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

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Abstract

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 likely 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 a freshwater detritivorous isopod (*Asellus aquaticus*), in terms 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), 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. In 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.

Key words

development; stoichiometry; elemental composition; fitness; life history; phenotypic plasticity; invertebrates; diet quality

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 environment 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 likely an important cause of developmental plasticity, because of its potentially large effects on the expression of morphological, physiological, and behavioral 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], mature earlier [29], or express 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 both in terms of 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

93 allowed us to investigate i) the extent of developmental plasticity in growth and pigmentation
94 caused by our diet manipulations, and ii) how such variation in developmental rates of growth and
95 pigmentation can jointly affect the survival of juveniles, in the absence of predators or their cues
96 [27,33]. Based on previous work regarding the physiological mechanisms of isopod development
97 [27,36,38], we expected to find higher pigmentation rates under high quality (=high protein) diet.
98 Moreover, we anticipated associations between developmental rates of growth and pigmentation,
99 partly because high quality diets often covary with pigmentation precursors - a covariation that we
100 attempted to disentangle with our manipulation of tryptophan. Our results confirm pronounced
101 developmental plasticity in pigmentation, and, to a lesser degree, in growth rates, and underscores
102 the need to consider diet- or resource-based developmental plasticity as an important source of
103 phenotypic variation, which may affect fitness before reproduction or selection from predation
104 later in life.
105
106 **Materials and Methods**
107 *Asellus aquaticus*
108 The freshwater isopod *A. aquaticus* is common in benthic communities across Europe and parts of
109 Asia [41]. The small crustaceans (mature animals are 4-15 mm, Fig. 1) are found in many different
110 microhabitats, like beds of *Chara tomentosa*, *Phragmites australis* (reed) or bare sand [33,34,36]
111 and are considered to play a significant role in freshwater food webs [33,36,41]. While *A.*
112 *aquaticus* can feed on fresh plant material, they often prefer substrates colonized with microbiota
113 (i.e. bacteria and fungi, Fig. 1D) on leaf litter or decaying macrophytes [36,42–44]. Feeding on
114 fungal and microbial biofilms may help alleviate stoichiometric mismatches between *A. aquaticus*
115 and their nutritionally poor detritial diets [36,43]. Moreover, the amino acid tryptophan, which is

116 essential for the main pigment in *A. aquaticus*, is known to vary strongly across various detrital
117 resources [40], but neither the effects of tryptophan or nutrition have been investigated in the
118 context of isopod life history and development. Here, we manipulate both diet quality and
119 tryptophan availability to explore the link between variation in developmental trajectories and
120 juvenile survival.
121
122 Common garden experiment
123 *Contrasts and food preparation* - Using a common garden experiment, we quantified the extent of
124 variation in developmental rates of growth and pigmentation, and their effects on survival in *A.*
125 *aquaticus* in response to diet composition (stoichiometric quality and tryptophan availability). To
126 do so, we exposed 1047 juvenile isopods from 29 families shortly after their birth (1-3 days) to
127 four different dietary contrasts: high elemental ratios (C:P and C:N, hereafter low quality [LQ]
128 diet) and low elemental ratios (hereafter high-quality diet [HQ]), as well as each of these diet
129 combinations crossed with a supplement (or not) of tryptophan. We measured growth,
130 pigmentation and survival of each individual over the course of 12 weeks. For each family, half of
131 the juveniles were randomly assigned to either low or high diet quality (full sib / split family
132 design). For the eight families with the highest number of offspring (50-60 juveniles), we crossed
133 the diet-quality treatment with a supplemental tryptophan treatment: in these eight families, 40
134 juveniles were randomly distributed among high- and low-quality treatments, and the remaining
135 10-20 individuals among the two treatments with tryptophan supplement. For the high-quality diet,
136 we used 80% dry yeast (*Saccharomyces cerevisiae*) and 20% potato starch that was autoclaved
137 together with agar and filtered lake water into a paste that was dried and cut into pellets (dry weight
138 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 (Fig. 1). Our tryptophan manipulation unintentionally lowered the C:P of this diet treatment (Fig 1E), but this effect was small relative to the overall diet contrast.

Experimental setup and procedure - We used juvenile isopods from a total of 29 successful matings (for details on isopod collection and breeding see 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 setup 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 ~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.

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, R2 = 0.97 [[27]]).

Statistical analyses

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 fit separate models each for body size (GAM1, Table1) 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, Table1). 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

185 developmental rates and starting conditions on lifespan under all diet treatment contrasts (See
186 supplement for details, Table S2). We applied both types of analysis in a complementary fashion:
187 with separate additive models, we accounted for the nonlinearity in developmental rates, and with
188 the path analysis we were able to disentangle complex interactions linking rearing conditions and
189 juvenile traits through development with survival variation.

190 To test for interactions between growth and pigmentation on survival, we also applied a
191 more complex multivariate GAM. To do so, we first converted measurements of body size and
192 pigmentation up until week 6 (dashed line in Fig. 2) to a single linear slope per individual isopod
193 (hereafter growth and pigmentation rate, respectively). We chose to calculate slopes from this time
194 frame, because pigmentation and growth increased linearly to this point, and isopod survival up to
195 this point was high. We then implemented an additive model (GAM4) with the "gam" function
196 from *mgcv*, using lifespan (in weeks) as the dependent variable, single thin plate spline terms for
197 growth and pigmentation rate, and a tensor smooth product term to test for the interaction (Table1).
198 The model included family as a random effect, and the spline and tensor term included diet as a
199 parametric component (See supplement for details).

200

201 **Results**

202 We found that growth rates were only weakly affected by diet quality and tryptophan supplement
203 (GAM 1, Table1, Fig. 2, Fig. 3), whereas rates of pigmentation were strongly affected by diet
204 quality. Tryptophan only resulted in significantly higher pigmentation rates under low quality diet
205 (significant interaction diet quality x tryptophan; Table 1, Fig. 2, Fig. 3). As indicated by the path
206 analysis (Fig. 3) and GAM2 (Table1, Fig. 2), pigmentation rates were lowest when juveniles were
207 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 (Table1, Fig. 2) and in the path analysis (Fig. 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, and supplementary Figure S2), the diet contrast resulted in clear divergence in the build-up of pigmentation through development (Fig. 2B). For a given body size, these diet-induced differences in pigmentation are comparable in magnitude to the observed habitat-specific variation in nature (Fig. 2D).

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, Table1, Fig. 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 (Fig. 3D). 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, Fig. 4, Table1). 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 (Fig. 4), as indicated by a significant nonlinear interaction of diet and rates (Table1). Specifically, survival on high quality diet peaked at intermediate growth rates and high rates of pigmentation (Fig. 4).

231

232 **Discussion**

233 Our experiment confirms and expands the results of a previous study [27] that found diet-based
234 developmental plasticity in pigmentation, and weak diet-based plasticity in growth in *A. aquaticus*.
235 In the current paper, we found that growth of juvenile isopods was only weakly affected by our
236 manipulation of diet stoichiometry and the tryptophan supplement (Fig. 2A, Fig. 3A, Table 1). The
237 growth rates we measured are comparable to previous rearing experiments that used naturally
238 occurring food items [36], confirming that the caloric content and nutritional balance of the pellets
239 that we provided ad libitum were an appropriate rearing environment. Maintaining high growth
240 rates on low quality food might be an important mechanism in natural habitats to escape
241 ("outgrow") gape limited predators (e.g. juvenile fish) or have a higher chance of escaping slow
242 moving invertebrate predators (e.g. odonate larvae) when they are larger [35]. Although our diet
243 contrast spanned beyond the range of natural food items that we measured in our study population
244 (Fig. 1), our treatments with high stoichiometric mismatch (i.e. high C:P/C:N) was sufficient near
245 natural growth [36] and pigmentation rates [27].

246 Pigmentation rates were strongly affected by our manipulation of diet stoichiometry (Fig.
247 2B, Fig. 3B, Table 1): when reared under high quality diet (low C:P, C:N) juvenile isopods from
248 a majority of families (22 out of 29, Fig. S2) showed greatly increased rates of pigmentation, and
249 also higher final levels pigmentation at the end of the experiment. This is in agreement with a
250 previous study [27] and provides additional support for plasticity of pigmentation during juvenile
251 development, which is irreversible for adult isopods [33]. Indeed, our dietary manipulations
252 recapitulated the entire phenotypic range of pigmentation for a given body size in the Lake Lucerne
253 population (see Fig. 1A-C, 2D and [27]). While variation among families in the extent of

254 phenotypic divergence likely results from a mixture of genetic and environmental factors, our
255 experimental design can neither quantify additive genetic variance of plasticity, nor test for
256 transgenerational plasticity (e.g. paternal effects). Even so, the high reproducibility of phenotypic
257 divergence within families exposed to contrasting diets provides strong evidence for diet-based
258 developmental plasticity in our study population.

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

268 A general result from our experiment was that juvenile survival depended strongly on the
269 developmental rates of both growth and pigmentation, albeit in complex ways. Both the significant
270 interaction in the path analysis (Fig. 3D) and the multivariate additive model (Fig. 4) suggest that
271 fast growing individuals had a lower likelihood of survival when they also had high rates of
272 pigmentation (Fig. 3D). Previous work has suggested that elevated growth rates in *A. aquaticus*
273 are associated with higher energy expenditure, and consequently, higher metabolism and resource
274 requirements [51], which may explain why fast-growing individuals have higher mortality rates.
275 Elevated dietary protein content has also been shown to reduce survival in other study systems
276 [52,53], which is thought to be caused by energetic expenditure associated with protein-digestion

277 and potentially harmful breakdown products [37,49]. Moreover, it is possible that a specific
278 composition of the gut microbial community is required to digest certain proteins [54]. Still, only
279 surprisingly little is known about the direct effects of protein consumption for aquatic isopods and
280 particularly *A. aquaticus*, given that many detrital food items may contain high amounts of protein
281 (Fig. 1).

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

296 Our experiment provided evidence for non-linear interactions between diet quality and
297 developmental rates that strongly affected juvenile survival. Specifically, under a low-quality diet
298 (high C:P, C:N), survival was constrained around a single peak centered at intermediate growth
299 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 (Fig. 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 *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 (Fig. 2D), but in the absence of predators or background variation. Additionally, we observe substantial variation in the slope and intercept of family level reaction norms (Fig. S2) and a negative relationship between developmental trajectories and survival (Fig. 3, Fig. 4, Table 1). This suggests an important link between factors affecting development, and the phenotypic evolution of cryptic body coloration. In 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* (Fig. 2D). 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 (Fig. 3D). 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 (Fig. 1, Fig. 2D, Fig. 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.

346

347 **Conflict of interests**

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

349

350 **Author contributions**

351 M. L. and B. M. conceived the idea and designed the experiment, M. L. conducted all experimental

352 and analytical work. Both authors contributed equally to writing the manuscript.

353

354 **Data availability statement**

355 A copy of all relevant data, code and instructions for reproduction of all shown results is available

356 on Dryad (https://doi.org/10.5061/dryad.1vhhmgqrt).

357

358 **Acknowledgement**

359 M. D. L. was funded by the Center for Adaptation to a Changing Environment (ACE) at ETH

360 Zürich, and by the Department of Aquatic Ecology at Eawag.

361

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Figures

Figure 1: Phenotypic variation in pigmentation in the freshwater isopod *Asellus 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 panel A-C.). B) Example of an isopod reared under 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 (Fig. 2D), [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 Table S1.

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 (Panel A, GAM1), log-transformed pigmentation (Panel B, GAM2) and survival (Panel 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 Panel A and B indicates the cutoff of values used for the

538 multivariate additive model (t1-t3, GAM4). Panel D shows the untransformed treatment-level
539 averages for length and pigmentation at each timepoint (same symbol and color coding as in Panel
540 A-C), and length and pigmentation of wild caught isopods from different habitats. Differences in
541 length and pigmentation due to the diet manipulation at the end of this experiment resembles
542 phenotypic variation in isopods from two different habitats in southern Sweden (SE, reed=black
543 points, *Chara tomentosa*=dark gray points). Moreover, developmental trajectories we measured in
544 this experiment fall within the range of phenotypes of isopods collected from Lake Lucerne in
545 Switzerland (CH, *Chara tomentosa*= light gray points).
546
547 Figure 3: Path analysis using Bayesian multilevel modelling to investigate the effects of diet
548 quality and tryptophan manipulation. Significant effects are indicated by colored arrows (green =
549 positive, red = negative, gray = not significant [overlap of the posterior with zero]), effect sizes
550 are given by number on arrows. Panels illustrate the effects of the factorial manipulation of
551 elemental composition (diet quality) and tryptophan on growth, pigmentation and survival rates
552 (panels A, B and C, respectively), as well as an interactive effect of growth and pigmentation rates
553 on survival across all diet manipulations (panel D - full three-way interaction between diet, growth
554 and pigmentation rates are analyzed by GAM 4 and shown in Fig. 4). Details on the path analysis
555 are given in the supplementary material (Table S2).
556
557 Figure 4: Survival landscapes modelled from the interaction of diet quality, growth rate and
558 pigmentation rate (GAM4). Each point denotes an individual isopod (black = quality contrast, gray
559 = tryptophan contrast). Diet specific surfaces are model estimates from GAM4 with survival
560 during experiment as the dependent and diet specific growth and pigmentation rates between start

and week 6 as the independent variable (see Table1 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 per day 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.

569 **Tables**

570 Table 1: Statistical results of generalized additive models. Models GAM1-GAM3 tested for an
571 effect of diet quality content on growth, pigmentation and survival (Fig. 2), GAM4 tested for
572 interactive effects of diet quality, growth rates, pigmentation rates on survival of isopods (Fig. 4).
573 Reported are results for linear (*Fixed effect)* and nonlinear (*Smooth term*) part of the model (tprs =
574 thin plate regression spline, tp = tensor product). For each model, the degrees of freedom for the
575 fixed effect term are 1, and the number of knots for each smooth function is 3. Significance of
576 Random effects was tested with a likelihood ratio test. Significant (<0.05) and marginally
577 significant (<0.1) results are in **bold**.

578

Model	*Response variable*	*Fixed effect*	*F*	*P value*	*Smooth term*	*Smooth function*	*edf*	*F*	*P value*	*Random effect*	*df*	*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 x 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 x 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 x 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 x growth rate	tprs	1.96	14.856	**>0.001**	Family	1	23.466	0.217
		Growth rate	652.88	**<0.002**	Low quality x growth rate	tprs	1.94	4.39	**0.014**	Block	1	60.419	**>0.001**
		Pigmentation rate	246.89	**<0.003**	High quality x pigmentation rate	tprs	1	23.212	**>0.001**				
		Diet x growth rate	108.07	**<0.004**	Low quality x pigmentation rate	tprs	1.98	6.501	**0.002**				
		Diet x pigmentation rate	66.537	**<0.005**	High quality x growth rate x pigmentation rate	tp	3.21	7.755	**>0.001**				

		Growth rate x pigmentation rate	2.709	0.1	Low quality + growth rate x pigmentation rate	tp	1	1.187	0.276				
		Diet x growth rate x pigmentation rate	2.778	**0.09**									

579