Received: 5 April 2018 | Accepted: 13 November 2018

DOI: 10.1111/1365-2656.12950

RESEARCH ARTICLE

Journal of Animal Ecology | BRITISH ECOLOGICAL SOCIETY

The role of plasticity in the evolution of cryptic pigmentation in a freshwater isopod

Moritz D. Lürig[1,2] | Rebecca J. Best[1,3] | Marek Svitok[1,4,5] | Jukka Jokela[2,6] | Blake Matthews[1]

[1]Swiss Federal Institute of Aquatic Science and Technology (Eawag Kastanienbaum), Kastanienbaum, Switzerland; [2]Center for Adaption to a Changing Environment (ACE), Institute of Integrative Biology, Zürich, Switzerland; [3]School of Earth and Sustainability, Northern Arizona University, Flagstaff, Arizona; [4]Department of Biology and General Ecology, Faculty of Ecology and Environmental Sciences, Technical University in Zvolen, Zvolen, Slovakia; [5]Department of Ecosystem Biology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic and [6]Department of Aquatic Ecology, Swiss Federal Institute of Aquatic Science and Technology, EAWAG, Dübendorf, Switzerland

Correspondence
Moritz D. Lürig
Email: moritz.luerig@eawag.ch

Funding information
Center for Adaptation to a Changing Environment (ACE) at ETH Zürich; Eawag Directorate; European Regional Development Fund, Grant/Award Number: CZ.02.1.01/0.0/0.0/15_003/0000441; Agentúra na Podporu Výskumu a Vývoja, Grant/Award Number: APVV-16-0236

Handling Editor: Stewart Plaistow

Abstract

1. Cryptic pigmentation of prey is often thought to evolve in response to predator-mediated selection, but pigmentation traits can also be plastic, and change with respect to both abiotic and biotic environmental conditions. In such cases, identifying the presence of, and drivers of trait plasticity is useful for understanding the evolution of crypsis.
2. Previous work suggests that cryptic pigmentation of freshwater isopods (*Asellus aquaticus*) has evolved in response to predation pressure by fish in habitats with varying macrophyte cover and coloration. However, macrophytes can potentially influence the distribution of pigmentation by altering not only habitat-specific predation susceptibility, but also dietary resources and abiotic conditions. The goals of this study were to experimentally test how two putative agents of selection, namely macrophytes and fish, affect the pigmentation of *A. aquaticus*, and to assess whether pigmentation is plastic, using a diet manipulation in a common garden.
3. We performed two experiments: (a) in an outdoor mesocosm experiment, we investigated how different densities of predatory fish (0/30/60 three-spined stickleback [*Gasterosteus aculeatus*] per mesocosm) and macrophytes (presence/absence) affected the abundance, pigmentation and body size structure of isopod populations. (b) In a subsequent laboratory experiment, we reared isopods in a common garden experiment on two different food sources (high/low protein content) to test whether variation in pigmentation of isopods can be explained by diet-based developmental plasticity.
4. We found that fish presence strongly reduced isopod densities, particularly in the absence of macrophytes, but had no effect on pigmentation or size structure of the populations. However, we found that isopods showed consistently higher pigmentation in the presence of macrophytes, regardless of fish presence or absence.

Our laboratory experiment, in which we manipulated the protein content of the isopods' diet, revealed strong plasticity of pigmentation and weak plasticity of growth rate.

5. The combined results of both experiments suggest that pigmentation of *A. aquaticus* is a developmentally plastic trait and that multiple environmental factors (e.g. macrophytes, diet and predation) might jointly influence the evolution of cryptic pigmentation of *A. aquaticus* in nature on relatively short time-scales.

KEYWORDS

Asellus aquaticus, computer vision, crypsis, divergent selection, macrophytes, phenotypic divergence, phenotypic plasticity, shallow lakes

1 | INTRODUCTION

Natural selection and plasticity often interactively shape the phenotypic distribution of natural populations. Developmental plasticity, where the environmental conditions experienced during juvenile development and growth produce lasting effects on adult phenotypes, can be an important source of phenotypic variation within a population. Such plasticity can be neutral, adaptive or maladaptive depending on the environmental context and on interactions with abiotic and biotic conditions. Phenotypic differences across populations are often explained by divergent natural selection (Calsbeek & Cox, 2010; Rundle & Nosil, 2005; Schluter, 2009), but the role of plasticity (developmental or otherwise) during adaptive population divergence is not well understood (Kingsolver & Pfennig, 2007; Pfennig et al., 2010; Schlichting, 2004). Sometimes phenotypic differences between environments can arise solely due to plasticity (Crispo, 2008) and be correlated or uncorrelated with fitness variation (Ghalambor, McKay, Carroll, & Reznick, 2007; Merilä, Laurila, Laugen, Räsänen, & Pahkala, 2000). Indeed, for many classic cases of adaptive population divergence (Table 1), it is often challenging to identify how multiple environmental differences can jointly affect the interaction between trait plasticity and natural selection (Nosil, Harmon, & Seehausen, 2009; Schmid & Guillaume, 2017).

During adaptive population divergence, multiple environmental differences (habitat, predation, resources, etc.) can potentially cause divergent plastic responses and influence the strength of divergent natural selection (Figure 1). Predators, for example, are capable of causing divergent selection (Bell, 2001; Bijleveld, Twietmeyer, Piechocki, van Gils, & Piersma, 2015; Moser, Roesti, & Berner, 2012; Quinn & Kinnison, 1999) and of inducing plastic responses (Scoville & Pfrender, 2010; Walsh et al., 2016). Similarly, plants can both affect the strength of divergent selection on grazing prey species through the food web (Carpenter & Lodge, 1986) and lead to plasticity by affecting light regimes (Miner & Kerr, 2011; Tollrian & Heibl, 2004) or nutrient dynamics (Hart & Lovvorn, 2003; Polunin, 1984). However, it is also possible that both biotic and abiotic environmental differences can interact to affect the distributions of phenotypes and fitness, and their covariance. Macrophytes can generate structural complexity (Kovalenko, Thomaz, & Warfe, 2011) and affect background coloration (Tavares, Pestana, Rocha, Schiavone, & Guillermo-Ferreira, 2018), to which not all prey phenotypes are equally well adapted (Lürig, Best, & Stachowicz, 2016). Thus, differences in macrophyte cover may affect the strength and direction of selection from predation (Merilaita, Lyytinen, & Mappes, 2001).

Rapid differentiation of cuticular pigmentation among populations of the benthic freshwater isopod *Asellus aquaticus* (L., Crustacea) was first documented in southern Sweden by Hargeby, Johansson, and Ahnesjö (2004). A subsequent survey among 29 Swedish lakes revealed that isopods are more pigmented in dark reed environments (Reed: *Phragmites australis*), less pigmented in lighter macrophyte environments (*Chara tomentosa*) and the least pigmented on light sand environments without macrophytes (Figure 2; Hargeby, Stoltz, & Johansson, 2005). In addition, fish predation trials in the laboratory have shown that darker isopods have higher survival in dark-coloured substrate, while lighter isopods have higher survival in environments with lighter substrates (Hargeby et al., 2004). Such results suggest that visual predation along an environmental gradient of background coloration is driving the rapid evolution of cryptic pigmentation of *A. aquaticus* (Hargeby et al., 2004). Importantly, macrophytes may alter predation susceptibility by making isopods more or less visible against their background, but also by altering the 3D structure of the habitat and the variety of refugia (Kovalenko et al., 2011; Tavares et al., 2018).

However, previous work has not emphasized how macrophytes might additionally influence the evolution of cryptic pigmentation of isopods, for example via their effects on food quality. It is known that macrophytes, and their associated epiphytes, periphyton and detritus can strongly affect the abundance and composition of invertebrate populations by altering resource quantity and quality (Diehl & Kornijów, 1998; Hart & Lovvorn, 2003; Jannot, Wissinger, & Lucas, 2008; Polunin, 1984; Sutcliffe, Carrick, & Willoughby, 1981). Previous work has demonstrated how such resource variation can affect life-history traits and development in *A. aquaticus* (Arakelova, 2001; Marcus, Sutcliffe, & Willoughby, 1978). There is also a functional link between the quality of macrophyte detritus

TABLE 1 Select examples of studies on adaptive population divergence in animals from field observations and laboratory experiments, ordered alphabetically. In all of these examples, at least two studies have found that different environmental factors may affect phenotypes through putative agents of selection and plasticity. We searched for studies using the Paperpile (Google Chrome browser Extension) literature search, using the words "Adaptive divergence" and "Phenotypic plasticity"

Organism	Traits	Putative agents of selection	References (selection)	Putative agents of plasticity	References (plasticity)
Barnacle (*Semibalanus balanoides*)	Cirral length	Temperature, diet, salinity	Flight, Schoepfer, and Rand (2010)	Microhabitat (wave action)	Marchinko (2003)
Bivalve (*Cerastoderma edule*)	Shell mass	Predation	Bijleveld et al. (2015)	Microhabitat (wave action)	De Montaudouin (1996)
Daphnia (*Daphnia* spp.)	Pigmentation	Predation, UV radiation	Miner and Kerr (2011); Scoville and Pfrender (2010)	Predation, UV radiation	Scoville and Pfrender (2010); Tollrian and Heibl (2004)
Guppy (*Poecilia reticulata*)	Life history	Predation	Reznick, Shaw, Helen Rodd, and Shaw (1997)	Food	Reznick and Yang (1993)
Lizard (*Anolis* spp.)	Limb length	Predation	Calsbeek and Cox (2010)	Microhabitat (shelter shape)	Losos, Schoener, Warheit, and Creer (2001)
Moor frog (*Rana arvalis*)	Body length, tail length, maximum body depth, maximum tail muscle depth and maximum tail depth	Predation, acidity	Egea-Serrano, Hangartner, Laurila, and Räsänen (2014)	Predation, acidity	Teplitsky and Räsänen (2007)
Snail (*Littorina saxatilis*)	Shell shape	Predation, microhabitat (wave action)	Garcia (2014); Johannesson and Johannesson (1996); Westram et al. (2014)	Predation, microhabitat (wave action)	Hollander and Butlin (2010)
Stickleback (*Gasterosteus aculeatus*)	Size and age at maturity	Predation, competition	Leinonen, Herczeg, Cano, and Merilä (2011)	Food	Lucek, Sivasundar, Roy, and Seehausen (2013)

and isopod pigmentation: the essential amino acid tryptophan is the precursor molecule in the developmental pathway of *A. aquaticus'* ommochrome-based pigmentation (Needham & Brunet, 1957; Shamim, Ranjan, Pandey, & Ramani, 2014), and because it cannot be synthesized by animals, it must be acquired through feeding, for example on macrophytes (Muztar, Slinger, & Burton, 1978). Building on this previous work, and the results of our mesocosm experiment (see below), we hypothesized that pigmentation of *A. aquaticus* could be developmentally plastic and influenced by diet (Figure 1, Figure 2).

In this study, we used two experiments to investigate the underlying causes of phenotypic variation in the freshwater isopod *A. aquaticus*. First, using an outdoor mesocosm experiment we tested how survival, body size and pigmentation of isopods depended on fish density and macrophyte presence/absence. Second, in a laboratory common garden experiment, we tested how diet (high and low protein content) affected the build-up of pigmentation throughout isopod development. Taken together, our experiments test two specific hypotheses: (I) fish and macrophytes jointly affect patterns of (cryptic) isopod pigmentation and (II) isopod pigmentation is a developmentally plastic trait influenced by differences in diet.

2 | MATERIALS AND METHODS

2.1 | Study system

Asellus aquaticus is a freshwater isopod that is common in water bodies across Europe and parts of Asia (Sworobowicz et al., 2015). *A. aquaticus* can have a semelparous uni- or bivoltine reproductive cycle, depending on geographic and local conditions (Arakelova, 2001; Økland, 1978). It occurs in many different microhabitats, for example dense patches of *Elodea canadensis* (Marcus et al., 1978), stands of *Chara* and reed (Hargeby et al., 2004) and sandy substrates (Hargeby et al., 2005). *A. aquaticus* is mainly a detritivore (Hargeby et al., 2004; Marcus et al., 1978) and an important prey item for invertebrate predators and fish (Hargeby et al., 2004; Hart & Gill, 1992). As such, it plays a significant role in freshwater food webs (Jeppesen, Sondergaard, Sondergaard, & Christoffersen, 1998). The distinctive pigmentation of isopods is composed of melanins (Needham, 1970), which are subcutaneous and therefore remain in the integument during moulting. Consistent with developmentally plastic traits, loss or gain of pigmentation after reaching maturity has not been reported.

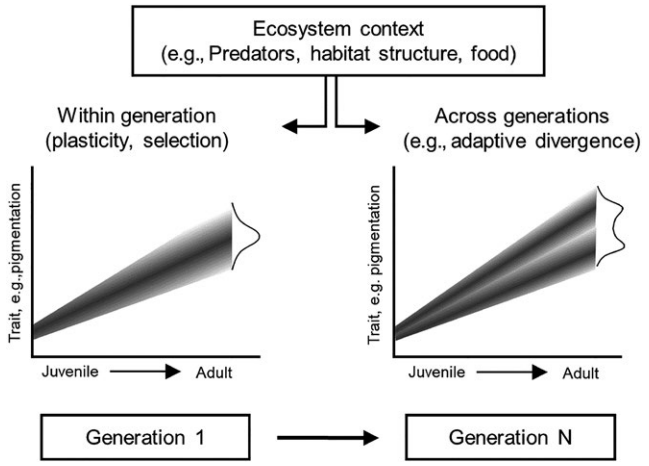

FIGURE 1 The ecosystem context during organismal development and growth can determine how the phenotype distribution in a population both develops within generations (i.e. due to plasticity and selection) and evolves across generations. Different evolutionary outcomes across generations are possible (e.g. via adaptive divergence) that can also be influenced by the ecosystem context

2.2 | Effects of fish and macrophytes on isopods (mesocosm experiment)

In 2015, we set up 50 outdoor mesocosms (1,000 L) at Eawag Kastanienbaum in a randomized block design that included factorial combinations of macrophytes (presence/absence) and fish (three-spined stickleback, *Gasterosteus aculeatus*) in densities of 0, 30 and 60 individuals per tank. To establish the experiment in early May 2015, we filled each mesocosm with water from Lake Lucerne, added a 2-cm-thick layer of gravel (2–4 mm grain size) and a 1-cm-thick layer of fine sediment from Lake Lucerne, consisting of silt and organic material. On 26 May 2015, we then planted two species of common macrophytes, *Chara tomentosa* (hereafter *Chara*) collected from Lake Lucerne, and *Myriophyllum spicatum* (hereafter *Myriophyllum*) collected from a stream in the Lake Constance watershed (Oberriet, St. Gallen).

All collected plant material from either location was divided into 60 equal portions by visual partitioning, of which 50 were randomly assigned to mesocosms and 10 were used to measure initial plant biomass (Supporting Information Table S1) and to count and phenotype isopods at the start (see below). In the 25 mesocosms designated as "macrophyte tanks," both plant species were placed at the bottom of the tank and allowed to root. The other 25 mesocosms designated as "no macrophyte tanks" received invertebrates associated with the macrophytes, including *A. aquaticus*. We accomplished this by thoroughly washing the plant material into the water and then temporarily suspending it in large mesh enclosures for 2.5 weeks. In this process, only very little *Chara* detritus was released into the "no macrophyte" tanks (low *Chara* biomass in "no macrophyte" tanks, see Supporting Information Table S1).

Isopods were introduced to the mesocosms by planting or washing plant material into the water (see above): on average, 159 ± 29 (mean ± *SD*) isopods were introduced to each mesocosm separately by planting or suspending both macrophyte species. We counted and phenotyped isopods coming from the 10 aliquots of both macrophyte species. Approximately 50% of the isopods were introduced from *Myriophyllum* (80 ± 34, mean ± *SD*) and 50% from *Chara* macrophytes (79 ± 26, mean ± *SD*). The isopods were exposed to experimental conditions for six months (May–October), which corresponds to the presence of two to three generations, and experienced fish predation for 3 months (August–October).

On 8 August 2015, we added fish (three-spined stickleback) to 40 mesocosms at a density of either 30 or 60 individuals per tank. The stickleback were laboratory-reared juveniles (3 months old) that we bred from wild-caught stickleback from the Lake Constance region. In each tank, the fish were either a mixture of lake and stream ecotypes, or their hybrids. Thus, both the macrophytes and the fish predators represented a diverse mixture from both lake and stream habitats.

We terminated the experiment on 22 October, after six months, and sampled the isopods from all mesocosms by dragging a net with a 28 × 28 cm opening and 100-μm mesh size across the bottom (sampling ⅓ of the benthic environment). We preserved all sampled isopods in the freezer for subsequent phenotypic analysis. At the end of the experiment, we quantified total macrophyte biomass of all species (Supporting Information Table S1) and the nutrient concentrations of each species (*Myriophyllum*, *Chara* and filamentous algae) with an elemental analyser (PYRO cube and Isoprime; Elementar, Supporting Information Table S2).

2.3 | Effects of diet on development of pigmentation (laboratory experiment)

In the following year (2016), we set up a laboratory experiment to test for developmental plasticity of pigmentation in *A. aquaticus* by manipulating dietary nutrient composition (ratios of N, P and C) during development and measuring rates of pigmentation change and growth over 100 days. For the high-nutrient diet, we mixed a substrate containing 80% dry yeast (Saccharomyces cerevisiae) and 20% potato starch with agar and filtered lake water. The low-nutrient diet was prepared in the same way, but with 20% yeast and 80% starch. Individual isopods from a total of 11 families were reared in a full-sib, split family design: half of each family was reared with a low-nutrient diet and the other half with a high-nutrient diet. To obtain the families, we collected >500 adult *A. aquaticus* from *Chara* vegetation in Lake Lucerne (47°00′06.8″N 8°20′02.7″E) and established them in a single aquarium (160 L with lake water) in the laboratory. We maintained this population with *Chara* plant material as substrate, at 20°C with a 12:12-hr light/dark cycle. These isopods were allowed to mate freely in the tank, and brooding females were isolated and reared in separate containers until their juveniles were ready for the experiment (5–10 days). Once a mother released her juveniles, we randomly distributed single individuals into 50-ml polyethylene tubes. The tubes were filled with filtered lake water and contained a pellet of one of the food types. We placed the racks that held the tubes in a water bath at 20°C to buffer against temperature changes

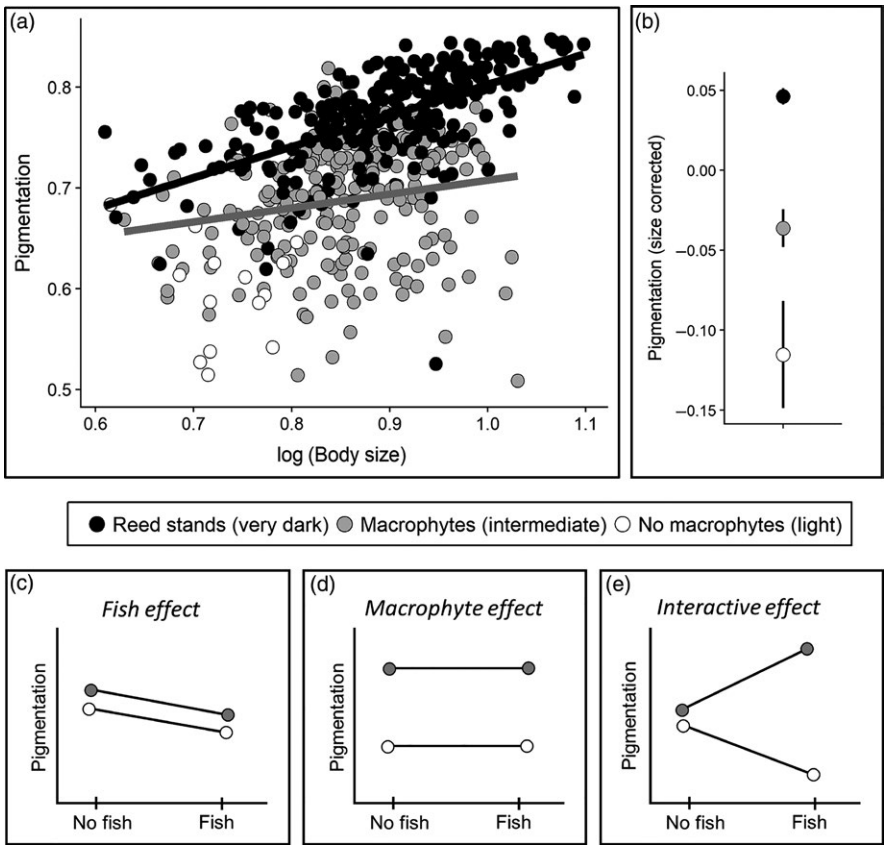

FIGURE 2 (a) The relationship of pigmentation and body size of *Asellus aquaticus* in microhabitats with different backgrounds (from dark to light): reed (*Phragmites australis*), macrophytes (*Chara tomentosa*) and no macrophytes (sandy substrate). The data include six lakes from southern Sweden and were collected from Hargeby et al. (2005) using *WebPlotDigitizer* (Rohatgi, 2010). Each data point is an individual; the lines are estimates of pigmentation from a linear mixed-effects model with vegetation as main effect, body size as the covariate and lake as the random effect (main effect of vegetation $p = 0.005$). (b) Size-corrected pigmentation (mean ± *SD*) per microhabitat. We corrected pigmentation for body size using the equation of a linear regression analysis including data from all lakes and microhabitats. (c–e) Schematic illustrations of how phenotypic differentiation in *A. aquaticus* may depend on different ecosystem contexts. (c) Across all macrophyte microhabitats, fish may selectively forage on larger individuals, which may result in larger number of small isopods, which are developmentally less pigmented. (d) Across all predation intensities from fish, differences in macrophytes may lead to differences in pigmentation, for example through food or light. (e) Fish and macrophytes may interact in their effect on pigmentation; for example, fish may remove more dark isopods in light environments or vice versa, and thus could select for pigmentation that matches the background of a microhabitat

and with a 12:12-hr light/dark cycle. Whenever a food pellet was fully consumed by an isopod, we replaced it with a one of the same kind. We changed half of the water in each tube every two weeks.

2.4 | Isopod phenotyping

In the mesocosm experiment, we imaged thawed isopods with a modified flatbed scanner (Epson) in high resolution (2,400 dpi). Individuals were placed inside a water film on the scanner to minimize reflectance and artefacts during the scanning (Figure 3b). We included greyscale card and millimetre reference cards in all pictures to ensure reproducible brightness conditions and magnification.

In the plasticity experiment, we took pictures of live isopods using a camera stand with a digital single-lens reflex camera (Canon) and a 100-mm macro lens (Tamron). We placed a single isopod on a white plastic bowl underneath the camera that was illuminated with an LED spot ring (Leica). We took a picture of every individual isopod at the start of the experiment and every two weeks over the course of the experiment.

We measured pigmentation and body size of isopods in both experiments by using computer vision techniques that analysed digital pictures of the specimens. Pigmentation and body size of isopods were extracted from all images with a self-written python package (https://github.com/mluerig/phenopype [Lürig, 2018]). The package uses thresholding algorithms and segmentation to locate isopods in the image and extract the phenotypic information from the areas marked as the animal (dorsal region of isopod torso = carapace, excluding legs and antennae). The greyscale values from these pixels are then extracted, 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, using the same pixels from the marked area. Results produced with

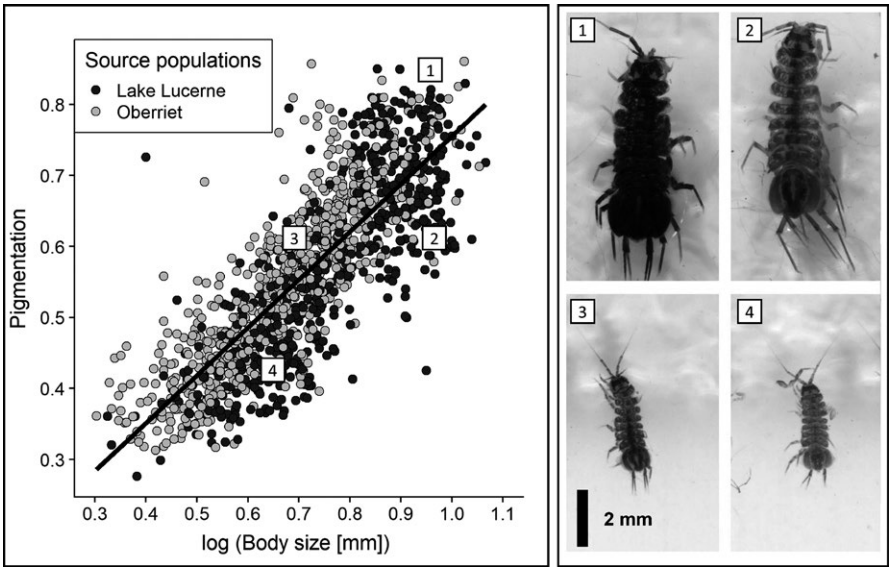

FIGURE 3 Pigmentation and body size in the source populations of *Asellus aquaticus* are positively related: linear regression coefficient = 0.671 (linear model of pigmentation and logarithmic body size $p < 0.001$). We used the linear equation of this regression analysis to size-correct pigmentation of isopods collected from the mesocosms after the experiment. Isopods from both populations were equally represented in the mesocosm at the start of the experiment. The four pictures show example images of scanned *A. aquaticus*, and the numbers indicate their position among the range of phenotypes. 1: dark adult, 2: light adult, 3: dark juvenile and 4: light juvenile (all from Lake Lucerne)

this method were not different from measurements of the same images using ImageJ (Supporting Information Figure S1, linear correlation between methods: 0.97, $p = 0.0291$ [Schindelin et al., 2012]).

Pigmentation in isopods is strongly dependent on body size, such that bigger isopods are more pigmented than smaller isopods in both our source populations (Figure 3). To explore how pigmentation might vary among treatments independently of body size, we size-corrected pigmentation using a linear regression of pigmentation and log-transformed body size in the source populations (Figure 3: intercept = 0.082, slope = 0.671). Hereafter, we refer to size-corrected pigmentation as "pigmentation."

2.5 | Data analysis

We used a series of linear mixed models (LMMs) to test for treatment effects on isopods in both the mesocosm and the laboratory experiments (Table 2). All LMMs were run using the R-package nlme (Pinheiro, Bates, DebRoy, & Sarkar, 2017) with normal error distributions.

In the mesocosm experiment, we used a LMM to test for differences in three response variables at the tank level: isopod abundance (Model M1), size-corrected pigmentation (Model M2) and body size (Model M3). The response variables in M2 and M3 were tank averages. For M1-M3, the fixed effects were macrophyte presence, fish density (0, 30 or 60 individuals) and their interaction, and the random effect was spatial block. Because of the unbalanced experimental design (10 tanks without fish, 20 tanks with 30 fish, 20 tanks with 60 fish), we parameterized the models with sum-to-zero constraints and performed all tests based on type III sum of squares (Quinn & Keough, 2002). Results of *F* tests and likelihood-ratio tests are reported for fixed and random effects, respectively. Additionally, to test for differences in isopod densities between fish presence and absence (0 vs. 30 and 60) we used a post hoc analysis (Tukey's all paired comparisons from R-package multcomp [Hothorn, Bretz, & Westfall, 2008]). Finally, we also tested for interactions between body size and treatment at the individual level (M4). For this model, uncorrected pigmentation was the response variable, and the fixed effects were body size, macrophytes, fish and their interactions. We also added tank identity to the random effects, by nesting tanks inside blocks.

In the laboratory experiment, we tested for the effect of dietary nutrient concentration on the development of pigmentation (Model M5) and body size (Model M6). For each model, the fixed effects were time (days since start) and diet type. To account for repeated measurements of the same individuals, we included individuals nested within families as random effects. We focused on the linear rate of growth and pigmentation accumulation over the first 70 days, because after this time, mortality rates were too high (fewer than 50% of individuals were still alive) to accurately quantify variation in nonlinear patterns (Supporting Information Figure S2). To test for overall differences in survival between individuals across families and between diet types, we used a log-rank test (R-package survminer [Kassambara & Kosinski, 2017]).

Residuals of all models were checked for normality and homoscedasticity using diagnostic plots. The models involving repeated measurements (M5 and M6) were also screened for the presence of temporal autocorrelation using correlograms. In the case of heteroscedasticity, we included an appropriate variance function to model the variance structure of the errors (grouped or power variance function [Pinheiro & Bates, 2000]). All analyses were performed in the programming language R (R Core Team, 2017).

TABLE 2 Statistical significance of isopod density, pigmentation and body size in the two experiments (mesocosm and laboratory). M1–M3 test for tank-level effects of macrophytes and fish, M4 tests for interactive effects of body size and treatment on individuals, M5 and M6 test the effect of diet on individuals. All models are linear mixed-effects models using type III sum of squares. Significant *p*-values (<0.05) are in bold

Model	Response variable	Fixed effects	*df*	*F*	*p*	Random effect	*df*	X^2	*p*
Mesocosm experiment									
M1	Density	Macrophytes	1, 40	0.048	0.762	Block	1	5.876	**0.015**
		Fish density	2, 40	10.183	**<0.001**				
		Macrophytes × fish density	2, 40	5.864	**0.006**				
M2	Pigmentation (size corrected)	Macrophytes	1, 40	4.990	**0.031**	Block	1	0.017	0.897
		Fish	2, 40	0.235	0.791				
		Macrophytes × fish density	2, 40	0.100	0.906				
M3	Body size	Macrophytes	1, 40	0.272	0.605	Block	1	0.293	0.588
		Fish	2, 40	0.352	0.705				
		Macrophytes × fish density	2, 40	0.389	0.680				
M4	Pigmentation	Body size	1, 2795	8531.919	**<0.001**	Block	1	45.178	**<0.001**
		Macrophytes	1, 40	15.654	**<0.001**	Tank	1	198.174	**<0.001**
		Fish density	2, 40	0.924	0.405				
		Body size × macrophytes	1, 2795	0.081	0.776				
		Body size × fish density	2, 2795	1.287	0.276				
		Macrophytes × fish density	2, 40	0.236	0.791				
		Body size × macrophytes × fish density	2, 2795	0.594	0.552				
Laboratory experiment									
M5	Pigmentation (size corrected)	Diet	1, 85	3.305	0.073	Family	1	109.780	**<0.001**
		Time	1, 333	188.311	**<0.001**	Individual	1	99.358	**<0.001**
		Diet × time	1, 333	89.549	**<0.001**				
M6	Body size	Diet	1, 85	2.604	0.110	Family	1	14.940	**0.002**
		Time	1, 333	562.316	**<0.001**	Individual	1	184.337	**<0.001**
		Diet × time	1, 333	4.120	**0.043**				

3 | RESULTS

3.1 | Mesocosm experiment

At the end of the experiment, isopod densities were significantly lower when fish were present in the mesocosms than when fish were absent. This effect, however, was dependent on the presence of macrophytes, which increased isopod survival, particularly at high density (Figure 4, M1: interactive effect). In the absence of fish, isopod densities in some mesocosms without macrophytes were very high, but the mean density was not significantly different from mesocosms with macrophytes. Isopod pigmentation was higher in the presence of macrophytes, regardless of fish density (Figure 5a; Table 2: M2). In addition, the population of isopods in the mesocosms tended to be less pigmented than the population used to inoculate the experiment starting population (Figure 5a solid line). Body size did not differ among the treatments (Figure 5b; no effect of macrophytes or fish density in Table 2: M3), and average size did not change relative to the starting population (Figure 5b, solid line). Furthermore, there were no interactive effects of any of the treatments and body size on pigmentation (Table 2, M4), but instead the significant effect of macrophytes on pigmentation was confirmed. Finally, we confirmed that the biomass of our planted macrophytes (*Myriophyllum* and especially *Chara*) was higher in the macrophyte treatment than the no macrophyte treatment, despite some growth from fragments in the sediment growth from the sediment (Supporting Information Table S1). The *Chara* plants in our experiment also had a higher phosphorus and nitrogen content relative to other sources of detritus in the mesocosms (Supporting Information Table S2).

3.2 | Plasticity experiment

In the laboratory experiment, the dietary manipulation of phosphorus and nitrogen content (Supporting Information Table S2) had

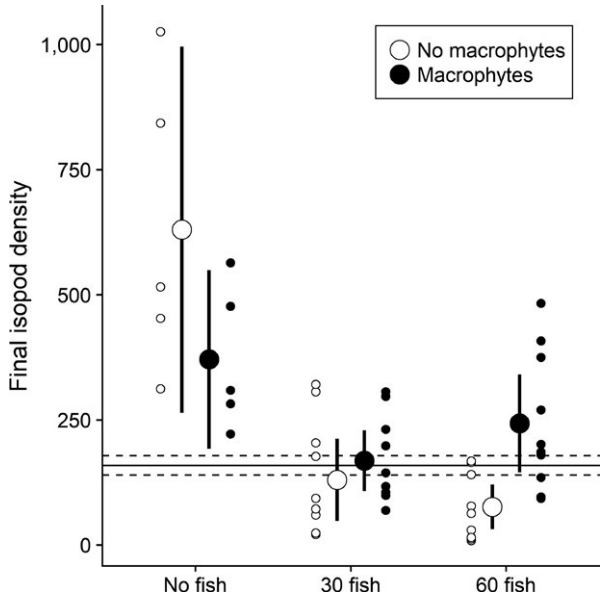

FIGURE 4 Fish presence significantly reduced isopod densities (post hoc contrasts: 0 vs. 30 fish and 0 vs 60 fish both significant [$p < 0.001$]). However, this interacted with macrophyte presence. Each small point represents a mesocosm tank; the large points are mean ± 95% confidence interval (CI). At the beginning of the experiment, all mesocosms were stocked with 159 ± 29 (mean ± *SD*; solid and dashed lines, respectively) specimens of *Asellus aquaticus*

strong effects on the rate of pigmentation development through time in *A. aquaticus* (Table 2: M5). Compared to the low-nutrient diet, the high-nutrient diet yielded higher pigmentation across all families (Figure 6a). The high-nutrient diet also marginally increased growth rates (Table 2: M6), but responses differed strongly among families (Figure 6b). Furthermore, death rate increased towards the end of the experiment (after day 70, Supporting Information Figure S2), but with no significant difference in survival among diet treatments (log-rank test: $p = 0.58$). Among the survivors, we observed notable effects of diet quality on fecundity: a marsupium developed in 11 females reared under high-nutrient diet but only one female on a low-nutrient diet.

4 | DISCUSSION

Both experiments are consistent with the hypothesis that isopod pigmentation is a developmentally plastic trait, which is likely influenced by food resources. In the mesocosm experiment, isopods collected from tanks with macrophytes had stronger pigmentation than isopods from macrophyte-free mesocosms (Figure 5a). Although we expected interactive effects of fish predation and macrophytes (i.e. hypothesis I), the effect of macrophytes on pigmentation persisted independent of the large range of fish density in our experiment. Furthermore, our laboratory diet manipulation experimentally confirmed plasticity of pigmentation (Figure 6a), and, to a lesser extent, plasticity in the somatic growth rate of isopods (Figure 6b). Below, we elaborate on potential mechanisms that might explain these outcomes and discuss the interactions between food availability, selection by predators and the role of plasticity during adaptive divergence of natural populations.

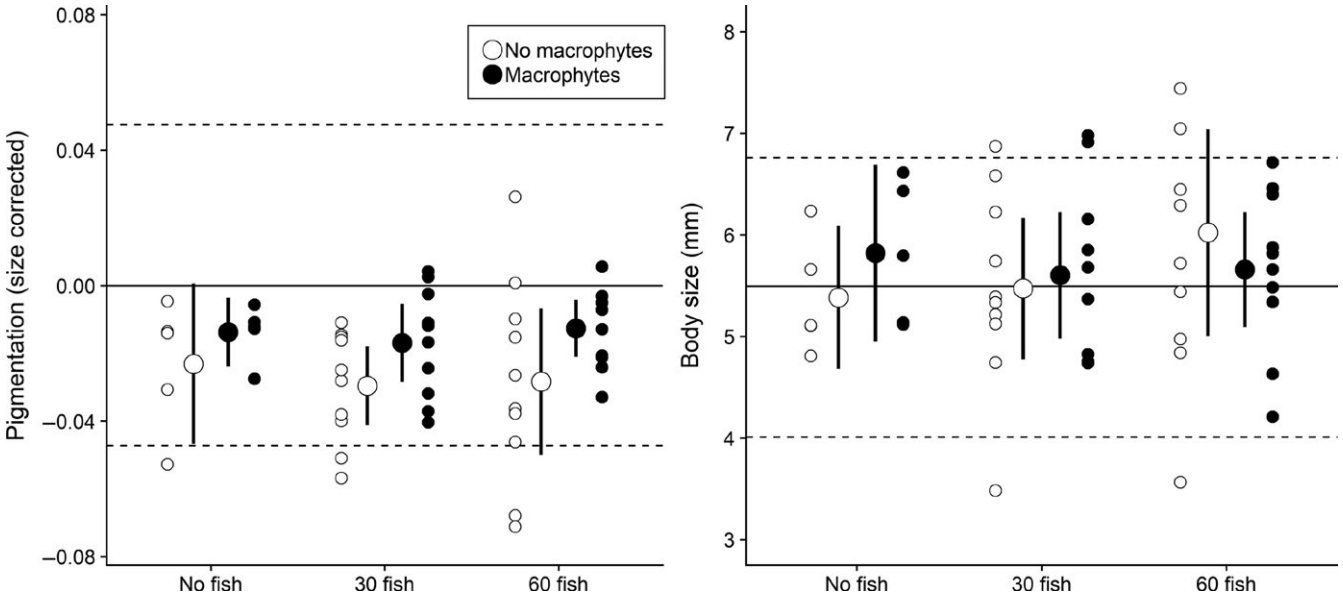

FIGURE 5 Macrophyte presence yielded higher pigmentation (left panel) in isopods than macrophyte-free tanks (significant main effect of macrophytes in M2, $p = 0.002$). Values are size-corrected using the linear equation of the regression shown in Figure 3. Body size (right panel) of isopod specimens retrieved from the mesocosms after the experiment was not affected by any of the treatments. In both panels, each data point represents the average response for one mesocosm and the large dots with error bars are mean ± CI per treatment across all mesocosms. The solid line indicates the mean starting condition, and the dashed lines show mean and *SD* of the starting populations, respectively

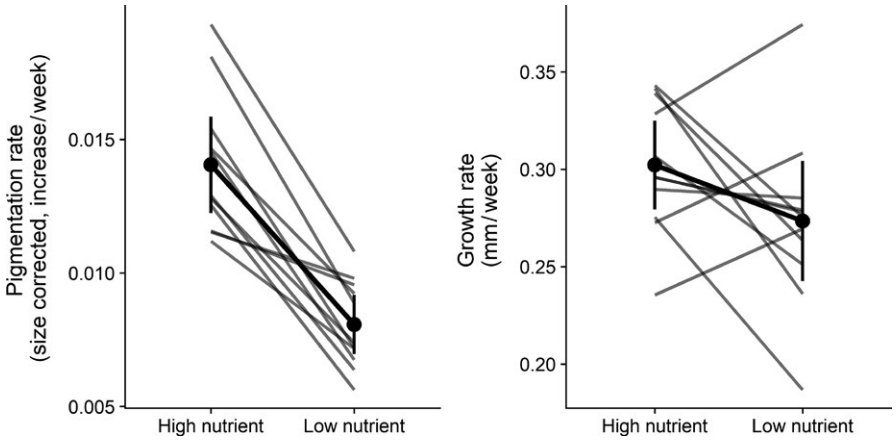

FIGURE 6 Rates of increase in pigmentation (left panel) and body size (right panel) of *Asellus aquaticus* were higher under high-nutrient diet (significant main interactive effect of time and diet in M4 and M5). Points are the weekly average change in pigmentation or body size of individuals across all families (mean ± CI), and grey lines indicate family-level reaction norms

Over the course of the six-month experiment, isopod density in mesocosms without fish predators increased significantly (between 100% and 200%), regardless of the macrophyte presence. In the presence of fish, isopod population size declined by 25% relative to initial densities, consistent with studies showing that stickleback are effective visual predators of *A. aquaticus* (Salvanes & Hart, 1998). However, when fish were present, isopods densities were higher in mesocosms with macrophytes than in macrophyte-free tanks, suggesting that macrophytes can reduce predation pressure by stickleback (Diehl & Kornijów, 1998). This could occur because macrophytes generate structural habitat complexity (Kovalenko et al., 2011; Lürig et al., 2016; Warfe, Barmuta, & Wotherspoon, 2008), making it difficult for fish to find and capture any isopods, or because they alter the intensity and heterogeneity of the light environment (Baker & Ball, 1995; Verweij et al., 2006).

Isopods in mesocosms from our macrophyte treatment exhibited darker pigmentation than in our treatment without added macrophytes, regardless of fish density, suggesting that the effects of macrophytes on pigmentation were independent of fish predation. This was surprising, given the findings of previous work (Eroukhmanoff, Hargeby, & Svensson, 2009; Hargeby et al., 2004, 2005), but matches one of the scenarios we proposed ("Macrophyte effect," Figure 2d). One possible explanation for stronger pigmentation in the presence of macrophytes could be the influence of macrophytes on the light environment. In most tanks, *Myriophyllum* extended its canopy to the water surface, substantially reducing the amount of incoming light. Isopods born into a darker environment could also develop more pigments to be less conspicuous. This phenomenon may also be a reasonable explanation for why isopods in our experiment were generally lighter than the isopods we collected from the wild: in Lake Lucerne and the Oberriet creek, macrophyte cover was higher than in the mesocosms, potentially inducing a much darker environment during isopod development. However, macrophytes that are blocking incoming light may also reduce the amount of UV radiation that organisms are exposed to, which typically increases pigmentation in aquatic organisms (Miner & Kerr, 2011; Tollrian & Heibl, 2004). Given such complexities, we suggest further work could investigate how experimental manipulations of the light environment could influence isopod pigmentation, growth and survival during development. This would complement the interpretation of our results, showing how dietary manipulations affected the development of pigmentation.

Over the course of the entire experiment, there was a clear difference in the dietary resources among the treatments that was available for detritivorous isopods (Table S1). In the mesocosms where macrophytes were planted, there was significantly higher biomass of *Chara* and *Myriophyllum*. Submerged plants are also often covered with epiphytes (Jeppesen et al., 1998), which, beside the plant itself, are part of *A. aquaticus*' dietary spectrum (Graca, Maltby, & Calow, 1993; Marcus et al., 1978). Furthermore, a substantial portion of the initially planted *Chara* biomass was converted over the season to consumable detritus (lower final living biomass than input biomass). *Chara* has a relatively high P content relative to its carbon content (i.e. low C:P ratio). Low C:P food resources are often associated with higher growth efficiencies of macroinvertebrates (Elser et al., 2000), while high C:P ratios may hinder growth and other developmental processes (Lee et al., 2008). While we did not find any effects of the macrophyte presence on the body size spectrum, it is possible that nutrient-rich detritus may increase the development of pigmentation in isopods. The biosynthesis of the ommochrome pigments in *A. aquaticus* results from a potentially costly physiological pathway (Needham, 1970; Needham & Brunet, 1957) that may require a high-quality diet, that is with high nutrient concentrations, to function properly. Additionally, macrophyte detritus may have provided the essential compounds required for the biosynthesis. The ommochrome pathway starts with the essential amino acid tryptophan as the precursor molecule (Shamim et al., 2014). *Myriophyllum* and *Chara* are both natural sources for tryptophan (Muztar et al., 1978), and so increased macrophyte detritus may have provided additional tryptophan that supported the biosynthesis of pigments.

Plasticity due to variation in resources is common in natural populations. Notable examples include plastic morphology and behaviour in fishes (perch [*Perca fluviatilis*]: (Olsson, Svanbäck, & Eklöv, 2007); Arctic charr [*Salvelinus alpinus*]: (Andersson, 2003)), life history in echinoids (Reitzel & Heyland, 2007) and *Drosophila* (Lee et al., 2008), and growth rates and sexual traits in amphipods (Cothran, Stiff, Jeyasingh, & Relyea, 2012; Sutcliffe et al., 1981). Both of our

experiments suggest a strong role for diet-based developmental plasticity of isopod pigmentation. As discussed above, resource-based plasticity could partly explain the consistent differences in pigmentation between mesocosms with and without macrophytes (Figure 5). Furthermore, across multiple families it was clear that isopods reared on a diet with more nutrients developed pigmentation faster for a given growth. The difference in intercepts of the reaction norms among families (Figure 6; Table 2: M5 family effect) suggests there is some genetic variation in the pigmentation of *A. aquaticus*. Growth rates were also significantly affected by diet, but the effect was much smaller and the relative differences among family-level responses were greater than for rates of pigmentation development (Figure 6). Interestingly, three families showed a positive growth rate when reared on the low-nutrient diet, but further experiments would be necessary to understand the extent of family-level variation in isopod development and to identify other involved key drivers of plasticity of isopod pigmentation and growth in natural populations.

Our study shows that differentiation in pigmentation in *A. aquaticus*, a process primarily thought to be driven by selection from predation (Eroukhmanoff et al., 2009; Hargeby et al., 2004, 2005), may also be influenced by developmental plasticity in response to different diets and macrophyte environments. Our results do not preclude the possibility for selection on cryptic pigmentation from fish predation, which is a previously suggested driver of phenotypic diversification of *A. aquaticus*. It is possible that the plastic response in our experiment was stronger than any selective effects of fish predation or that the experiment was not long enough to observe predator-mediated selection. Overall, our results illustrate that the same environmental factor (macrophytes) known to impact divergent selection for cryptic coloration can also drive phenotypic plasticity in pigmentation via diet. Such cases might be common in natural populations, because the putative agents of selection on a trait might also affect plasticity of the same trait (Table 1). Such complexities highlight the need for more comparative and experimental studies of (mal)adaptive developmental plasticity in general (Scoville & Pfrender, 2010), and its role during adaptive divergence in particular.

ACKNOWLEDGEMENTS

We thank Gilles Antoniazza, Emil Birnstiel, Laetitia Catalano, Andreas Taverna, Erwin Schäffer, Doris Hohmann, Daniel Steiner and Jaime M. Anaya-Rojas for major contributions to fish breeding and care and mesocosm set-up, maintenance and sampling. Furthermore we thank Katja Räsänen for helpful discussions throughout the project. Serge Robert, Patrick Kathriner and Beat Kienholz provided laboratory facilities and infrastructure support. M.S. received financial support from the Slovak Research and Development Agency (APVV-16-0236) and from European Regional Development Fund-Project "Mechanisms and dynamics of macromolecular complexes: from single molecules to cells" (No. CZ.02.1.01/0.0/0.0/15_003/0000441). The Eawag Directorate provided financial support for R.J.B. M.D.L. was funded by the Center for Adaptation to a Changing Environment (ACE) at ETH Zürich.

AUTHORS' CONTRIBUTIONS

M.D.L. and B.M. conceived the ideas and designed methodology; M.D.L., R.J.B. and M.S. collected the data; M.D.L. and M.S. analysed the data; M.D.L. and B.M. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

All isopod abundances, phenotypic data and plant biomass from the experiment, as well as phenotypic data collected from Hargeby et al. (2005) have been deposited in the Dryad Digital Repository with the https://doi.org/10.5061/dryad.cf225bk (Lürig, Best, Svitok, Jokela, & Matthews, 2018). Furthermore, a snapshot of the python package used for the phenotyping has been archived at Zenodo with the https://doi.org/10.5281/zenodo.1490236 (Lürig, 2018).

ORCID

Moritz D. Lürig https://orcid.org/0000-0002-8175-6234
Rebecca J. Best https://orcid.org/0000-0003-2103-064X
Marek Svitok https://orcid.org/0000-0003-2710-8102
Jukka Jokela https://orcid.org/0000-0002-1731-727X
Blake Matthews https://orcid.org/0000-0001-9089-704X

REFERENCES

Andersson, J. (2003). Effects of diet-induced resource polymorphism on performance in arctic charr (*Salvelinus alpinus*). *Evolutionary Ecology Research*, *5*, 213–228.

Arakelova, K. S. (2001). The evaluation of individual production and scope for growth in aquatic sow bugs (*Asellus aquaticus*). *Aquatic Ecology*, *35*, 31–42. https://doi.org/10.1023/A:1011446224456

Baker, R. L., & Ball, S. L. (1995). Microhabitat selection by larval *Chironomus tentans* (Diptera: Chironomidae): Effects of predators, food, cover and light. *Freshwater Biology*, *34*, 101–106. https://doi.org/10.1111/j.1365-2427.1995.tb00427.x

Bell, M. A. (2001). Lateral plate evolution in the threespine stickleback: Getting nowhere fast. *Genetica*, *112–113*, 445–461. https://doi.org/10.1023/A:1013326024547

Bijleveld, A. I., Twietmeyer, S., Piechocki, J., van Gils, J. A., & Piersma, T. (2015). Natural selection by pulsed predation: Survival of the thickest. *Ecology*, *96*, 1943–1956. https://doi.org/10.1890/14-1845.1

Calsbeek, R., & Cox, R. M. (2010). Experimentally assessing the relative importance of predation and competition as agents of selection. *Nature*, *465*, 613–616. https://doi.org/10.1038/nature09020

Carpenter, S. R., & Lodge, D. M. (1986). Effects of submersed macrophytes on ecosystem processes. *Aquatic Botany*, *26*, 341–370. https://doi.org/10.1016/0304-3770(86)90031-8

Cothran, R. D., Stiff, A. R., Jeyasingh, P. D., & Relyea, R. A. (2012). Eutrophication and predation risk interact to affect sexual trait expression and mating success. *Evolution*, *66*, 708–719. https://doi.org/10.1111/j.1558-5646.2011.01475.x

Crispo, E. (2008). Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. *Journal of Evolutionary Biology*, *21*, 1460–1469. https://doi.org/10.1111/j.1420-9101.2008.01592.x

De Montaudouin, X. (1996). Factors involved in growth plasticity of cockles *Cerastoderma edule* (L.), identified by field survey and transplant experiments. *Journal of Sea Research*, *36*, 251–265. https://doi.org/10.1016/S1385-1101(96)90794-7

Diehl, S., & Kornijów, R. (1998). Influence of submerged macrophytes on trophic interactions among fish and macroinvertebrates. In E. Jeppesen, M. Søndergaard, M. Søndergaard, & K. Christoffersen (Eds.), *The structuring role of submerged macrophytes in lakes* (pp. 24–46). New York, NY: Springer New York. https://doi.org/10.1007/978-1-4612-0695-8

Egea-Serrano, A., Hangartner, S., Laurila, A., & Räsänen, K. (2014). Multifarious selection through environmental change: Acidity and predator-mediated adaptive divergence in the moor frog (*Rana arvalis*). *Proceedings of the Royal Society of London Series B: Biological Sciences*, *281*, 20133266. https://doi.org/10.1098/rspb.2013.3266

Elser, J. J., Fagan, W. F., Denno, R. F., Dobberfuhl, D. R., Folarin, A., Huberty, A., & Sterner, R. W. (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature*, *408*, 578–580. https://doi.org/10.1038/35046058

Eroukhmanoff, F., Hargeby, A., & Svensson, E. I. (2009). Rapid adaptive divergence between ecotypes of an aquatic isopod inferred from F-Q analysis. *Molecular Ecology*, *18*, 4912–4923. https://doi.org/10.1111/j.1365-294X.2009.04408.x

Flight, P. A., Schoepfer, S. D., & Rand, D. M. (2010). Physiological stress and the fitness effects of Mpi genotypes in the acorn barnacle *Semibalanus balanoides*. *Marine Ecology Progress Series*, *404*, 139–149. https://doi.org/10.3354/meps08504

Garcia, C. (2014). The divergence between ecotypes in a *Littorina saxatilis* hybrid zone is aligned with natural selection, not with intra-ecotype variation. *Evolutionary Ecology*, *28*, 793–810. https://doi.org/10.1007/s10682-014-9695-x

Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, *21*, 394–407. https://doi.org/10.1111/j.1365-2435.2007.01283.x

Graca, M. A. S., Maltby, L., & Calow, P. (1993). Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus*. 1. Feeding strategies. *Oecologia*, *93*, 139–144. https://doi.org/10.1007/BF00321203

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. https://doi.org/10.1111/j.0014-3820.2004.tb01575.x

Hargeby, A., Stoltz, J., & Johansson, J. (2005). Locally differentiated cryptic pigmentation in the freshwater isopod *Asellus aquaticus*. *Journal of Evolutionary Biology*, *18*, 713–721. https://doi.org/10.1111/j.1420-9101.2004.00837.x

Hart, P. J. B., & Gill, A. B. (1992). Constraints on prey size selection by the three-spined stickleback: Energy requirements and the capacity and fullness of the gut. *Journal of Fish Biology*, *40*, 205–218. https://doi.org/10.1111/j.1095-8649.1992.tb02567.x

Hart, E. A., & Lovvorn, J. R. (2003). Algal vs. macrophyte inputs to food webs of inland saline wetlands. *Ecology*, *84*, 3317–3326. https://doi.org/10.1890/02-0629

Hollander, J., & Butlin, R. K. (2010). The adaptive value of phenotypic plasticity in two ecotypes of a marine gastropod. *BMC Evolutionary Biology*, *10*, 333. https://doi.org/10.1186/1471-2148-10-333

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*, 346–363. https://doi.org/10.1002/(ISSN)1521-4036

Jannot, J. E., Wissinger, S. A., & Lucas, J. R. (2008). Diet and a developmental time constraint alter life-history trade-offs in a caddis fly (Trichoptera: Limnephilidae). *Biological Journal of the Linnean Society Linnean Society of London*, *95*, 495–504. https://doi.org/10.1111/j.1095-8312.2008.01061.x

Jeppesen, E., Sondergaard, M., Sondergaard, M., & Christoffersen, K. (1998). *The structuring role of submerged macrophytes in lakes*. New York, NY: Springer. https://doi.org/10.1007/978-1-4612-0695-8

Johannesson, B., & Johannesson, K. (1996). Population differences in behaviour and morphology in the snail *Littorina saxatilis*: Phenotypic plasticity or genetic differentiation? *Journal of Zoology*, *240*, 475–493. https://doi.org/10.1111/j.1469-7998.1996.tb05299.x

Kassambara, A., & Kosinski, M. (2017). survminer: Drawing Survival Curves using "ggplot2" (Version 0.4.1).

Kingsolver, J. G., & Pfennig, D. W. (2007). Patterns and power of phenotypic selection in nature. *BioScience*, *57*, 561–572. https://doi.org/10.1641/B570706

Kovalenko, K. E., Thomaz, S. M., & Warfe, D. M. (2011). Habitat complexity: Approaches and future directions. *Hydrobiologia*, *685*, 1–17. https://doi.org/10.1007/s10750-011-0974-z

Lee, K. P., Simpson, S. J., Clissold, F. J., Brooks, R., Ballard, J. W. O., Taylor, P. W., & Raubenheimer, D. (2008). Lifespan and reproduction in *Drosophila*: New insights from nutritional geometry. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 2498–2503. https://doi.org/10.1073/pnas.0710787105

Leinonen, T., Herczeg, G., Cano, J. M., & Merilä, J. (2011). Predation-imposed selection on threespine stickleback (*Gasterosteus aculeatus*) morphology: A test of the refuge use hypothesis. *Evolution*, *65*, 2916–2926. https://doi.org/10.1111/j.1558-5646.2011.01349.x

Losos, J. B., Schoener, T. W., Warheit, K. I., & Creer, D. (2001). Experimental studies of adaptive differentiation in Bahamian *Anolis* lizards. *Genetica*, *112*, 113–399.

Lucek, K., Sivasundar, A., Roy, D., & Seehausen, O. (2013). Repeated and predictable patterns of ecotypic differentiation during a biological invasion: Lake-stream divergence in parapatric Swiss stickleback. *Journal of Evolutionary Biology*, *26*, 2691–2709. https://doi.org/10.1111/jeb.12267

Lürig, M. (2018). phenopype – a phenotyping pipeline for python (Version 0.4.5). http://doi.org/10.5281/zenodo.1490236

Lürig, M. D., Best, R. J., & Stachowicz, J. J. (2016). Microhabitat partitioning in seagrass mesograzers is driven by consistent species choices across multiple predator and competitor contexts. *Oikos*, *125*, 1324–1333. https://doi.org/10.1111/oik.02932

Lürig, M. D., Best, R. J., Svitok, M., Jokela, J., & Matthews, B. (2018). Data from: The role of plasticity in the evolution of cryptic pigmentation in a freshwater isopod. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.cf225bk

Marchinko, K. B. (2003). Dramatic phenotypic plasticity in barnacle legs (*Balanus glandula*, Darwin): Magnitude, age dependence, and speed of response. *Evolution*, *57*, 1281–1290. https://doi.org/10.1111/j.0014-3820.2003.tb00336.x

Marcus, J. H., Sutcliffe, D. W., & Willoughby, L. G. (1978). Feeding and growth of *Asellus aquaticus* (Isopoda) on food items from the littoral of Windermere, including green leaves of *Elodea canadensis*. *Freshwater Biology*, *8*, 505–519. https://doi.org/10.1111/j.1365-2427.1978.tb01473.x

Merilä, J., Laurila, A., Laugen, A. T., Räsänen, K., & Pahkala, M. (2000). Plasticity in age and size at metamorphosis in *Rana temporaria* – comparison of high and low latitude populations. *Ecography*, *23*, 457–465. https://doi.org/10.1111/j.1600-0587.2000.tb00302.x

Merilaita, S., Lyytinen, A., & Mappes, J. (2001). Selection for cryptic coloration in a visually heterogeneous habitat. *Proceedings of the Royal Society of London Series B: Biological Sciences*, *268*, 1925–1929. https://doi.org/10.1098/rspb.2001.1747

Miner, B. E., & Kerr, B. (2011). Adaptation to local ultraviolet radiation conditions among neighbouring *Daphnia* populations. *Proceedings of the Royal Society of London Series B: Biological Sciences*, *278*, 1306–1313. https://doi.org/10.1098/rspb.2010.1663

Moser, D., Roesti, M., & Berner, D. (2012). Repeated lake-stream divergence in stickleback life history within a Central European lake basin. *PLoS ONE*, *7*, e50620. https://doi.org/10.1371/journal.pone.0050620

Muztar, A. J., Slinger, S. J., & Burton, J. H. (1978). The chemical composition of aquatic macrophytes. II. Amino acid composition of the protein and non-protein fractions. *Canadian Journal of Plant*

Science. Revue Canadienne de Phytotechnie, *58*, 843–849. https://doi.org/10.4141/cjps78-123
Needham, A. E. (1970). The integumental pigments of some isopod crustacea. *Comparative Biochemistry and Physiology*, *35*, 509–534. https://doi.org/10.1016/0010-406X(70)90970-9
Needham, A., & Brunet, P. C. (1957). The integumental pigment of *Asellus*. *Cellular and Molecular Life Sciences: CMLS*, *13*, 207–209. https://doi.org/10.1007/BF02157167
Nosil, P., Harmon, L. J., & Seehausen, O. (2009). Ecological explanations for (incomplete) speciation. *Trends in Ecology & Evolution*, *24*, 145–156. https://doi.org/10.1016/j.tree.2008.10.011
Økland, K. A. (1978). Life history and growth of *Asellus aquaticus* (L.) in relation to environment in a eutrophic lake in Norway. *Hydrobiologia*, *59*, 243–259. https://doi.org/10.1007/BF00036504
Olsson, J., Svanbäck, R., & Eklöv, P. (2007). Effects of resource level and habitat type on behavioral and morphological plasticity in Eurasian perch. *Oecologia*, *152*, 48–56. https://doi.org/10.1007/s00442-006-0588-8
Pfennig, D. W., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D., & Moczek, A. P. (2010). Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology & Evolution*, *25*, 459–467.
Pinheiro, J. C., & Bates, D. M. (2000). *Mixed-effects models in S and S-PLUS*. New York, NY: Springer. https://doi.org/10.1007/978-1-4419-0318-1
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2017). nlme: Linear and Nonlinear Mixed Effects Models (Version 3.1-131).
Polunin, N. V. C. (1984). The decomposition of emergent macrophytes in fresh water. In A. MacFadyen & E. D. Ford (Eds.), *Advances in ecological research* (Vol. 14, pp. 115–166). London, UK: Academic Press. https://doi.org/10.1016/s0065-2504(08)60170-1
Quinn, G. P., & Keough, M. J. (2002). *Experimental design and data analysis for biologists*. Cambridge, UK: Cambridge University Press. https://doi.org/10.1017/CBO9780511806384
Quinn, T. P., & Kinnison, M. T. (1999). Size-selective and sex-selective predation by brown bears on sockeye salmon. *Oecologia*, *121*, 273–282. https://doi.org/10.1007/s004420050929
R Core Team. (2017). *R: A language and environment for statistical computing* (Version 3.4.3). Vienna, Austria: R Foundation for Statistical Computing.
Reitzel, A. M., & Heyland, A. (2007). Reduction in morphological plasticity in echinoid larvae: Relationship of plasticity with maternal investment and food availability. *Evolutionary Ecology Research*, *9*, 109–121.
Reznick, D. N., Shaw, F. H., Helen Rodd, F., & Shaw, R. G. (1997). Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science*, *275*, 1934–1937. https://doi.org/10.1126/science.275.5308.1934
Reznick, D., & Yang, A. P. (1993). The influence of fluctuating resources on life history: Patterns of allocation and plasticity in female guppies. *Ecology*, *74*, 2011–2019. https://doi.org/10.2307/1940844
Rohatgi, A. (2010). WebPlotDigitizer – Copyright 2010–2017 Ankit Rohatgi. Retrieved September 6, 2018, from https://apps.automeris.io/wpd/
Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, *8*, 336–352. https://doi.org/10.1111/j.1461-0248.2004.00715.x
Salvanes, A. G. V., & Hart, P. J. B. (1998). Individual variability in state-dependent feeding behaviour in three-spined sticklebacks. *Animal Behaviour*, *55*, 1349–1359. https://doi.org/10.1006/anbe.1997.0707
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, *9*, 676–682. https://doi.org/10.1038/nmeth.2019
Schlichting, C. D. (2004). *The role of phenotypic plasticity in diversification* (Vol. 209, pp. 191–200). Cambridge, UK: The Company of Biologists Ltd. https://doi.org/10.1242/jeb.02324
Schluter, D. (2009). Evidence for ecological speciation and its alternative. *Science*, *323*, 737–741. https://doi.org/10.1126/science.1160006
Schmid, M., & Guillaume, F. (2017). The role of phenotypic plasticity on population differentiation. *Heredity*, *119*, 214–225. https://doi.org/10.1038/hdy.2017.36
Scoville, A. G., & Pfrender, M. E. (2010). Phenotypic plasticity facilitates recurrent rapid adaptation to introduced predators. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 4260–4263. https://doi.org/10.1073/pnas.0912748107
Shamim, G., Ranjan, S. K., Pandey, D. M., & Ramani, R. (2014). Biochemistry and biosynthesis of insect pigments. *European Journal of Entomology*, *54*, 646. https://doi.org/10.14411/eje.2014.021
Sutcliffe, D. W., Carrick, T. R., & Willoughby, L. G. (1981). Effects of diet, body size, age and temperature on growth rates in the amphipod *Gammarus pulex*. *Freshwater Biology*, *11*, 183–214. https://doi.org/10.1111/j.1365-2427.1981.tb01252.x
Sworobowicz, L., Grabowski, M., Mamos, T., Burzynski, A., Kilikowska, A., Sell, J., & Wysocka, A. (2015). Revisiting the phylogeography of *Asellus aquaticus* in Europe: Insights into cryptic diversity and spatio-temporal diversification. *Freshwater Biology*, *60*, 1824–1840. https://doi.org/10.1111/fwb.12613
Tavares, R. I. S., Pestana, G. C., Rocha, A. D., Schiavone, D. C., & Guillermo-Ferreira, R. (2018). Come to the dark side: Habitat selection of larval odonates depends on background visual patterns: Background preference in dragonflies. *Ecological Entomology*, *43*, 640–646. https://doi.org/10.1111/een.12643
Teplitsky, C., & Räsänen, K. (2007). Adaptive plasticity in stressful environments: Acidity constrains inducible defences in *Rana arvalis*. *Evolutionary Ecology*, *9*, 447–458.
Tollrian, R., & Heibl, C. (2004). Phenotypic plasticity in pigmentation in Daphnia induced by UV radiation and fish kairomones. *Functional Ecology*, *18*, 497–502. https://doi.org/10.1111/j.0269-8463.2004.00870.x
Verweij, M. C., Nagelkerken, I., De Graaff, D., Peeters, M., Bakker, E. J., & Van der Velde, G. (2006). Structure, food and shade attract juvenile coral reef fish to mangrove and seagrass habitats: A field experiment. *Marine Ecology Progress Series*, *306*, 257–268. https://doi.org/10.3354/meps306257
Walsh, M. R., Castoe, T., Holmes, J., Packer, M., Biles, K., Walsh, M., … Post, D. M. (2016). Local adaptation in transgenerational responses to predators. *Proceedings of the Royal Society of London Series B: Biological Sciences*, *283*, 20152271. https://doi.org/10.1098/rspb.2015.2271
Warfe, D. M., Barmuta, L. A., & Wotherspoon, S. (2008). Quantifying habitat structure: Surface convolution and living space for species in complex environments. *Oikos*, *117*, 1764–1773. https://doi.org/10.1111/j.1600-0706.2008.16836.x
Westram, A. M., Galindo, J., Alm Rosenblad, M., Grahame, J. W., Panova, M., & Butlin, R. K. (2014). Do the same genes underlie parallel phenotypic divergence in different *Littorina saxatilis* populations? *Molecular Ecology*, *23*, 4603–4616. https://doi.org/10.1111/mec.12883

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Lürig MD, Best RJ, Svitok M, Jokela J, Matthews B. The role of plasticity in the evolution of cryptic pigmentation in a freshwater isopod. *J Anim Ecol*. 2019;88:612–623. https://doi.org/10.1111/1365-2656.12950