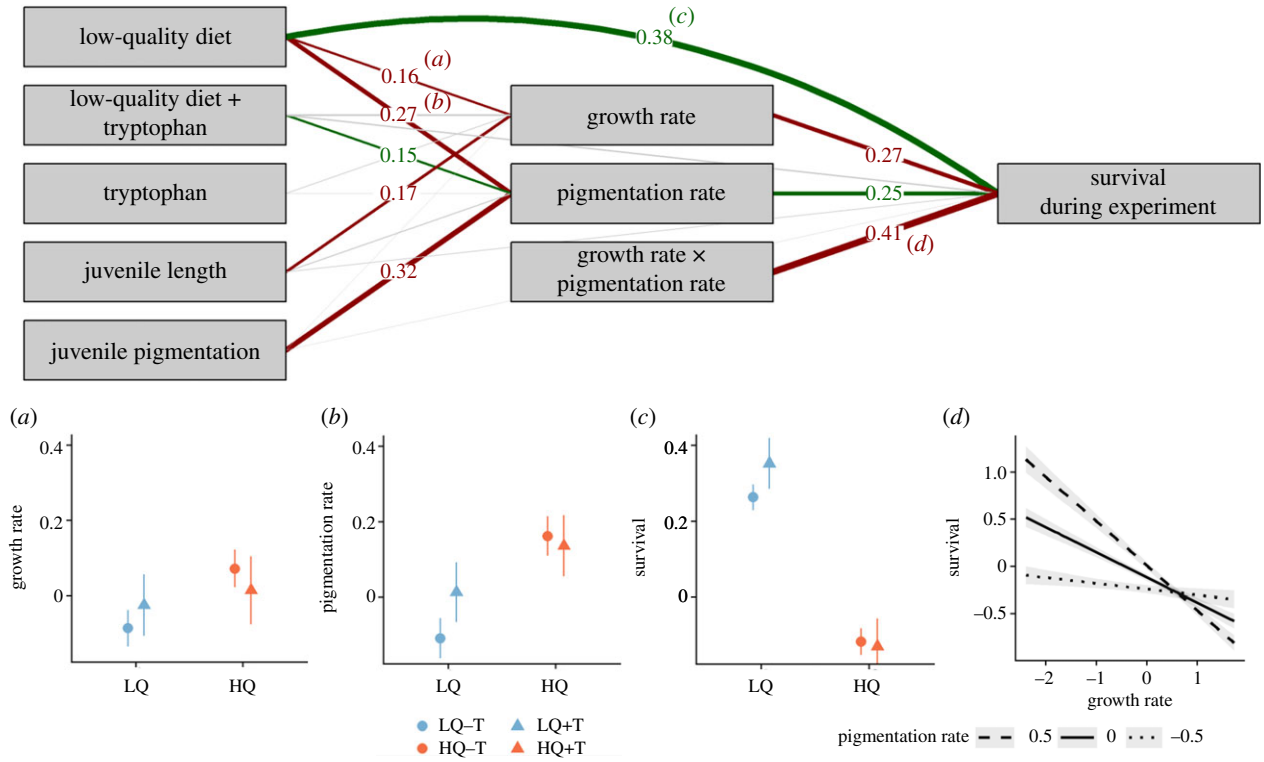

Figure 3. Path analysis using Bayesian multilevel modelling to investigate the effects of diet quality and tryptophan manipulation. Significant effects are indicated by coloured arrows (green, positive; red, negative; grey, not significant (overlap of the posterior with zero)), effect sizes are given by number on arrows. Panels illustrate the effects of the factorial manipulation of elemental composition (diet quality) and tryptophan on growth, pigmentation and survival rates (*a–c*, respectively), as well as an interactive effect of growth and pigmentation rates on survival across all diet manipulations (*d*—full three-way interaction between diet, growth and pigmentation rates are analysed by GAM 4 and shown in figure 4). Details on the path analysis are given in electronic supplementary material (table S2). (Online version in colour.)

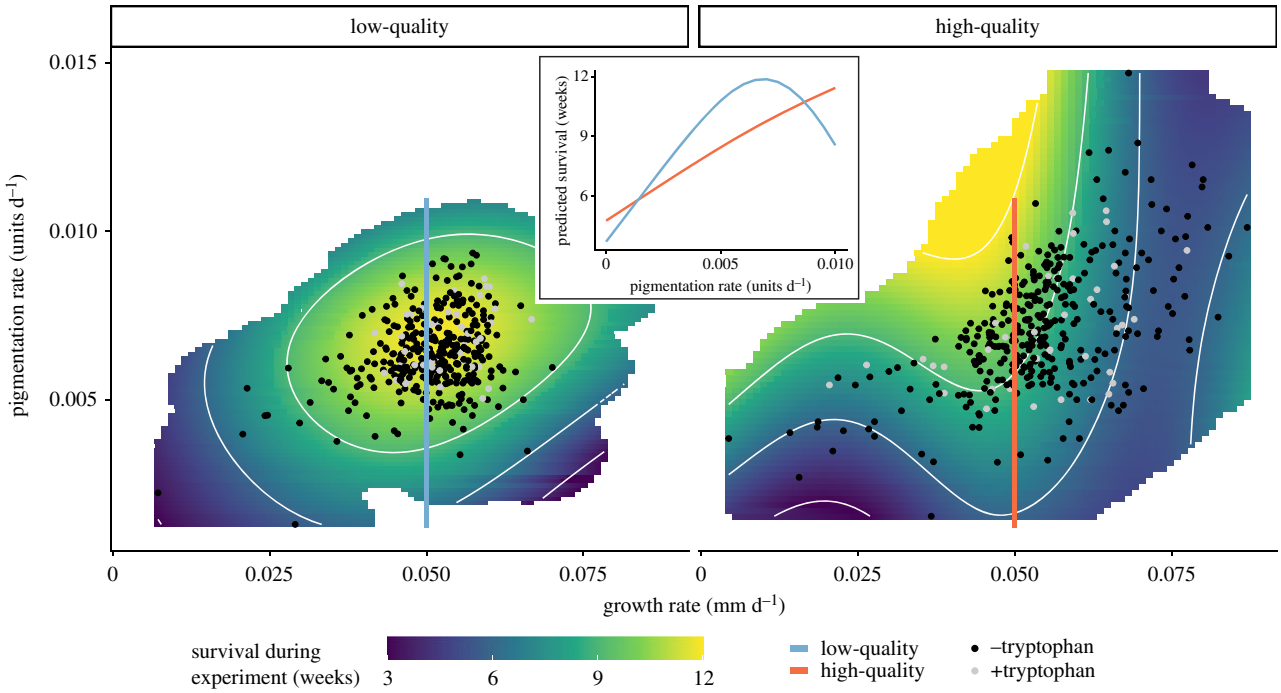

Figure 4. Survival landscapes modelled from the interaction of diet quality, growth rate and pigmentation rate (GAM4). Each point denotes an individual isopod (black, quality contrast; grey, tryptophan contrast). Diet-specific surfaces are model estimates from GAM4 with survival during experiment as the dependent and diet-specific growth and pigmentation rates between start and week 6 as the independent variable (see table 1 for details, GAM4). The blue (low-protein) and orange lines (high-protein) show the predicted survival for a fixed growth rate of 0.05 mm d^{-1} over a range of pigmentation rates: under low-protein diet, a peak for high survival is forming at intermediate growth and pigmentation rates, whereas under high-protein diet, survival increases linearly with pigmentation rate. (Online version in colour.)

variance of plasticity nor test for transgenerational plasticity (e.g. paternal effects). Even so, the high reproducibility of phenotypic divergence within families exposed to contrasting diets provides strong evidence for diet-based developmental plasticity in our study population.

Our supplement of tryptophan to both high- and low-quality diets showed small, but significant positive effects on pigmentation rates, but only for isopods reared on low-quality diet (figure 3*b* and table 1). It is well known that the addition of tryptophan to diets can increase pigmentation in insects. For example, larvae of cabbage butterflies (*Pieris brassicae*) reared on tryptophan-limited artificial foods have reduced wing pigmentation compared to larvae reared on tryptophan-rich foods [48]. Typically, organisms acquire tryptophan from protein-rich diets [49], and the yeast we used to create the high-quality diet (i.e. *S. cerevisiae*) is known to contain tryptophan [50]. Therefore, the faster development of pigmentation we observed in the low C : P diet could be partly explained by higher levels of tryptophan originating from yeast.

A general result from our experiment was that juvenile survival depended strongly on the developmental rates of both growth and pigmentation, albeit in complex ways. Both the significant interaction in the path analysis (figure 3*d*) and the multivariate additive model (figure 4) suggest that fast-growing individuals had a lower likelihood of survival when they also had high rates of pigmentation (figure 3*d*). Previous work has suggested that elevated growth rates in *A. aquaticus* are associated with higher energy expenditure, and consequently, higher metabolism and resource requirements [51], which may explain why fast-growing individuals have higher mortality rates. Elevated dietary protein content has also been shown to reduce survival in other study systems [52,53], which is thought to be caused by energetic expenditure associated with protein digestion and potentially harmful breakdown products [37,49]. Moreover, it is possible that a specific composition of the gut microbial community is required to digest certain proteins [54]. Still, only surprisingly little is known about the direct effects of protein consumption for aquatic isopods and particularly *A. aquaticus*, given that many detrital food items may contain high amounts of protein (figure 1).

Decreased survival under high developmental rates may also be due to resource competition antagonisms within the developing organism [15], namely if isopods experience physiological costs of maintaining high rates of both growth and pigmentation [13,17,18]. The relative consistency of growth rates across all treatment combinations suggests that the development of body size is more conserved than pigmentation [27]. Indeed, somatic growth, the correlated development of thoracic and other tissues during early ontogeny and before reaching maximum body size, is one main dimension of resource allocation in animals, followed by physiological maintenance and reproduction [1,9,55]. However, depending on the resources available during early ontogeny, development of secondary characteristics like ornaments, weapons or pigmentation can vary in comparison to body size, due to the necessity to develop fully sized body parts and organs to ensure their functionality [56,57]. It is possible that during early ontogeny of *A. aquaticus*, resource allocation to growth is prioritized over the development of isopod pigmentation when stoichiometric mismatches between consumers and their diet are high [15,19,25].

Our experiment provided evidence for nonlinear interactions between diet quality and developmental rates that strongly affected juvenile survival. Specifically, under a low-quality diet (high C : P, C : N), survival was constrained around a single peak centred at intermediate growth and pigmentation rates. By comparison, under a high-quality diet (low C : P, C : N), high survival was observed over a broader range of growth and pigmentation rates, albeit with a tendency for high survival at intermediate growth rates. Previous work on other organisms has also observed broader survival landscapes on high versus low-quality food [13,16,53]. However, this was not the case in our study (figure 4 inset): high-fitness under low-quality diet was constrained to a single peak of moderate growth and pigmentation rates, whereas high-quality diet did not show a distinct high-fitness peak. This could either be due to the aforementioned negative consequences of protein breakdown, or to physiological stress from accelerated rates of development [13,58].

Previous work on populations of *A. aquaticus* in southern Sweden has proposed that visual predation by predators is an important agent of selection, driving rapid evolution of cryptic body coloration in *A. aquaticus* [33,34]. Specifically, in shallow lakes, visual predators are thought to cause the evolution of darker isopods in dark stands of reed, and lighter isopods in light beds, of *Chara tomentosa*. However, the phenotypic differences stemming from our diet manipulation caused pigmentation differences as large as the phenotypic differentiation observed in southern Sweden populations (figure 2*d*), but in the absence of predators or background variation. Additionally, we observe substantial variation in the slope and intercept of family-level reaction norms (electronic supplementary material, figure S2) and a negative relationship between developmental trajectories and survival (figures 3 and 4 and table 1). This suggests an important link between factors affecting development, and the phenotypic evolution of cryptic body coloration. In the light of this work, we need more direct tests of the putative agents of selection driving phenotypic evolution and their mechanisms (e.g. macrophytes as diet and shelter).

The fact that we found elevated pigmentation rates under low elemental ratios and tryptophan supplement adds complexity to our understanding about how visual predators might mediate the evolution of pigmentation in *A. aquaticus* (figure 2*d*). Certain macrophytes contain tryptophan in relatively high levels [40], but the breakdown of proteins containing tryptophan and their digestions may result in toxicity [37,49]. Ommochrome synthesis may be a mechanism to bind excess tryptophan to pigment granules, while isopods can take advantage of any high-quality biomass instead of feeding selectively. Such 'local excretion' (i.e. the formation of inert pigments from soluble tryptophan) might be adaptive in arthropods to avoid toxicity of high-protein/low-elemental-ratio diets [37]. Although not a direct test, our path analysis provides some support for this hypothesis, as it shows higher survival under high pigmentation rates and lower growth rates (figure 3*d*). Such mechanisms do not exclude the possibility for the evolution of cryptic pigmentation, but we need a better understanding of sources of tryptophan in natural diets, and the associated costs of acquiring and using tryptophan to synthesize xanthommatin. Parasites, although known to affect pigmentation in *A. aquaticus* [39], unlikely played a role in our study because the isopods were reared in filtered lake water and the diets were autoclaved during their preparation.

In our study, we explored the links between variation in stoichiometric composition of diet, plasticity of developmental rates and fitness of juveniles (figures 1, 2*d* and 4). Diet stoichiometry and its potential mismatch with organisms' nutritional requirements is increasingly acknowledged to play a fundamental role in shaping life history and development [12,24,59]. Our study illustrates the environmental dependence of links between developmental rates and fitness variation in a natural population of detritivores. Such experiments, particularly if they are designed to test elemental stoichiometry and nutritional geometry theory [6,12], could be particularly insightful for consumers, including detritivores [60–62], that are likely to encounter stoichiometric mismatches through development [21–23]. Ultimately, such approaches could improve our understanding about the underlying sources and fitness consequences of developmental plasticity in natural populations.

Data accessibility. All relevant data, code and instructions for reproduction of all shown results are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.1vhhmgqrt [63].

Authors' contributions. M.D.L. and B.M. conceived the idea and designed the experiment, M.D.L. conducted all experimental and analytical work. Both authors contributed equally to writing the manuscript.

Competing interests. The authors declare that no conflicts of interest affected the work on this paper.

Funding. M.D.L. was funded by the Center for Adaptation to a Changing Environment (ACE) at ETH Zürich, and by the Department of Aquatic Ecology at Eawag.

References

1. Stearns SC. 1992 *The evolution of life histories*. Oxford, UK: Oxford University Press.
2. West-Eberhard MJ. 2005 Developmental plasticity and the origin of species differences. *Proc. Natl Acad. Sci. USA* **102**(Suppl. 1), 6543–6549. (doi:10.1073/pnas.0501844102)
3. Nettle D, Bateson M. 2015 Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve? *Proc. R. Soc. B* **282**, 20151005. (doi:10.1098/rspb.2015.1005)
4. Fischer S, Bohn L, Oberhummer E, Nyman C, Taborsky B. 2017 Divergence of developmental trajectories is triggered interactively by early social and ecological experience in a cooperative breeder. *Proc. Natl Acad. Sci. USA* **114**, E9300–E9307. (doi:10.1073/pnas.1705934114)
5. Groothuis TGG, Taborsky B. 2015 Introducing biological realism into the study of developmental plasticity in behaviour. *Front. Zool.* **12**(Suppl. 1), S6. (doi:10.1186/1742-9994-12-S1-S6)
6. Lee W-S, Monaghan P, Metcalfe NB. 2010 The trade-off between growth rate and locomotor performance varies with perceived time until breeding. *J. Exp. Biol.* **213**, 3289–3298. (doi:10.1242/jeb.043083)
7. Niewiarowski PH, Angilletta Jr MJ. 2008 Countergradient variation in embryonic growth and development: do embryonic and juvenile performances trade off? *Funct. Ecol.* **22**, 895–901. (doi:10.1111/j.1365-2435.2008.01441.x)
8. Chen E-H, Hou Q-L, Wei D-D, Jiang H-B, Wang J-J. 2017 Phenotypic plasticity, trade-offs and gene expression changes accompanying dietary restriction and switches in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Sci. Rep.* **7**, 1988. (doi:10.1038/s41598-017-02106-3)
9. Reznick D. 2013 Evolution of life histories. In *The Princeton guide to evolution* (eds JB Losos, DA Baum, DJ Futuyma, HE Hoekstra, RE Lenski, AJ Moore, CL Peichel, D Schluter, MC Whitlock), pp. 268–275. Princeton, NJ: Princeton University Press.
10. van Bergen E, Beldade P. 2019 Seasonal plasticity in anti-predatory strategies: matching of color and color preference for effective crypsis. *Evol. Lett.* **3**, 313–320. (doi:10.1002/evl3.113)
11. Edelaar P, Jovani R, Gomez-Mestre I. 2017 Should I change or should I go? Phenotypic plasticity and matching habitat choice in the adaptation to environmental heterogeneity. *Am. Nat.* **190**, 506–520. (doi:10.1086/693345)
12. Lee KP, Simpson SJ, Clissold FJ, Brooks R, Ballard JWO, Taylor PW, Soran N, Raubenheimer D. 2008 Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proc. Natl Acad. Sci. USA* **105**, 2498–2503. (doi:10.1073/pnas.0710787105)
13. Lee W-S, Monaghan P, Metcalfe NB. 2013 Experimental demonstration of the growth rate—lifespan trade-off. *Proc. R. Soc. B* **280**, 20122370. (doi:10.1098/rspb.2012.2370)
14. Naguib M, Podos J, Simmons LW, Barrett L, Healy SD, Zuk M (eds). 2017 *Advances in the study of behavior*, vol. 49. Amsterdam, The Netherlands: Elsevier Science. See https://www.sciencedirect.com/bookseries/advances-in-the-study-of-behavior/vol/49/suppl/C.
15. West-Eberhard MJ. 2003 *Developmental plasticity and evolution*. Oxford, UK: Oxford University Press. See Https://market.android.com/details?id=book-7DQNTPYaHIYC.
16. Verberk WCEP, Siepel H, Esselink H. 2008 Life-history strategies in freshwater macroinvertebrates. *Freshw. Biol.* **53**, 1722–1738. (doi:10.1111/j.1365-2427.2008.02035.x)
17. Metcalfe NB, Monaghan P. 2001 Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* **16**, 254–260. (doi:10.1016/S0169-5347(01)02124-3)
18. Metcalfe NB, Monaghan P. 2003 Growth versus lifespan: perspectives from evolutionary ecology. *Exp. Gerontol.* **38**, 935–940. (doi:10.1016/S0531-5565(03)00159-1)
19. Elser JJ *et al*. 2000 Nutritional constraints in terrestrial and freshwater food webs. *Nature* **408**, 578–580. (doi:10.1038/35046058)
20. Sperfeld E, Wagner ND, Halvorson HM, Malishev M, Raubenheimer D. 2017 Bridging ecological stoichiometry and nutritional geometry with homeostasis concepts and integrative models of organism nutrition. *Funct. Ecol.* **31**, 286–296. (doi:10.1111/1365-2435.12707)
21. Martinson HM, Schneider K, Gilbert J, Hines JE, Hambäck PA, Fagan WF. 2008 Detritivory: stoichiometry of a neglected trophic level. *Ecol. Res.* **23**, 487–491. (doi:10.1007/s11284-008-0471-7)
22. Frainer A, Jabiol J, Gessner MO, Bruder A, Mckie BG. 2016 Stoichiometric imbalances between detritus and detritivores are related to shifts in ecosystem functioning. *Oikos* **125**, 861–871. (doi:10.1111/oik.02687)
23. Halvorson HM, Sperfeld E, Evans-White MA. 2017 Quantity and quality limit detritivore growth: mechanisms revealed by ecological stoichiometry and co-limitation theory. *Ecology* **98**, 2995–3002. (doi:10.1002/ecy.2026)
24. Acharya K, Kyle M, Elser JJ. 2004 Biological stoichiometry of *Daphnia* growth: an ecophysiological test of the growth rate hypothesis. *Limnol. Oceanogr.* **49**, 656–665. (doi:10.4319/lo.2004.49.3.0656)
25. Leal MC, Seehausen O, Matthews B. 2017 The ecology and evolution of stoichiometric phenotypes. *Trends Ecol. Evol.* **32**, 108–117. (doi:10.1016/j.tree.2016.11.006)
26. Klepsatel P, Knoblochová D, Girish TN, Dircksen H, Gáliková M. 2020 The influence of developmental diet on reproduction and metabolism in *Drosophila*. *BMC Evol. Biol.* **20**, 93. (doi:10.1186/s12862-020-01663-y)
27. Lürig MD, Best RJ, Svitok M, Jokela J, Matthews B. 2019 The role of plasticity in the evolution of cryptic pigmentation in a freshwater isopod. *J. Anim. Ecol.* **88**, 612–623. (doi:10.1111/1365-2656.12950)
28. Morris M, Rogers SM. 2013 Overcoming maladaptive plasticity through plastic compensation. *Curr. Zool.* **59**, 526–536. (doi:10.1093/czoolo/59.4.526)
29. Plaistow SJ, Lapsley CT, Beckerman AP, Benton TG. 2004 Age and size at maturity: sex, environmental variability and developmental thresholds. *Proc. R. Soc. B* **271**, 919–924. (doi:10.1098/rspb.2004.2682)

30. Kodric-Brown A, Sibly RM, Brown JH. 2006 The allometry of ornaments and weapons. *Proc. Natl Acad. Sci. USA* **103**, 8733–8738. (doi:10.1073/pnas.0602994103)
31. Nalepa CA, Bignell DE, Bandi C. 2001 Detritivory, coprophagy, and the evolution of digestive mutualisms in Dictyoptera. *Insectes Soc.* **48**, 194–201. (doi:10.1007/PL00001767)
32. Senior AM, Nakagawa S, Lihoreau M, Simpson SJ, Raubenheimer D. 2015 An overlooked consequence of dietary mixing: a varied diet reduces interindividual variance in fitness. *Am. Nat.* **186**, 649–659. (doi:10.1086/683182)
33. Hargeby A, Johansson J, Ahnesjö J. 2004 Habitat-specific pigmentation in a freshwater isopod: adaptive evolution over a small spatiotemporal scale. *Evolution* **58**, 81–94. (doi:10.1111/j.0014-3820.2004.tb01575.x)
34. Hargeby A, Stoltz J, Johansson J. 2005 Locally differentiated cryptic pigmentation in the freshwater isopod *Asellus aquaticus*. *J. Evol. Biol.* **18**, 713–721. (doi:10.1111/j.1420-9101.2004.00837.x)
35. Eroukhmanoff F, Hargeby A, Arnberg NN, Hellgren O, Bensch S, Svensson EI. 2009 Parallelism and historical contingency during rapid ecotype divergence in an isopod. *J. Evol. Biol.* **22**, 1098–1110. (doi:10.1111/j.1420-9101.2009.01723.x)
36. Marcus JH, Sutcliffe DW, Willoughby LG. 1978 Feeding and growth of *Asellus aquaticus* (Isopoda) on food items from the littoral of Windermere, including green leaves of *Elodea canadensis*. *Freshw. Biol.* **8**, 505–519. (doi:10.1111/j.1365-2427.1978.tb01473.x)
37. Linzen B. 1974 The Tryptophan → Ommochrome pathway in insects. In *Advances in insect physiology* (eds JE Treherne, MJ Berridge, VB Wigglesworth), pp. 117–246. New York, NY: Academic Press.
38. Needham AE, Brunet PC. 1957 The integumental pigment of *Asellus*. *Cell. Mol. Life Sci.* **13**, 207–209. (doi:10.1007/BF02157167)
39. Oetinger DF, Nickol BB. 1981 Effects of Acanthocephalans on pigmentation of freshwater isopods. *J. Parasitol.* **67**, 672–684. (doi:10.2307/3280441)
40. Muztar AJ, Slinger SJ, Burton JH. 1978 The chemical composition of aquatic macrophytes. II. Amino acid composition of the protein and non-protein fractions. *Can. J. Plant Sci.* **58**, 843–849. (doi:10.4141/cjps78-123)
41. Sworobowicz L, Grabowski M, Mamos T, Burzyński A, Kilikowska A, Sell J, Wysocka A. 2015 Revisiting the phylogeography of *Asellus aquaticus* in Europe: insights into cryptic diversity and spatiotemporal diversification. *Freshw. Biol.* **60**, 1824–1840. (doi:10.1111/fwb.12613)
42. Rossi L. 1985 Interactions between invertebrates and microfungi in freshwater ecosystems. *Oikos* **44**, 175–184. (doi:10.2307/3544059)
43. Graça MA, Maltby L, Calow P. 1993 Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus*. I. Feeding strategies. *Oecologia* **93**, 139–144. (doi:10.1007/BF00321203)
44. Bohmann I. 2005 Coarse detritus in oligotrophic lake littoral zones—utilization by invertebrates and contribution to carbon flow. PhD thesis, University of Kalmar, Sweden.
45. Lürig MD. 2018 phenopype—a phenotyping pipeline for python. See https://doi.org/10.5281/zenodo.3483222.
46. Wood SN. 2011 Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J. R. Stat. Soc. B* **73**, 3–36. (doi:10.1111/j.1467-9868.2010.00749.x)
47. Bürkner P-C. 2018 Advanced Bayesian multilevel modeling with the R package brms. *R J.* **10**, 395–411. (doi:10.32614/RJ-2018-017)
48. Kayser H. 1979 Ommochrome formation and kynurenine excretion in *Pieris brassicae*: relation to tryptophan supply on an artificial diet. *J. Insect Physiol.* **25**, 641–646. (doi:10.1016/0022-1910(79)90113-6)
49. Arganda S, Bouchebti S, Bazazi S, Le Hesran S, Puga C, Latil G, Simpson SJ, Dussutour A. 2017 Parsing the life-shortening effects of dietary protein: effects of individual amino acids. *Proc. R. Soc. B* **284**, 20162052. (doi:10.1098/rspb.2016.2052)
50. Miozzari G, Niederberger P, Hütter R. 1978 Tryptophan biosynthesis in *Saccharomyces cerevisiae*: control of the flux through the pathway. *J. Bacteriol.* **134**, 48–59. (doi:10.1128/JB.134.1.48-59.1978)
51. Peeters ETHM, Camu JM, Beijer JAJ, Scheffer M, Gardeniers JJP. 2002 Response of the waterlouse *Asellus aquaticus* to multiple stressors: effects of current velocity and mineral substratum. *J. Aquat. Ecosyst. Stress Recov.* **9**, 193–203. (doi:10.1023/A:1021218721123)
52. Fontana L, Partridge L. 2015 Promoting health and longevity through diet: from model organisms to humans. *Cell* **161**, 106–118. (doi:10.1016/j.cell.2015.02.020)
53. Le Couteur DG, Solon-Biet S, Cogger VC, Mitchell SJ, Senior A, de Cabo R, Raubenheimer D, Simpson SJ. 2016 The impact of low-protein high-carbohydrate diets on aging and lifespan. *Cell. Mol. Life Sci.* **73**, 1237–1252. (doi:10.1007/s00018-015-2120-y)
54. Madsen L, Myrmel LS, Fjære E, Liaset B, Kristiansen K. 2017 Links between dietary protein sources, the gut microbiota, and obesity. *Front. Physiol.* **8**, 1047. (doi:10.3389/fphys.2017.01047)
55. Mousseau TA, Fox CW. 1998 *Maternal effects as adaptations*. Oxford, UK: Oxford University Press.
56. Bonduriansky R, Day T. 2003 The evolution of static allometry in sexually selected traits. *Evolution* **57**, 2450–2458. (doi:10.1111/j.0014-3820.2003.tb01490.x)
57. Frankino WA, Zwaan BJ, Stern DL, Brakefield PM. 2005 Natural selection and developmental constraints in the evolution of allometries. *Science* **307**, 718–720. (doi:10.1126/science.1105409)
58. McCarthy ID, Houlihan Dominic F, Carter CG. 1994 Individual variation in protein turnover and growth efficiency in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Proc. R. Soc. Lond. B* **257**, 141–147. (doi:10.1098/rspb.1994.0107)
59. Elser JJ, O'brien WJ, Dobberfuhl DR, Dowling TE. 2000 The evolution of ecosystem processes: growth rate and elemental stoichiometry of a key herbivore in temperate and arctic habitats. *J. Evol. Biol.* **13**, 845–853. (doi:10.1046/j.1420-9101.2000.00215.x)
60. Rietsma CS, Valiela I, Sylvester-Serianni A. 1982 Food preferences of dominant salt marsh herbivores and detritivores. *Mar. Ecol.* **3**, 179–189. (doi:10.1111/j.1439-0485.1982.tb00382.x)
61. Friberg N, Jacobsen D. 1994 Feeding plasticity of two detritivore-shredders. *Freshw. Biol.* **32**, 133–142. (doi:10.1111/j.1365-2427.1994.tb00873.x)
62. Bloor MC. 2011 Dietary preference of *Gammarus pulex* and *Asellus aquaticus* during a laboratory breeding programme for ecotoxicological studies. *Int. J. Zool.* **2011**, 1–5. (doi:10.1155/2011/294394)
63. Lürig MD, Matthews B. 2021 Data from: Dietary-based developmental plasticity affects juvenile survival in an aquatic detritivore. Dryad Digital Repository. (doi:10.5061/dryad.1vhhmgqrt)