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SPECIES INTERACTIONS - FROM PHENOTYPES TO ECOSYSTEMS

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*Ich widme diese Arbeit meinen lieben Eltern,
und Adrien, den ich sehr vermisse.*

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Summary

English summary

Interactions between species can structure populations and communities, affect the flow of energy and matter within and across ecosystem boundaries, and shape the biotic and abiotic environment. Thereby, species interactions can also feed back on the participating species themselves, and on other members of the community. Complex interaction networks can arise that may influence the stability of ecosystems and potentially make them resilient against external perturbation. However, species interactions are not static: through endlessly recurring interplay with their environment, species, and thus, their interactions with other species, are subject to evolutionary change. It is therefore widely acknowledged that species interactions are not only the foundation for the functioning of all ecosystems, but also contribute to the emergence and maintenance of biological diversity on earth. In the face of rapid global environmental change it is critical to learn more about the nature of species interactions, for example, in the context of their strength (effect sizes), temporal stability (variation, within and between generations) or dimensionality (number of, interactions per species). For this dissertation I have conducted a series of experiments to explore the role of species interactions within different levels of ecological organization and across a range of ecological contexts. Specifically, I investigated i) how species interactions can shape phenotypic distributions of populations, ii) how species interactions shape developmental trajectories of phenotypes, and iii) how species interactions affect resistance and resilience of ecosystems in response to external disturbance. In four chapters I addressed these questions with a series of outdoor and laboratory experiments, which provided

compelling evidence for strong effects of species interactions on phenotypes, populations, communities and ecosystems.

German summary

Wechselwirkungen zwischen Arten können Populationen und Gemeinschaften strukturieren, den Energie- und Materialfluss innerhalb und über die Ökosystemgrenzen hinweg beeinflussen, und die biotische und abiotische Umgebung modifizieren. Dabei können Wechselwirkungen auch auf die beteiligten Arten selbst, sowie auf andere Mitglieder der Gemeinschaft zurückwirken und komplexe Netzwerke aus Wechselwirkungen bilden. Durch diese Wechselwirkungen können vorhandene Arten die Stabilität ihrer Ökosysteme beeinflussen, und sie möglicherweise widerstandsfähig gegen Störungen von außen machen. Wechselwirkungen zwischen Arten sind jedoch nicht statisch: durch das endlos wiederkehrende Zusammenspiel mit ihrer Umwelt unterliegen intra- und, interspezifische Wechselwirkungen mit anderen Arten evolutionären Dynamiken. Somit bilden Wechselwirkungen zwischen Arten nicht nur die Grundlage für das Funktionieren aller Ökosysteme, sondern tragen auch massgeblich zur Entstehung und Erhaltung der biologischen Vielfalt auf der Erde bei. Angesichts kontemporärer globaler Umweltveränderungen ist es wichtig mehr über die verschiedenen Typen der Wechselwirkungen zwischen den Arten zu erfahren: zum Beispiel im Zusammenhang mit ihrer Stärke (Effektgrößen), ihrer zeitlichen Variabilität (innerhalb und zwischen Generationen) oder ihrer Dimensionalität (Anzahl der Wechselwirkungen pro Spezies). Für diese Doktorarbeit habe ich eine Reihe von Experimenten durchgeführt, um die Rolle von Wechselwirkungen zwischen Arten auf verschiedenen Ebenen der ökologischen Organisation, und in verschiedenen ökologischen Kontexten zu untersuchen. Insbesondere habe ich betrachtet i) wie Wechselwirkungen zwischen Arten die

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phänotypische Verteilung von Populationen steuern können, ii) wie Wechselwirkungen zwischen Arten die Entwicklungsverläufe von Phänotypen beeinflussen, und iii) wie Wechselwirkungen zwischen Arten die Resistenz und Widerstandsfähigkeit, von Ökosystemen gegen äußere Störungen definieren. In vier Kapiteln bin ich diesen Fragen mit einer Reihe von Freiland- und, Labor-Experimenten nachgegangen, die belastbare Beweise für starke Auswirkungen von Wechselwirkungen zwischen Arten auf Phänotypen, Populationen, Gemeinschaften und Ökosysteme geliefert haben.

General introduction

Ecosystems as biological networks

Networks of biological interactions that are embedded within ecological communities are one of the most complex entities that natural scientists attempt to understand. Interactions between species can structure populations and communities (McCann et al. 1998; Bruno et al. 2003), affect the flow of energy and matter within and across ecosystem boundaries (Goudard and Loreau 2008; Kéfi et al. 2012), and shape the biotic and abiotic environment (Harmon et al. 2009; Matthews et al. 2014). These effects can also feed back on the participating species themselves and on other members of the community, thereby forming complex interaction networks (Olf et al. 2009) that influence the stability of ecosystems and potentially make them resilient against external perturbation (Ruiter et al. 1995; Donohue et al. 2016). In general, ecosystems that harbor a more diverse array of species, and thus, species interactions, are considered to be more resistant in the face of external perturbation through redundancy and compensation (Hillebrand and Matthiessen 2009). However, the nature of species interactions is not static: through endlessly recurring interplay with their surrounding, including con- and heterospecifics, species, and thus their interactions with other species, are subject to evolutionary change (Thompson 1999; Abrams 2000). Thus, biological interactions are not only the foundation for the functioning of all ecosystems, but also contribute to the emergence and maintenance of biological diversity on earth. The ongoing rapid loss of biodiversity, and thereby, of interactions related to ecosystem function, is threatening the stability and health of terrestrial and aquatic ecosystems

worldwide (Smith 2003; Reitsema et al. 2018), with potentially dramatic consequences also for us humans. Therefore it is critical to learn more about the nature of species interactions, for example, in the context of their strength (effect sizes), temporal stability (variation within and between generations) or dimensionality (number of interactions per species). For my dissertation I have conducted a series of experiments to explore the role of different types of species interactions on different levels of ecological organization and across a range of ecological contexts.

Consumer-resource interactions

Consumer-resource interactions, which are essential to all species, govern the flow of energy and matter within and across ecosystem boundaries (Tilman 1982; Olff et al. 2009). In the context of species interactions, resources are anything that an organism consumes to grow, maintain, or reproduce itself (Tilman 1982). On the one hand, two species are in a consumer-resource relationship when one species ingests the whole or parts of an organism of a second species, for example, plant biomass grazed by herbivores, prey consumed by predators, or carrion consumed by scavengers. Such trophic interactions can link together in long, interconnected chains that form food webs (Ruiter et al. 1995; McCann et al. 1998). On the other hand, resources for one species can also be what a second species has produced, like oxygen by plants or mineral nutrients that are recycled by fungi and bacteria. Such interactions can be sometimes be difficult to determine, because they are asynchronous, and may include “legacy” effects of organisms, like the transformation of living biomass into particulate organic material after an organism’s death (Olff et al. 2009). Detritivores, for example, mainly utilize organic material, stemming from species that have been alive in the past, and make the nutrients it contains available to the rest of the community

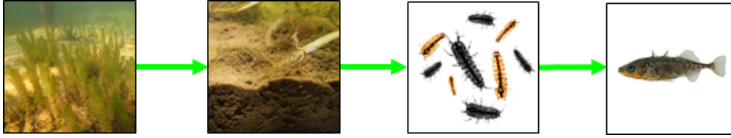


FIGURE 1: Simple cascade of consumer-resource species interactions (i.e. food-chain) with organisms used in this thesis. After their death, aquatic macrophytes decay and get converted into detritus, which is an important resource for detritivores, like the freshwater isopod *Asellus aquaticus*. Depending on the amount of detritus biomass, detritivorous organisms can reach enormous densities, which attracts benthic predators like threespine stickleback (*Gasterosteus aculeatus*)

through decomposition (Wallace and Webster 1996; Adey and Loveland 2007). Furthermore, not all resources are ingestible, but of spatial nature: aquatic macrophytes, for example, can provide microhabitat and shelter for zooplankton, or harbor periphyton that grows on the surface (Jeppesen et al. 1998). Macrophytes can also modify fluxes of abiotic resources that are consumed by other species, like the amount of light that penetrates the water column or the concentration of dissolved organic carbon that is utilized by bacteria (Carpenter and Lodge 1986; Reitsema et al. 2018). Indeed, autotrophic organisms often provide more than one resource to other community members, making them an integral part of most ecosystems (Tilman 1982; Ellison 2019). However, understanding such potentially complex interaction webs around single species or assemblages of ecologically similar species remains challenging (Berlow et al. 2004; Brophy et al. 2017). Factorial experiments manipulating the presence and absence of the species in question can provide insight into the role of species interactions on populations, communities, and ecosystems.

Key species interactions and ecosystem stability

In some cases, the presence of a single species, and its interactions with other members of the community, contributes disproportionately to the functioning of the ecosystem (Dayton 1972; Ellison et al. 2005). Such interactions can be trophic, for example, when a herbivore or a predator keeps the abundance of another species in check (Ellison 2019). Often the species that are being ingested would have high abundances or even dominate their respective habitat, so that their removal may open up physical space to be utilized by weaker competitors. Thus the presence of “keystone” predators or herbivores (Paine 1966; Menge et al. 1994) can modify community composition directly (through ingestion) and indirectly (through competitive release), which may increase the biodiversity of ecosystems. Furthermore, many ecologically important species can define ecosystem dynamics through facilitative non-trophic interactions, whose outstanding role in interaction networks is increasingly being acknowledged (Kéfi et al. 2012; Ellison 2019). A very common form of such key facilitative interactions is the creation of habitats for communities to thrive in (Angelini et al. 2011; Ellison 2019). Assemblage of trees or corals, for example, form physical structure that can shield against physical stress or provide refuges from predation (Stachowicz 2001; Bruno et al. 2003), but also modify the the flow of biotic or abiotic materials, energy, and nutrients (Ellison 2019). Due to their foundational role at the center of interaction networks embedded in ecosystems, such species are termed “foundation species” (Stachowicz 2001; Ellison et al. 2005; Ellison 2019).

The pivotal role of keystone and foundation species becomes especially apparent in the face of disturbance, particularly where ecosystems can shift to alternate states. External perturbations can mediate the effect of such important species on ecosystems, for example, by

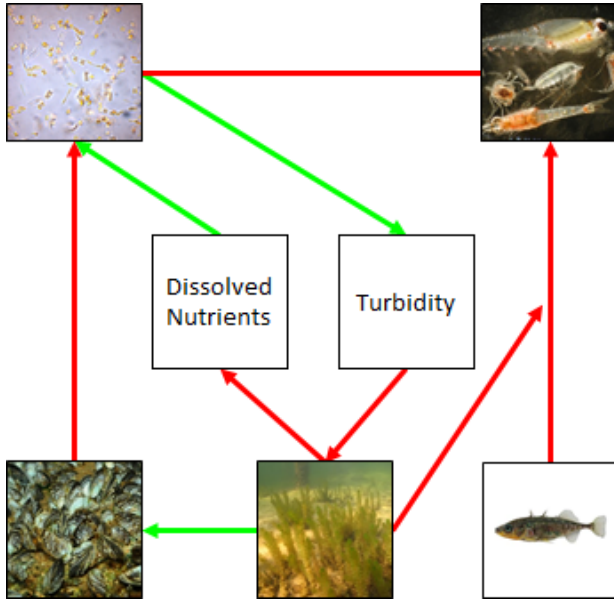


FIGURE 2: Simple interaction networks in shallow lakes; green arrows denote positive, red arrows negative interactions. Assemblages of macrophyte can stabilize shallow lake ecosystems in a clear water state by competing with phytoplankton for dissolved nutrients and light, thereby reducing water turbidity in a positive feedback loop. Macrophytes also provide shelter for zooplankton from predation, and substrate for benthic filter feeders, which both may enhance water clarity.

reducing their densities or by interfering with their interactions with other species (Suttle et al. 2007). Should this reduce or neutralize the effect of key species interactions on the overall community and ecosystem functioning, it can fundamentally alter the nature of an ecosystem towards a different state (Ellison et al. 2005; Kéfi et al. 2016; Morone et al. 2019). A very well studied case of alternative ecosystem states is the transition from clear to turbid water states under increased nutrient loading in shallow lakes (Scheffer et al. 1993; Scheffer 2013). There, rooted macrophytes compete with phytoplankton for dissolved nutrients and light, which, even under elevated nutrient perturbation, can stabilize the ecosystem at a clear water state. A positive feedback between light transmission and macrophyte biomass is mediated by several species interactions, including competition for inorganic nutrients (Carpenter and Lodge 1986; Jeppesen et al. 1998), shelter provisioning for zooplankton grazers (Jeppesen et al. 1997), and allelopathic chemical production (Hilt and Gross 2008). However, ponds that experience prolonged perturbation by nutrient will cross the “critical turbidity” threshold above which macrophytes are negatively affected and will eventually die off. This marks the transition to a turbid water state, where macrophytes are absent and with low overall invertebrate and fish diversity (Scheffer et al. 1993). The lack of experimental evidence for such transitions contributes to the uncertainty in estimating resistance and resilience of ecosystems with different species composition to perturbation (Morone et al. 2019). Hence, without empirical tests of the most important species interactions under a given disturbance regime, forecasting ecosystem responses to increasing anthropogenic disturbances will remain challenging.

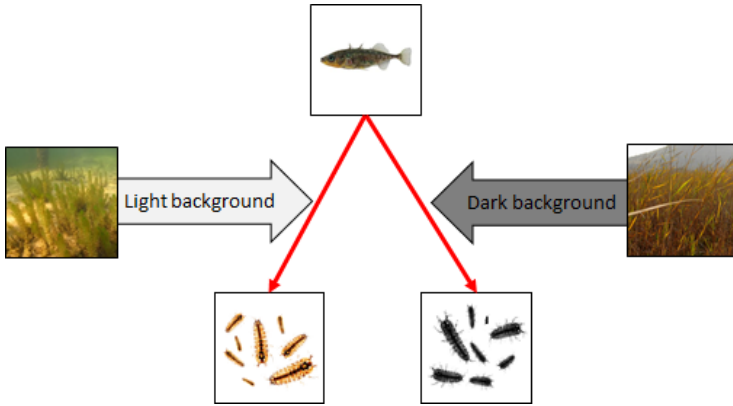


FIGURE 3: Example of how species interactions can affect phenotypic evolution: visual predation along a macrophyte gradient can select for lighter or darker pigmentation in isopods (*A. aquaticus*) via background matching. Differently coloured macrophytes modify the selective pressure emanating from a visual predator towards isopods with either lighter or darker body pigmentation. However, in this case the role of macrophytes and their detritus as a food source during the development is unclear.

Species interactions and phenotypic evolution

Interactions between species underlie similar evolutionary and coevolutionary dynamics as the species themselves (Thompson 1999). Not all interactions that species are engaged in will influence their reproductive success, but the ones that do should select for phenotypes that maximize their fitness in the context of that interaction (Stearns 2013). For example, prey species commonly have phenotypes that reduce predation pressure, e.g. through camouflage (Lürig et al. 2016; Stevens 2016; Duarte et al. 2017) or armor plating (Bell 2001; Leinonen et al. 2011), whereas predator species have traits that maximize foraging success like a hydrodynamic body shape (Langerhans 2009).

Thus, the dynamics of both, species interactions and natural selection, are tied to the same entity: the phenotype. Phenotypic evolution can be very rapid and occur on timescales of just a few generations (Thompson 1998), during which a single interaction between can affect the distribution of phenotypes in populations and communities (Grant and Grant 1995; Hairston et al. 2005; Becks et al. 2012; Turcotte et al. 2012). Due to their importance and often clear mechanistic underpinnings, eco-evolutionary dynamics of consumer-resource interactions between species are well researched (Abrams 2000; Yoshida et al. 2003). Prominent examples of rapid interaction mediated evolution are beak size and shape in Darwin finches in response to seeds (Grant and Grant 1995), clumping-ability in phytoplankton in response to predators (Becks et al. 2012), or host-parasite coevolution in *Daphnia* (Duffy et al. 2008).

Rapid population wide trait changes in response to species interactions can also be influenced by phenotypic plasticity; the ability of a single genotype to form different phenotypes, continuously or discontinuously, depending on the environment (West-Eberhard 2003). During development, phenotypic plasticity can increase organismal fitness in the context of a specific environment or interaction. *Daphnia*, for example, are plastic in the expression of defenses (Agrawal 2001) or pigmentation, depending on the presence of predators (Tollrian and Heibl 2004). Especially resource quality and quantity often have large effects on the development of morphological, physiological, and behavioral traits of individuals. Throughout development, organisms need to balance the allocation of acquired resources to maintenance, growth, and reproduction (Stearns 2013; West-Eberhard 2003). Depending on available resources, organisms can, for example, grow faster or slower (Metcalf and Monaghan 2001), or change time of maturity (Lee et al. 2013; Plaistow et al. 2004). Such consumer-resource mediated plasticity can lead to patterns that are similar to the signal of natural selection, and it is often challenging to separate the effect

of both processes on phenotypes. For example, two species of macrophytes in shallow lakes are thought to each select for darker or lighter pigmentation in a benthic isopod (*A. aquaticus*), which is driven by visual predation in differently coloured backgrounds (dark and light) (Hargeby et al. 2004; Hargeby et al. 2005). However, there is some evidence for plasticity of pigmentation in isopod size and pigmentation that, depending on macrophyte microhabitat, may be driven by local resource quality and quantity (Needham 1970; Marcus et al. 1978). This example highlights the complexity that is underlying species interaction networks, and emphasizes need for appropriate experimental systems that can disentangle the effects of natural selection and phenotypic plasticity in natural populations.

Goals and structure of this dissertation

The main goals of this thesis were threefold:

- i) How do species interaction shape phenotypic distributions of populations, and what is the role of phenotypic plasticity in this context?
- ii) How can species interactions shape developmental trajectories of phenotypes and survival during early life stages?
- iii) How do species interaction affect resistance and resilience of ecosystems in response to external perturbation?

To address these questions I conducted a series of experiments where I manipulated the presence and absence of different species (or their effect) to test how their interactions affect phenotypes (Chapter 1 and 3), populations (Chapter 1), and ecosystems (Chapter 2 and 4).

Chapter 1 - In the first chapter I conducted a mesocosm experiment, where I manipulated the presence and absence of submerged

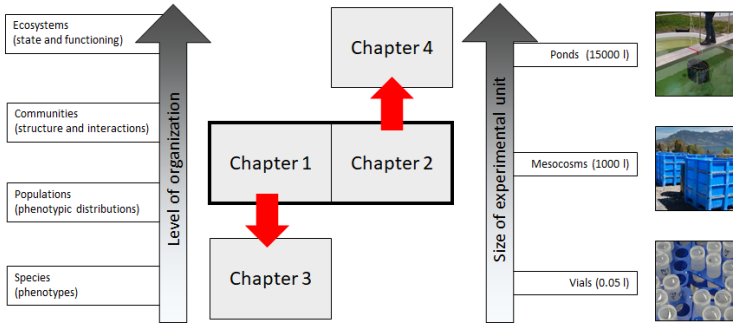


FIGURE 4: Structure of this dissertation. Chapter 1 and 2 were conducted as a single experiment, but with two different foci. Following from this first experiment, new scientific questions emerged that led to additional experiments I conducted in Chapters 3 and 4.

macrophytes (*Myriophyllum spicatum* and *Chara tomentosa*) to investigate how their interaction with a visual predator (*Gasterosteus aculeatus*) affects survival and phenotypic distributions in populations of a benthic isopod (*Asellus aquaticus*). In an adjunct laboratory experiment I reared juvenile isopods on different diets to test for plasticity of pigmentation. After phenotyping over 4000 isopods collected from the mesocosms I found that macrophytes, independent of fish presence, positively affect isopod pigmentation, which, according to our lab experiment, is a highly plastic trait. This chapter is published in *Journal of Animal Ecology* (Lürig et al. 2019).

Chapter 2 - Using the same experimental setup as in Chapter 1, I tested how the presence and absence of submerged macrophytes affects ecosystem dynamics in the absence of external disturbances. Using automated sensor platforms I quantified a suite of ecosystem parameters in high resolution to better understand how macrophytes moderate the response of ecosystems to abiotic variability and seasonal forcing. I found that macrophytes affected a wide range of biotic

and abiotic properties (phytoplankton abundance, dissolved organic substances, metabolism) and can help stabilize ecosystems in a clear water state. This chapter is currently under review at *Limnology and Oceanography*.

Chapter 3 - Following up the findings of Chapter 1, I conducted a large scale laboratory experiment in which I manipulated the concentration of protein and the amino acid tryptophan in an artificial diet to test the effect of variation in dietary resources on development of *A. aquaticus*. I phenotyped over 1000 isopods from 29 different families up to five times during their juvenile development, and found that, depending on the diet, developmental trajectories for pigmentation and body size can strongly affect isopod survival. In line with manipulations of dietary protein in other study systems, I found that isopods reared under lower protein concentrated showed increased survival, but decreased rates of pigmentation. This chapter is currently under review at *Evolution*.

Chapter 4 - Extending the work from Chapter 2, I participated in a large scale pond experiment to investigate how interactions between two important foundation species, macrophytes (*Myriophyllum spicatum*) and mussels (*Dreissena polymorpha*) can affect ecosystem dynamics in response to perturbation with nutrient. Using the same high resolution sensor array I found that when by themselves, macrophytes and mussels can reduce phytoplankton abundances and stabilize the ecosystems in clear water state. However, in the ecosystems where both species were together we found strong evidence for non-additive antagonistic interactions between the two species. This chapter is in manuscript form.

Chapter 1

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

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Abstract

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 cryptic. 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. We performed two experiments: In an outdoor mesocosm experiment, we investigated how different densities of predatory fish (0 / 30 / 60 threespine stickleback [*Gasterosteus aculeatus*] per mesocosm) and macrophytes (presence / absence) affected the abundance, pigmentation and body size structure of isopod populations. 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. 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. 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 timescales.

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 inclusive of interactions with abiotic and biotic conditions. Phenotypic differences across populations are often explained by divergent natural selection (Rundle and Nosil 2005; Schluter 2009; Calsbeek and Cox 2010), but the role of plasticity (developmental or otherwise, Figure 1) during adaptive population divergence is not well understood (Schlichting 2004; Kingsolver and Pfennig 2007; Pfennig et al. 2010). Sometimes phenotypic differences between environments can arise solely due to plasticity (Crispo 2008) and be correlated or uncorrelated with fitness variation (Merilä et al. 2000; Ghalambor et al. 2007). 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 et al. 2009; Schmid and 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

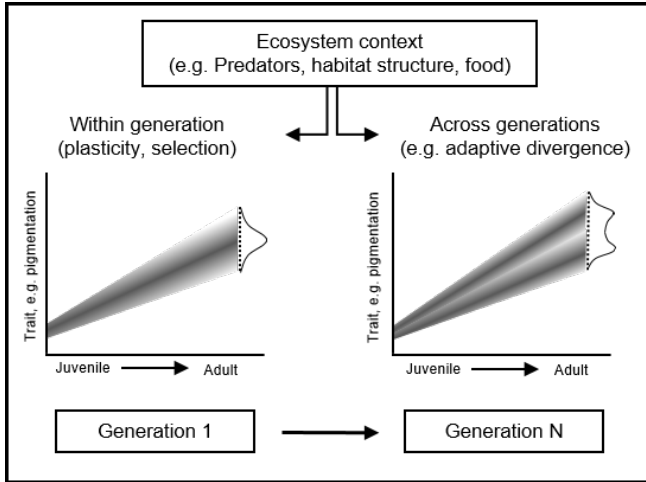


FIGURE 1: The ecosystem context during organismal development and growth can determine how the phenotype distribution in a population both develops within generations (i.e. due to plasticity and selection) and evolves across generations. Different evolutionary outcomes across generations are possible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

natural selection. Predators, for example, are capable of causing divergent selection (Quinn and Kinnison 1999; Bell 2001; Moser et al. 2012; Bijleveld et al. 2015) and of inducing plastic responses (Scoville and 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 and Lodge 1986) and can lead to plasticity by affecting light regimes (Tollrian and Heibl 2004; Miner and Kerr 2011) or nutrient dynamics (Polunin 1984; Hart and Lovvorn 2003). 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 et al. 2012) and affect background coloration (Tavares et al. 2018), to which not all prey phenotypes are equally well adapted (Lürig et al. 2016). Thus, differences in macrophyte cover may affect the strength and direction of selection from predation (Merilaita et al. 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 & Ahnesjö Hargeby et al. (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 1 [Hargeby et al. 2005]). In addition, fish predation trials in the laboratory have shown that darker isopods have higher survival in dark-colored 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. 2012; Tavares et al. 2018).

However, previous work has not emphasized how macrophytes might additionally influence the evolution of cryptic pigmentation of isopods, e.g. 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 (Sutcliffe et al. 1981;

Polunin 1984; Diehl and Kornijów 1998; Hart and Lovvorn 2003; Jannot et al. 2008). Previous work has demonstrated how such resource variation can affect life history traits and development in *A. aquaticus* (Marcus et al. 1978; Arakelova 2001). There is also a functional link

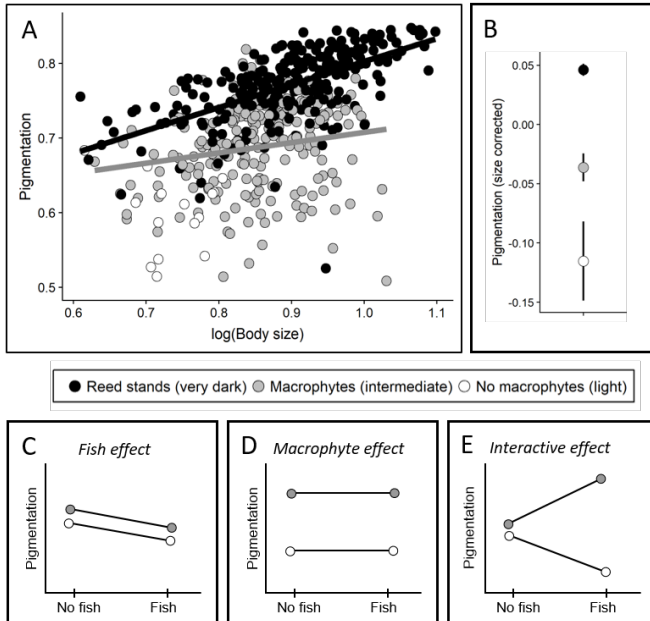


FIGURE 2: A - The relationship of pigmentation and body size of *A. aquaticus* in microhabitats with different backgrounds (from dark to light): reed (*Phragmites australis*), macrophytes (*Chara tomentosa*) and no macrophytes (sandy substrate). The data includes six lakes from Southern Sweden, and was collected from Hargeby, Stoltz & Johansson Hargeby et al. (2005) using *WebPlotDigitizer* (Rohatgi 2010). Each data point is an individual; the lines are estimates of pigmentation from a linear mixed effect model with vegetation as main effect, body size as the covariate, and lake as the random effect (main effect of vegetation $P=0.005$).

FIGURE 2: (*continued*) B) Size corrected pigmentation (mean \pm 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, e.g. through food or light. E) Fish and macrophytes may interact in their effect on pigmentation, e.g.: 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.

between the quality of macrophyte detritus and isopod pigmentation: the essential amino acid tryptophan is the precursor molecule in the developmental pathway of *A. aquaticus*' ommochrome based pigmentation (Needham and Brunet 1957; Shamim et al. 2014), and because it cannot be synthesized by animals it must be acquired through feeding, e.g. on macrophytes (Muztar et al. 1978). Building on this previous work, and the results of our mesocosm experiment, we hypothesized that pigmentation of *A. aquaticus* could be developmentally plastic, and influenced by diet (Figure 1).

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

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

TABLE 1: (See caption on next page)

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 Extension) literature search, using the words “Adaptive divergence”, and “Phenotypic plasticity”

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.

Materials and methods

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 (Økland 1978; Arakelova 2001). It occurs in many different microhabitats, e.g. 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 (Marcus et al. 1978; Hargeby et al. 2004) and an important prey item for invertebrate predators and fish (Hargeby et al. 2004; Hart and Gill 1992). As such, it plays a significant role in freshwater food webs (Jeppesen et al. 1998). The distinctive pigmentation of isopods is composed of melanins (Needham 1970),

which are subcutaneous and therefore remain in the integument during molting. Consistent with developmentally plastic traits, loss or gain of pigmentation after reaching maturity has not been reported.

Effects of fish and macrophytes on isopods (mesocosm experiment)

In 2015, we set up 50 outdoor mesocosms (1000 L) at Eawag Kastanienbaum in a randomized block design that included factorial combinations of macrophytes (presence / absence) and fish (threespine 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 May 26th 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 (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 Table S1).

Isopods were introduced to the mesocosms by planting or washing plant material into the water (see above): on average 159 ± 29 (mean \pm 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 \pm SD) and 50% from *Chara* macrophytes (79 ± 26 , mean \pm SD). The isopods were exposed to experimental conditions for six months (May-Oct), which corresponds to the presence of 2 - 3 generations, and experienced fish predation for 3 months (Aug-Oct). On August 8th 2015, we added fish (threespine 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 fish predators represented a diverse mixture from both lake and stream habitats.

We terminated the experiment on Oct 22nd, after six months, and sampled the isopods from all mesocosms by dragging a net with a 28×28 cm opening and 100- μ m mesh size across the bottom (sampling approx 30% 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 (Table S1), and the nutrient concentrations of each species (*Myriophyllum*, *Chara*, and filamentous algae) with an elemental analyzer (Pyro-cube and Isoprime, Elementar, [Table S1]).

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

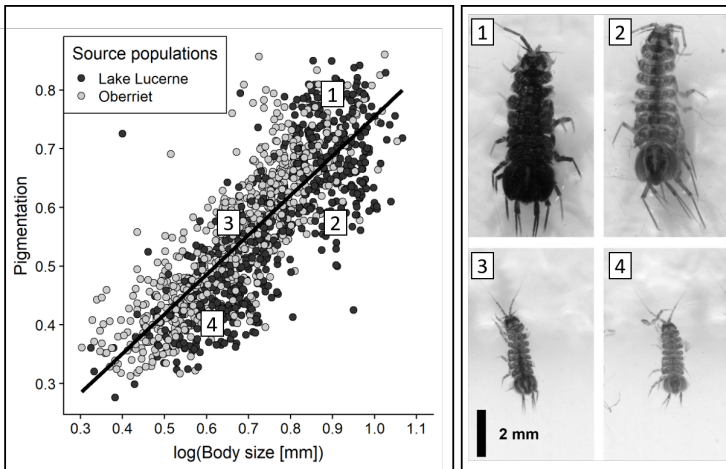


FIGURE 3: Pigmentation and body size in the source populations of *Asellus aquaticus* are positively related: linear regression coefficient = 0.671 (linear model of pigmentation and logarithmic body size $P < 0.001$). We used the linear equation of this regression analysis to size correct pigmentation 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*, the numbers indicate their position among the range of phenotypes. 1: dark adult, 2: light adult, 3: dark juvenile, 4: light juvenile (all from Lake Lucerne)

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 (160L with lake water) in the laboratory. We maintained this population with *Chara* plant material as substrate, at 20°C with a 12:12 hour 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 and with a 12:12 h light / dark cycle. Whenever a food pellet was fully consumed by an isopod, we replaced it with one of the same kind. We changed half of the water in each tube every two weeks.

Isopod phenotyping

In the mesocosm experiment, we imaged thawed isopods with a modified flatbed scanner (Epson V39) in high resolution (2400 dpi). Individuals were placed inside a water film on the scanner to minimize reflectance and artifacts during the scanning (Figure 3). We included gray scale card and millimeter 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 analyzed digital pictures of the specimens. Pigmentation and body size of isopods were extracted from all images with the self-written python package *phenotype* (Lürig 2018). The package uses thresholding algorithms and segmentation to locate isopods in the image and extract the phenotypic information from the area marked as the animal (dorsal region of isopod torso = carapace excluding legs and antennae). The gray scale values from these pixels are then extracted, averaged and converted to a pigmentation scale from 0 (gray scale value of 255) to 1 (gray scale 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 this method were not different from measurements of the same images using *ImageJ* (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”.

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 et al. 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 parametrized the models with sum-to-zero constraints and performed all tests based on type III sum-of-squares (Quinn and 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 posthoc analysis (Tukey all paired comparisons from R-package *multcomp*, [Hothorn et al. 2008]). Finally, we also tested for interactions between body size and treatment at the individual level (Model 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 non-linear patterns (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 and 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 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 et al. 2017]). All analyses were performed in the programming language R (R Core Team 2017).

Results

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; Table 2, 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

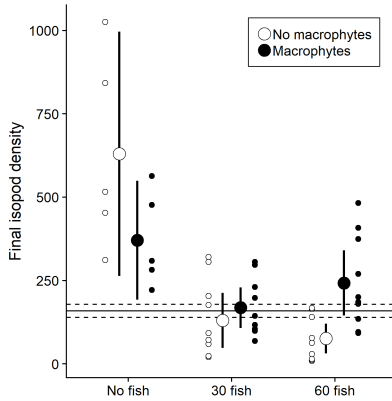


FIGURE 4: Fish presence significantly reduced isopod densities (post hoc contrasts: 0 vs.30 fish and 0 vs 60 fish both significant [$p < 0.001$]). However, this interacted with macrophyte presence. Each small point represents a mesocosm tank; the large points are mean \pm 95% confidence interval (CI).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

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 (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 (Table S2).

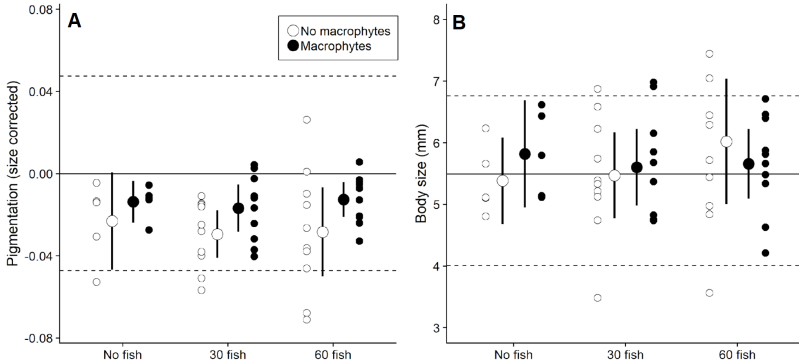


FIGURE 5: A) Macrophyte presence yielded higher pigmentation in isopods than macrophyte free tanks (significant main effect of macrophytes in M2 $P=0.002$). Values are size corrected using the linear equation of the regression shown in Figure 3. B) Body size of isopod specimens retrieved from the mesocosms 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 \pm 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.

Plasticity experiment

In the laboratory experiment the dietary manipulation of phosphorus and nitrogen content (Table S1), had 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 toward the end of the experiment (after day 70,

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.

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. 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.

Over the course of the six-month experiment, isopod density in mesocosms without fish predators increased significantly (between 100% and 200%), regardless of 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 and 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 and Kornijów 1998). This could occur because macrophytes generate

Model	Response variable	Fixed effects	df	F	p	Random effect	df	X ²	p
M1	Density	Macrophytes	1, 40	0.048	0.762	Block	1	5.876	0.015
		Fish density	2, 40	10.183	<0.001				
		Macrophytes x 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 x 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 x fish density	2, 40	0.389	0.680				
M4	Pigmentation	Body size	1, 2795	8531.9	<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				
M5	Pigmentation (size corrected)	Body size x macrophytes	1, 2795	0.081	0.776				
		Body size x fish density	2, 2795	1.287	0.276				
		Macrophytes x fish density	2, 40	0.236	0.791				
		Body size x macrophytes x fish density	2, 2795	0.594	0.552				
		Diet	1, 85	3.305	0.073	Family	1	109.780	<0.001
		Time	1, 333	188.3	<0.001	Individual	1	99.358	<0.001
M6	Body size	Diet x time	1, 333	89.55	<0.001				
		Diet	1, 85	2.604	0.110	Family	1	14.940	0.002
		Time	1, 333	562.3	<0.001	Individual	1	184.337	>0.001
		Diet x time	1, 333	4.120	0.043				

TABLE 2: (See caption on next page)

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 tests the effect of diet on individuals. All models are linear mixed effect models using type III sum of squares. Significant p-values (<0.05) are in **bold**.

structural habitat complexity (Kovalenko et al. 2012; Warfe et al. 2008; Lürig et al. 2016), making it difficult for fish to find and capture any isopods, or because they alter the intensity and heterogeneity of the light environment (Baker and 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 (Hargeby et al. 2004; Hargeby et al. 2005; Eroukhanoff et al. 2009b), 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 collection 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 (Tollrian and Heibl 2004; Miner and Kerr 2011). 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

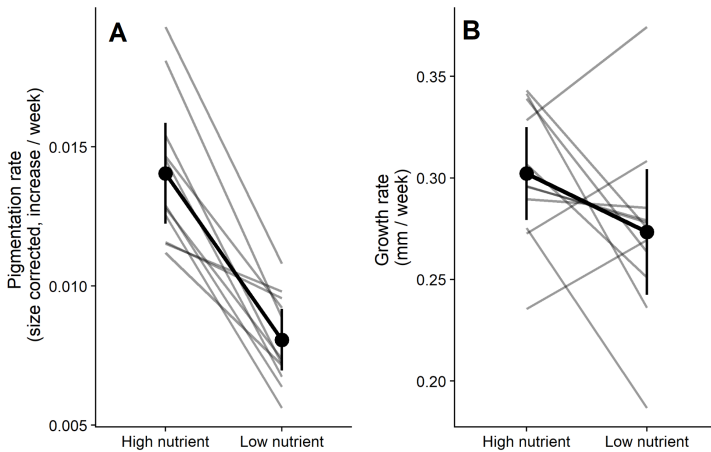


FIGURE 6: Rates of increase in pigmentation (A) and body size (B) of *A. aquaticus* were higher under high nutrient diet (significant main interactive effect of time and diet in M4 and M5). Points are the weekly average change in pigmentation or body size of individuals across all families (mean \pm CI), gray lines indicate family level reaction norms.

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 (Marcus et al. 1978; Graca et al. 1993). 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* which 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 macro-invertebrates (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 macrophyte presence on the body size spectrum, it is possible that nutrient rich detritus may increase the development of pigmentation in isopods. The bio-synthesis of the ommochrome pigments in *A. aquaticus* results from a potentially costly physiological pathway (Needham and Brunet 1957; Needham 1970) that may require a high quality diet, i.e. with high nutrient concentrations, to function properly. Additionally, macrophyte detritus may have provided the essential compounds required for the bio-synthesis. 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 bio-synthesis of pigments.

Plasticity due to variation in resources is common in natural populations. Notable examples include plastic morphology and behavior in fishes (perch [*Perca fluviatilis*]: (Olsson et al. 2007); arctic charr [*Salvelinus alpinus*]: (Andersson 2003)), life history in echinoids (Reitzel and Heyland 2007) and *Drosophila* (Lee et al. 2008), and growth rates and sexual traits in amphipods (Cothran et al. 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 (Hargeby et al. 2004; Hargeby et al. 2005; Eroukhmanoff et al. 2009b), 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 and

Pfrender 2010), and its role during adaptive divergence in particular.

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Supplement

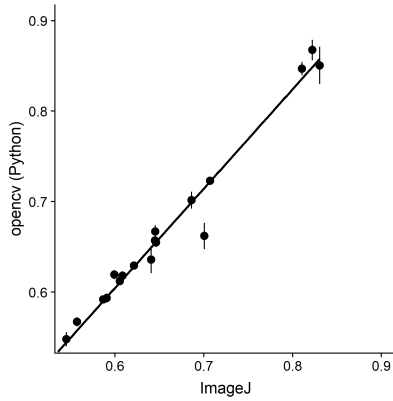


FIGURE S1: Comparison of the performance of the phenotyping package *phenotype* in Python (Lürig 2018) with manual measurements in **ImageJ**.

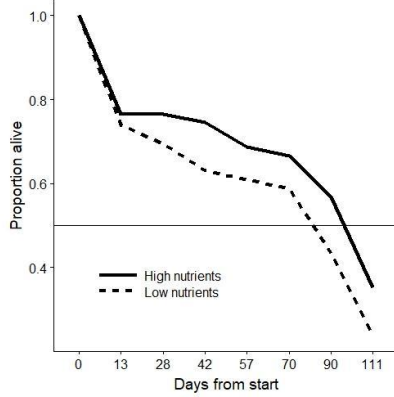


FIGURE S2: Proportion of live isopods over the course of the laboratory experiment. We included only data in our analysis up to day 70, before in one of the treatments fewer than 50% (solid line) of the isopods were alive.

Chapter 1

TABLE S1: Plant biomass at the beginning and end of the mesocosm experiment. We used 10 aliquots (out of 60) to measure initial biomass in the macrophyte treatments. At the end of the experiment we collected all plant material of the entire benthic substrate. In the “no macrophyte” tanks both *Chara* and *Myriophyllum* were growing from the sediment but at much lower densities. In addition, all tanks were colonized by prostrate filamentous algae (which was growing in tanks of the “macrophyte” treatment as well).

Phase of experiment	Macrophyte treatment	Species	N	Dry weight g (mean ± SD)
Start	Macrophytes	<i>Chara</i>	10	165.1 ± 21.65
		<i>Myriophyllum</i>		2.84 ± 0.54
	No macrophytes	<i>Chara</i>	0	0
		<i>Myriophyllum</i>		0
End	Macrophytes	<i>Chara</i>	25	11.06 ± 10.44
		<i>Myriophyllum</i>		11 ± 2.74
		Filamentous algae		17.78 ± 19.16
	No macrophytes	<i>Chara</i>	25	2.69 ± 3.29
		<i>Myriophyllum</i>		1.7 ± 0.76
	Filamentous algae		36.2 ± 32.89	

Chapter 1

TABLE S2: Elemental composition of macrophytes collected after the experiment and of the diet pellets used in the laboratory experiment.

	C (%)	N (%)	P (%)	C:N	C:P
<i>Chara</i>	21.59	1.42	0.08	15.24	273.67
<i>Myriophyllum</i>	27.76	1.29	0.03	21.46	815.40
Filamentous algae	20.70	1.24	0.05	16.65	434.41
Low nutrient diet	1.09	32.50	0.16	29.93	202.97
High nutrient diet	4.03	40.72	0.48	10.11	84.49

Chapter 2

Submerged macrophytes affect the variability of aquatic ecosystems

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Abstract

Macrophytes are important foundation species that can strongly influence the structure and functioning of aquatic ecosystems. However, only little is known about the temporal dynamics and dimensionality of macrophyte-ecosystem interactions, i.e. how fast and how diverse they can be. Here, we used mesocosm ecosystems (1000 L) with and without macrophytes and sampled algal biomass, dissolved organic matter fluorescence, oxygen, and temperature with high-resolution sensors (15 min intervals) over several months (94 days from spring to fall). We found that macrophytes lowered the mean but increased the variance of algal biomass; an effect that matches theoretical expectations from a model we implemented where macrophytes and algae differentially compete for nutrients and light. Moreover, the presence of macrophytes increased dissolved organic matter fluorescence and decreased UV transmission. Furthermore, we detected elevated primary productivity in the presence of macrophytes, and, depending on seasonality, higher productivity to respiration ratios in the presence of macrophytes. We show that high resolution sampling can reveal new insights into how assemblages of macrophytes affect the mean and variance of phytoplankton dynamics in ecosystems. Overall our findings confirm the strong, but seasonally dependent effects that assemblages of macrophytes can have on a wide range of parameters in aquatic ecosystems.

Introduction

Decades of research on submerged macrophytes have documented how they can influence a suite of ecosystem properties and processes (Carpenter and Lodge 1986; Jeppesen et al. 1997; Huss and Wehr 2004; Reitsema et al. 2018). Macrophytes affect their environment through a combination of both trophic and non-trophic interactions,

percolating through ecosystem networks (Jeppesen et al. 1997; Olff et al. 2009). Acting as foundation species (Dayton 1972; Ellison et al. 2005), macrophytes create and maintain habitats for other species, affect species interactions, and influence the dynamics of matter and energy in ecosystems (Carpenter and Lodge 1986; Jeppesen et al. 1997). Populations of individual macrophytes species, as well as species assemblages, can also influence how aquatic ecosystems respond to environmental change, and the propensity of ecosystems to shift between alternative stable states in shallow lakes (Scheffer et al. 1993; Faafeng and Mjelde 1998; Blindow et al. 1998). However, while the net ecosystem effects of macrophytes are often well studied, much less is known about how they affect the temporal dynamics of ecosystems. Specifically, little is known about whether species interactions lead to gradual or abrupt transitions in phytoplankton populations, and other ecosystem metrics. Without well-resolved and long-term data on macrophyte-ecosystem interactions, it is challenging to test for the existence of alternative stable states (Scheffer et al. 2009), anticipate the response to a changing environment (Reitsema et al. 2018), and understand the importance of such interactions for ecosystem management (Spears et al. 2017).

The strong and persistent ecosystem effects of macrophyte communities are linked to their competitive interactions with phytoplankton communities for dissolved nutrients and light (Carpenter and Lodge 1986; Scheffer et al. 1993). In shallow lakes, rooted macrophytes can dominate when light transmission is not substantially reduced by phytoplankton growth (Scheffer et al. 1993; Blindow et al. 1998; Berg et al. 1998). Submerged macrophytes are sensitive to changes in water transparency, but the positive feedback between light transmission and macrophyte biomass is an important reason why macrophytes help maintain a clear water state over a wide range of nutrient loading (Kéfi et al. 2016). Many types of macrophytes are efficient at taking up nutrients from the water and, if rooted, also

from the sediment, which can limit phytoplankton growth at low to intermediate nutrient loading (Yamamichi et al. 2018). Furthermore, macrophytes provide shelter for zooplankton from fish predation, which helps keeping phytoplankton abundances low via grazing (Jeppesen et al. 1997), and can also produce allelopathic chemicals that inhibit phytoplankton growth (Gross 2003; Hilt and Gross 2008; Nakai et al. 2012). Such mechanisms can contribute to the positive feedbacks that help maintain lakes in a clear water state, and underlie both medium-term (Kéfi et al. 2016; Iacarella et al. 2018) and long-term ecosystem stability and variability (Ibelings et al. 2007). However, surprisingly little is known about how macrophytes affect fine scale temporal dynamics – a knowledge gap that can be filled with high resolution quantification of ecosystem dynamics with automated sensors.

In addition to the effects of macrophytes on algal biomass dynamics, macrophytes are also known to affect ecosystem via their effects on dissolved organic matter (Reitsema et al. 2018), and this could also be an important dimension of their ecosystem impacts. Dissolved organic carbon (DOC), a subset of dissolved organic matter, is a diverse mixture of labile (low molecular weight) and refractory (high molecular weight) components (Bolan et al. 2011). Changes in both DOC concentration and composition can affect light transparency (Retamal et al. 2007) and ecosystem metabolism (Findlay and Sinsabaugh 2003). In the clear water state, macrophytes and algae both produce carbon as labile DOC components, mainly carbohydrates, that are byproducts of photosynthesis (Carpenter and Lodge 1986; Bolan et al. 2011; Reitsema et al. 2018). On the one hand, DOC originating from macrophytes can be utilized by some phytoplankton, potentially alleviating carbon limitation for some microalgae species (Huss and Wehr 2004; Fonseca and Bicudo 2010) and by bacteria (Catalán et al. 2014). DOC production by macrophytes might also affect water clarity via changes in composition of the dissolved molecules, but not much is known about how

this might affect algal dynamics in general, and feedbacks between light and macrophyte biomass in particular. In addition, macrophytes can also diminish DOC content in aquatic ecosystems through several mechanisms. For example, nutrient depletion from macrophytes may inhibit growth of other DOC producing organisms like phytoplankton, periphyton and filamentous algae (Findlay and Sinsabaugh 2003). Furthermore, oxygen release from macrophytes and the provisioning of carbon substrates may stimulate bacterial degradation of DOC (Catalán et al. 2014; Reitsema et al. 2018).

The effects of macrophytes might also extend to overall ecosystem metabolism via several biological mechanisms, including both competitive interactions with phytoplankton (Mitchell 1989) and bacteria (Wetzel and Søndergaard 1998) as well as effects on DOC dynamics associated with the growth and decay of macrophyte tissue (Kaenel et al. 2000) and rates and dynamics of DOC production (Findlay and Sinsabaugh 2003; Reitsema et al. 2018). In aquatic ecosystems, whole-ecosystem metabolism can be modeled from the dynamics of primary productivity and respiration, characterized by daily changes in dissolved oxygen (O_2). In the clear-water, macrophyte-dominated state of shallow lakes, ecosystem productivity is typically higher than in the turbid phytoplankton-dominated state for a similar nutrient load (Wetzel 1964; Carpenter and Lodge 1986; Brothers et al. 2013). Macrophytes are very efficient at photosynthesis (Kaenel et al. 2000), but also provide substrate for the growth of autotrophic periphyton (Wetzel and Søndergaard 1998; Brothers et al. 2013). In addition, macrophytes produce dissolved substances that are a substrate for bacteria and can influence the dynamics of DOC accumulation and decomposition (Wetzel and Søndergaard 1998). These effects can also extend to seasonal timescales, because macrophytes can affect the overall metabolic balance of lakes and influence shifts between net autotrophy and net heterotrophy (Mitchell and Rogers 1985; Madsen and Adams

1988; Nielsen et al. 2013). The culmination of such effects on productivity and respiration can cause lakes with dense macrophyte cover to experience larger diurnal fluctuations in O_2 concentration than lakes lacking macrophytes (Carpenter and Lodge 1986; Kaenel et al. 2000).

Here, we monitor the temporal dynamics of replicated clear water state ecosystems during one growing season in order to understand how macrophytes affect ecosystem properties and processes, and their temporal dynamics. Using outdoor mesocosms (1000L), we manipulated the presence and absence of a macrophyte assemblage consisting of two species (*Myriophyllum spicatum* and *Chara tomentosa*). We quantified several biotic (two phytoplankton pigments) and abiotic (temperature and conductivity, O_2 , fDOM) properties with very high temporal resolution (15 min), which allowed us to capture the dynamics of ecosystems with and without macrophytes in response to natural environmental variability and seasonal forcing, and to estimate ecosystem metabolism using a Bayesian modeling approach. Furthermore, to investigate a potential driver behind unexpected variance patterns in the biotic time series, we used a model of competitive interactions between microalgae and macrophytes. Using this setup, our goal was to test whether macrophytes would alter mean and variance of (i) algal biomass, (ii) properties of dissolved organic matter, and (iii) ecosystem metabolism. We compare our findings with previous empirical work and discuss the broad functional spectrum of macrophytes as foundation species in the context of shallow lake ecosystems.

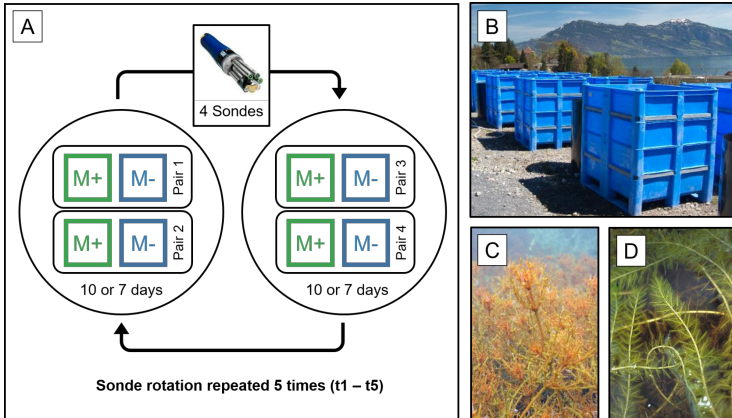


FIGURE 1: A: Scheme of the experimental procedure. experiment Because we were limited to four sondes, we could only measure two tank pairs of macrophyte (M+)/no macrophyte (M-) contrasts. To measure all eight tanks, we followed a rotation scheme in which every tank measure measured for 10 consecutive days before the sondes were moved to another tank (for details refer to Methods section). B: Picture of experimental site showing the set up mesocosms (1000L). C: *Chara tomentosa* (Photo credit: Gustav Johansson). D: *Myriophyllum spicatum* (Photo credit: Alison Fox).

Materials and methods

Experimental design and setup

In an outdoor mesocosm experiment, we established the presence or absence of an assemblage of macrophytes: *Myriophyllum spicatum* (hereafter *Myriophyllum*) and *Chara tomentosa* (hereafter *Chara*). We chose this assemblage because both species are common in Europe and other parts of the world, they commonly occur together in macrophyte assemblages, and their strong influence on lake ecosystems has been previously documented (Berg et al. 1998; Ibelings et al. 2007; Hilt and Gross 2008; Nakai et al. 2012). The species tend to occupy different microhabitats: *Chara* expands horizontally across the benthic surface, whereas *Myriophyllum* grows vertically towards the water surface.

We set up the experiment in four pairs of 1000L mesocosms (1 x 1 x 1 m) on a site next to Eawag Kastanienbaum (8 tanks total). Each pair consisted of one mesocosm with (M+) and one mesocosm without (M-) a macrophyte assemblage. To prepare the mesocosms, we first established a 2 cm thick layer of limestone gravel from a local quarry (2-4 mm grain size) and a 1 cm thick layer of fine sediment that we collected from Lake Lucerne. Afterwards the mesocosms were filled with water from Lake Lucerne, and the suspended sediment was allowed to settle for two weeks. On May 25th, 2015, we collected *Myriophyllum* from a stream in Oberriet, (Kanton St. Gallen), and the plants were kept overnight in two additional mesocosms next to the experimental facility. The following day we collected *Chara* from Lake Lucerne (47°00'06.8"N 8°20'02.7"E) and planted both species in the mesocosms. All collected plant material of either species was divided into 18 equal portions, of which 10 were used for quantification of initial plant biomass as dry weight. Four portions were placed at the bottom of the M+ tanks so the plants could take root. The remaining four portions were suspended in the M- tanks using large mesh

enclosures for two weeks so that these mesocosms would receive the macrophyte associated invertebrate and bacterial communities. On July 4th, two weeks before we started measuring the ecosystems, we added 20 $\mu\text{g/L}$ of P together with N in Redfield ratio (144.65 $\mu\text{g/L}$). During the experiment, we measured nutrient concentrations in the mesocosms on four occasions ([Figure S2](#)).

Ecosystem dynamics measurement using multiparameter sondes

We measured high-frequency ecosystem dynamics in the mesocosms from July 18th until Oct 20 2015, using four autonomous multi parameter instruments (EXO2 modular sensor platform [YSI-WTW], hereafter referred to as sondes) in the mesocosms. Additionally, we measured light in the spectrum of photosynthetically active radiation (PAR) in 15 min interval using a quantum sensor (Li-Cor) that was installed on the pond site at the height of the water surface in the mesocosms.

Sensors

The sondes were equipped with modular sensors that recorded the following ecosystem parameters at 15 minute intervals (see [Table 1](#) for details): temperature, chlorophyll A and phycocyanin (as proxies for algae and cyanobacteria biomass respectively), dissolved oxygen, fluorescent dissolved organic matter (fDOM) and specific conductivity. The sondes were equipped with an autonomous wiper that cleaned the glass sensor head once every hour. All sensors were thoroughly cleaned whenever the sondes were moved to another mesocosm (see contrasts and sampling design)

Calibration

Before placing the sondes in the mesocosms, we performed a 48h

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Parameter	Unit	Sensor type	Calibration
Chlorophyll A	mg/L	Optical, fluorescence	HPLC, cross
Conductivity	$\mu\text{S}/\text{cm}$	4-electrode nickel cell	Conductivity standard
Cyanobacteria	Raw fluorescence	Optical, fluorescence	cross
Dissolved organic matter	Raw fluorescence	Optical, fluorescence *	cross
Dissolved Oxygen	% saturation **	Optical, luminescence	Saturated air, cross
Temperature	$^{\circ}\text{C}$	Thermistor	cross

TABLE 1: Parameters measured in high frequency using autonomous sondes. Prior to the experiment we performed a cross-comparison trial with all four sondes, after which we corrected all sensors for relative differences among them (i.e., “cross” = calibrated against each other). Chlorophyll A sensors were additionally calibrated with samples taken during this trial that were analyzed for their chlorophyll A content with high pressure liquid chromatography (HPLC). Oxygen sensors were calibrated against water-saturated air. (*fDOM-sensors measures emission at 365 ± 5 and excitation at 480 ± 40 nm. **For metabolism modeling mg/L output were used.)

cross-comparison trial where we let all sondes measure water parameters inside a single tank. With this data we were able to correct differences among sensors of the same kind due to the manufacturing process. In other words, the sondes were calibrated against each other. During the cross-comparison trial we also quantified chlorophyll-a concentration by analyzing water samples with high performance liquid chromatography (HPLC, Jasco) and used these values to calibrate the optical sensors installed on the sondes in accordance with the manufacturer’s manual (YSI-WTW). Hence we report Chlorophyll A as mg/L, Phycocyanin and fDOM as raw fluorescence units. The oxygen sensors were calibrated against water-saturated air.

Contrasts and sampling design

Because we only had four sondes available, we sampled two pairs of tanks (each pair consists of one M+ and one M-) over a 10-day period, and then moved the sondes to the remaining two pairs for the following 10 days (Figure 1). Over the entire study we repeated this two-part cycle 5 times, yielding five distinct periods in which all tanks were sampled (Figure 3, t1-t5). On the third sampling period (t3) we reduced the length of the measurement period to 7 days per set of tanks due to battery issues with the Sondes. Between all transfers, we thoroughly cleaned the sondes by power washing the sondes and sensor bodies before placing them in the mesocosms again.

Ecosystem metabolism modeling

We estimated ecosystem metabolism with the *streamMetabolizer* package (Appling et al. 2018) in the programming language R (R Core Team 2017) by using temperature and oxygen measurements (mg/L) from the sondes and the PAR-measurements from the light sensor. The package applies inverse modeling to estimate daily rates of gross primary productivity (P), respiration (R) and gas exchange (K600) as $\text{O}_2 \text{ m}^{-2} \text{ d}^{-1}$. For every modeled rate we calculated the ratio of P and R. Prior to modeling we smoothed all input data with a 12 hour moving average window, to facilitate model convergence and for more conservative estimates. We used a Bayes-type model, and established appropriate parameters (pooled K600 for gas-exchange and informative log-normal priors [0,1])

DOC sampling

For each measurement period (i.e. every 10 days) we sampled water for DOC analysis (Table 3). Water samples were filtered through 47mm ashed GF/F filters (6 hours at 450°C), acidified with HCl 2 M and preserved at 4 C in the dark until analysis via high temperature

catalytic oxidation (TOC-VCS, Shimadzu), with a detection limit of 0.5 mg/L (± 0.5). Specific ultraviolet absorbances (SUVA) were obtained from scans (1 nm intervals) on a Shimadzu UV1700 spectrophotometer, using 1 cm quartz cuvettes. We selected absorbance at 254 nm (SUVA₂₅₄) as a proxy of aromaticity and reactivity of DOC (Weishaar et al. 2003). Furthermore we measured SUVA₃₅₀, which is an indicator for how much UV A radiation is absorbed in the water (Fischer et al. 2014). We normalized the SUVA measurements by dividing the sample absorbances by the total DOC concentration (Hansen et al., 2016). Finally we calculated spectral slope ratio (SSR) as the ratio of linear regressions of the log-transformed spectra of 275–295 nm and 350–400 nm (Helms et al. 2008; Hansen et al. 2016). SSR is a common proxy for DOC molecular weight, to which it should be inversely related. We were unable to analyze two samples over the course of the experiment (one on Oct 2nd and 16th). At the end of the experiment (Oct 20, 2015), we quantified total macrophyte biomass of both species as dry weight.

Statistical analysis

We tested for an effect of macrophytes on chlorophyll A, phycocyanin, O₂ and fDOM separately for each measurement period. By treating each period separately, we could account for any variation due to the sonde switching by specifying a random effect for that temporal block. After removing incomplete data and outliers (See Supplementary material i), we implemented a series of generalized additive models (GAM) using the R-package *mgcv* (Wood 2004). Each model used data from all eight tanks to test for differences in mean or CV, with the presence or absence of macrophytes as the independent variable. All models included penalized thin plate regression splines of each time point in a series, as well as pair and tank as random effects, and

a term that accounted for first order autocorrelation. For the ecosystem metabolism models, we used the same structure but with 8 or 5 estimates per period for P, R or P:R (coming from *streamMetabolizer* models) as the dependent and macrophyte presence as the independent variable. Furthermore, we calculated pairwise log response ratios (LRR) for macrophyte presence in all five periods for the high frequency measurements and metabolism. To do so we divided vectors of mean and CV (coming either from the sliding window for the water parameters or from the daily estimates of metabolism) for M+ by the corresponding vector of M- for each given pair of tanks. We then calculated the natural logarithm for these ratios for each measurement period and for each tank.

We used paired t-tests to test for differences in total DOC concentration, $SUVA_{280}$ and $SUVA_{350}$, and SSR between mesocosms with and without macrophytes. For each date (10 dates in total, see [Table 3](#)) we performed separate tests for all four metrics (n=8 tanks). t-tests were performed with the stats R-package (R Core Team 2017). We also calculated pair-wise LRRs for all four DOC metrics as described above, but on the 10 point measurements, which returns a single value rather than period-specific LRRs.

	t1			t2			t3			t4			t5		
Mean	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq
Chlorophyll A	1724	0.085	0.809	2.696	0.007	0.945	0.355	0.722	0.863	3.340	0.001	0.936	3.600	<0.001	0.927
Conductivity	2.064	0.039	0.968	0.112	0.911	0.939	-1.165	0.244	0.907	-0.533	0.594	0.875	-0.010	0.985	0.884
DOM	-0.302	0.762	0.641	-4.92	<0.001	0.889	-9.620	<0.001	0.963	-6.690	<0.001	0.983	-6.533	<0.001	0.966
O2	-0.877	0.380	0.753	1.163	0.245	0.779	-0.350	0.726	0.836	-2.03E	0.044	0.856	-3.265	0.001	0.892
Phycocyanin	0.311	0.756	0.748	0.637	0.524	0.752	-0.445	0.656	0.865	0.006	0.995	0.883	-0.727	0.467	0.875
Temperature	-0.082	0.934	0.448	0.386	0.699	0.734	-0.370	0.711	0.646	0.657	0.511	0.775	-0.118	0.910	0.901
CV	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq
Chlorophyll A	-2.041	0.041	0.754	-3.320	0.001	0.799	1.578	0.115	0.531	-0.388	0.698	0.661	-2.503	0.005	0.734
Conductivity	-0.278	0.781	0.339	-0.966	0.334	0.358	1.604	###	0.374	-0.989	0.323	0.464	-0.062	0.950	0.583
DOM	-0.052	0.958	0.508	1.119	0.263	0.357	4.036	<0.001	0.426	0.746	0.456	0.629	0.431	0.666	0.492
O2	0.244	0.808	0.617	1.186	0.236	0.558	0.949	0.343	0.329	0.566	0.571	0.363	0.312	0.755	0.404
Phycocyanin	-4.846	<0.001	0.668	-2.092	0.037	0.537	-1.854	0.176	0.621	-2.105	0.035	0.541	-1.836	0.059	0.696
Temperature	-0.233	0.816	0.324	-0.253	0.801	0.446	0.914	0.361	0.257	0.193	0.847	0.415	0.886	0.376	0.430
Metabolism	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq	t-value	p-value	Rsq
P	-3.653	<0.001	0.705	-1.165	0.249	0.461	-2.147	0.036	0.369	1.381	0.176	0.046	-3.395	0.002	0.406
R	-3.470	0.001	0.329	0.121	0.905	0.545	-0.367	0.360	0.456	-0.415	0.681	0.235	-0.346	0.340	0.230
P:R	0.160	0.874	-0.033	1.816	0.074	-0.005	-4.812	<0.001	0.090	-3.389	0.002	0.303	-0.650	0.520	0.119

TABLE 2: Statistical results of GAM-models testing time series of water parameters and metabolic rates. Results are from individual models (one model per parameter and measurement period). For mean and CV of water parameters, N per model is 768 for t1-t3 and 480 for t4 and t5. For metabolic rates, N per model is 8 for t1-t3 and 5 for t4 and t5. Trends ($p < 0.1$) indicated by bold font, significant results ($p < 0.05$) indicated by underlined bold font. t-value = model estimate / model estimate SD, Rsq = R squared of model fit. Positive t-values indicate that the parameter is significantly higher in M- tanks.

Results

Macrophyte biomass and nutrients

The overall biomass of the macrophyte community changed over the course of the experiment, decreasing in the M+ treatment and increasing slightly in the M- treatment. At the end of the experiment substantially less *Chara* biomass was present in the M+ mesocosms than at the beginning (from 165.1 ± 21.65 to 5.08 ± 7.6

g dry weight/mesocosm, [Table S1](#)), whereas *Myriophyllum* biomass increased threefold from 2.84 ± 0.54 g to 8.45 ± 1.6 g dry weight. In the M- treatment there was no *Myriophyllum*, but *Chara* biomass increased slightly due to growth from the sediment (from 0 to 0.27 ± 0.54 g dry weight). In both treatments, filamentous algae grew over the course of the experiment to a final biomass of 8.33 ± 10.54 g dry weight (M+) and 3.21 ± 5.46 g dry weight (M-). Throughout the experiment we observed no differences in concentrations of phosphate or nitrogen between mesocosms with and without macrophytes ([Figure S2](#)). The nutrients we supplied on July 4th were almost completely consumed by July 18th, and were consistently low ($<2\mu\text{g P/L}$, $<50\mu\text{gN/L}$) over the entire experiment. However, concentrations of both nutrients tended to increase towards the end of the experiment, likely due to decomposition of plant material (e.g. *Chara*).

Ecosystem dynamics

As expected, solar radiation and water temperature decreased strongly over the course of the experiment from July 18th to Oct 23rd ([Figure S1](#)). Several parameters differed between M+ and M- tanks over the course of the experiment, with the magnitude of the difference varying by period ([Figure 3](#) and [Figure 6](#), for P-values see [Table 2](#)). As expected, phytoplankton abundance (indicated by Chlorophyll-a concentrations) was significantly higher in tanks without macrophytes (M-) during three out of five measurement periods ([Table 2](#), t2, t4, and t5) and nearly so in an additional period (t1). In contrast, and unexpectedly, M+ tanks had higher variance in phytoplankton abundance (a significantly higher CV across the time series) during three periods (t1, t2, and t5, [Figure 4](#)), indicating higher variation in cyanobacteria population dynamics. The mean concentrations of phycocyanin were not significantly different between M+ and M-, but there was considerable variation in mesocosms

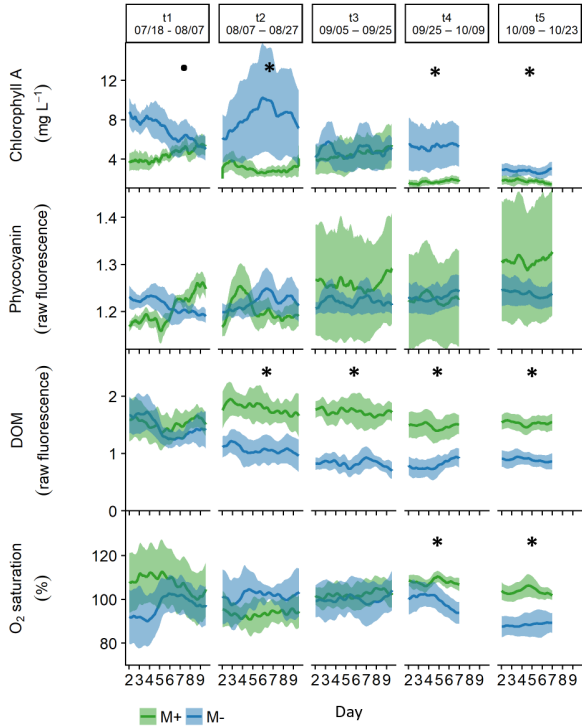


FIGURE 3: Sliding window (1-day window size) estimates of the Mean from high frequency measurements of water parameters. Lines show Mean \pm SE ($n = 8$ tanks), asterisks indicate significant differences. One model (Generalized additive model [GAM]) was used per period, including tank and the pair (see Figure 1) it was in as random effects. Here the sliding window time series of the Mean from both blocks are shown pooled for better illustration. Because the sliding window had a width of one day, only aggregate days 2-9 for each measurement are shown.

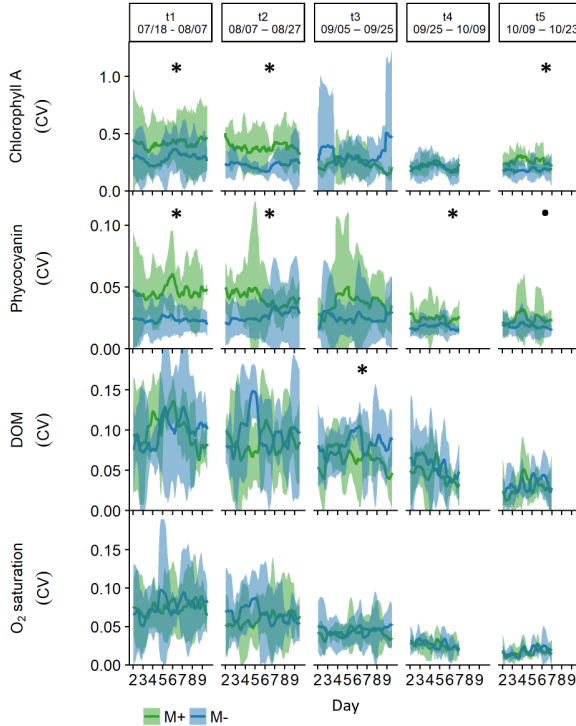


FIGURE 4: Sliding window (1-day window size) estimates of the CV from high frequency measurements of water parameters. Shown are Mean \pm SE ($n = 8$ tanks), asterisks indicate significant differences. One GAM was used per period, including both consecutive blocks as random variables. Here the sliding window time series of CV from both blocks are shown pooled for better illustration. Because the sliding window had a width of one day, only aggregate days 2-9 for each measurement are shown.

with macrophytes (Figure 3). However, phycocyanin variance was significantly higher during three periods (Figure 4, t1, t2 and t4) in the M+ treatment. Measurements of fDOM show consistently higher values in M+ during four out of five measurement periods (GAM, t2 - t5), and CV was only higher on one date (GAM, t3). The mean concentration of dissolved oxygen was significantly higher in M+, but only towards the end of the experiment (Figure 4, t4 and t5). In these two periods – likely due to decreasing irradiance (Figure S1) – M- became undersaturated with dissolved oxygen indicating net heterotrophy, while M+ remained supersaturated. During the entire experiment, there were no differences between M+ and M- in the CV of dissolved oxygen. Effect sizes of macrophyte presence on mean and variance of all parameters measured in high frequency are summarized in Figure 6.

Ecosystem metabolism

We found weak differences in ecosystem metabolism between mesocosms with and without macrophytes (Figure 5). In three measurement periods mesocosms with macrophytes had significantly higher gross primary productivity (t1, t3, and t5), although only to a small extent. During t1, mesocosms with macrophytes also had higher respiration (GAM, main effect of macrophytes, $P = 0.001$). In t2 there was a tendency for higher P:R ratio in mesocosms without macrophytes (GAM, main effect of macrophytes, $P=0.074$), but in t3 and t4 we found the opposite pattern with significantly higher P:R ratio in the presence of macrophytes (GAM, main effect of macrophytes, $P<0.001$ and $P=0.002$, respectively). Overall, P and R decreased significantly over the course of the experiment, likely due to seasonal dynamics (decreasing temperature and light, Figure S1) but the P:R ratio remained around one.

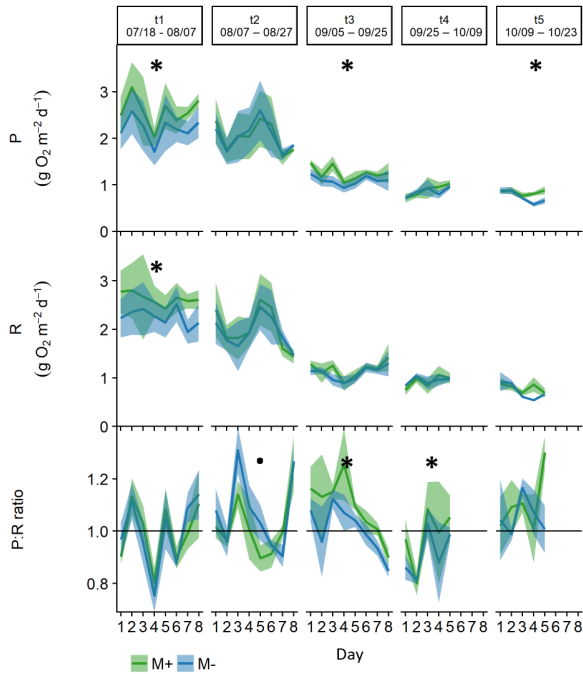


FIGURE 5: Ecosystem productivity (P), respiration (R) and P:R ratio calculated from high frequency measurements of O₂ saturation, temperature, light, and air pressure. Shown are Mean \pm SE (n= 8 tanks), dots indicate trends, asterisks indicate significant differences. One GAM was used per period, including both consecutive blocks as random variables. Here the time series of metabolic rates from both blocks are shown pooled for better illustration. The modeling procedure requires full days to be included, but because of the model parametrization to start each day 1 hour before sunrise, the last day is incomplete and thus cannot be modeled. Hence, only aggregate days 1-8 are shown.

	18-Jul	28-Jul	7-Aug	17-Aug	27-Aug	5-Sep	15-Sep	25-Sep	9-Oct	20-Oct
Total DOC	0.336	0.765	0.294	0.328	0.729	0.21	0.665	0.637	0.305	0.359
SUVA ₂₈₀	0.299	0.452	0.236	0.108	0.034	0.039	0.203	0.81	0.485	0.205
SUVA ₃₅₀	0.481	0.368	0.512	0.057	0.03	0.125	0.096	0.088	0.145	0.644
SSR _(0.75-2.95/350-400)	0.664	0.608	0.821	0.002	0.003	0.002	0.02	0.126	0.005	0.04

TABLE 3: Statistical results of DOC measurements. Reported are p-values from two-sided paired t-tests

DOC

Overall DOC concentration was not significantly different between M+ and M- mesocosms (Table 3): except for the first sampling time point, values of mean total DOC across mesocosms in both treatments ranged between 3.5 and 4.5 mg * L-1 (Figure S3). However, there were clear effects of macrophytes on chromophoric (impacting light transparency) DOC components, SUVA₂₈₀ and SUVA₃₅₀, were often higher in the presence of macrophytes (Table 3, Figure S3), indicating that less UV light was able to penetrate in these ecosystems. SSR diverged among treatments early in the experiment and remained higher in the -M treatment for most of the season (Figure S3), potentially indicating dissolved substances of lower molecular weight in the absence of macrophytes (e.g. sugars or amino acids).

Discussion

Over the course of our experiment, macrophytes affected a wide range of ecosystems parameters (for an overview see Figure 6). Most notably from those measured at high frequency, average biomass of algae (i.e. Chlorophyll pigments) was significantly reduced in the presence of macrophytes (Figure 3). This was expected, and in agreement with a large body previous work documenting the outcome of competition between macrophytes and phytoplankton for dissolved nutrients and

light (Sand-Jensen and Borum 1991; Scheffer et al. 1993; Faafeng and Mjelde 1998; Nes et al. 2007). The ability of macrophytes to keep phytoplankton biomass low is important for stabilizing the clear water state in response to nutrient additions (Scheffer et al. 1993; Ibelings et al. 2007), and understanding the timescale of competition for light and nutrients between these producers is critical to predicting this stability. One indication for nutrient competition being the driver of lower concentration of phytoplankton in the presence of macrophytes is the similar reduction in dissolved inorganic nutrients in both treatments (Figure S3).

While the low nutrient loading in our experiment would not be expected to, and did not, push these systems into a turbid state, we did observe unexpectedly higher variability of phytoplankton biomass in the presence of macrophytes (Figure 3). One mechanism for higher variability of phytoplankton abundance could be that under natural variation of nutrient additions (e.g. rainfall) and nutrient recycling (e.g. from sediment and organic matter [*Chara* decomposition, Table S1]), the higher variance in the M+ treatment could result from macrophytes consistently out-competing algae for seasonally available nutrients. If macrophytes and phytoplankton vary in their ability to compete for light and nutrients, phytoplankton might be able to respond more rapidly in ambient nutrient concentrations than macrophytes (Setaro and Melack 1984; Mitchell 1989; Eichel et al. 2014), and thus will quickly increase in abundance when dissolved nutrients are available. Rooted macrophytes, on the other hand, build up biomass over time and can also store nutrients (Faafeng and Mjelde 1998; Søndergaard and Moss 1998; Yamamichi et al. 2018); and thus prevented a high mean level of algal biomass, but instead repeatedly suppressed multiple bouts of phytoplankton growth.

In order to test and better understand how competitive interactions between macrophytes and algae might affect the mean and variance

of algal biomass, we used a simple model of macrophytes and phytoplankton to simulate dynamics in the presence and absence of macrophytes (Figure 5A). By assuming that phytoplankton and macrophytes compete for both light and nutrients, we found that the presence of macrophytes kept phytoplankton densities low, whereas in the absence of macrophytes, the system reaches a high phytoplankton density state (Figure 5B). Interestingly, while the mean phytoplankton density is lower in the presence of macrophytes, the model predicts

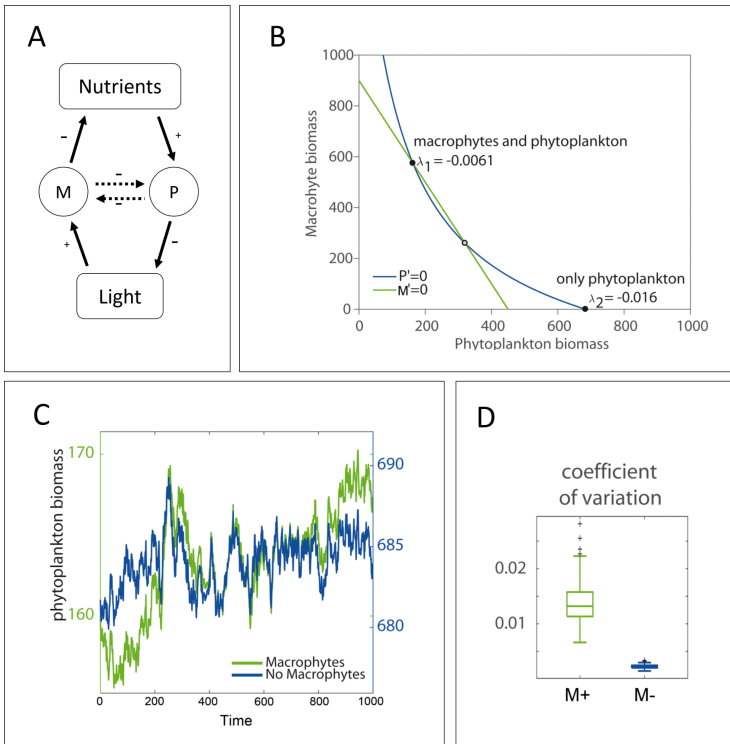


FIGURE 5: A simple model of competition for light and nutrients between macrophytes and phytoplankton (caption continues next page).

FIGURE 5: (*continued*) A) Schematic of interactions between macrophytes (M) and phytoplankton (P). Macrophytes consume nutrients, which has a negative indirect effect on phytoplankton. If phytoplankton biomass becomes too high, it reduces light levels such that there is a negative indirect effect on macrophytes. Thus, macrophytes are more strongly limited by light, and phytoplankton by nutrients. B) Zero-growth curves of macrophytes (red line) and phytoplankton (blue line). Black points mark the 2 alternative stable equilibria of either a macrophyte-and-phytoplankton state or an only-phytoplankton state. Although these two states exist for the same level of nutrients in the lake, their stability (measured as the dominant eigenvalue λ) differs: the only-phytoplankton is more stable than the macrophyte-and-phytoplankton state. C) Simulated time series of phytoplankton biomass in the presence (green) and in the absence (blue – note second y-axis) of macrophytes for the same level of nutrients in the lake. D) Coefficient of variation of phytoplankton biomass estimated from 200 simulated sets (model details and parameters used can be found in Supplement).

a lower variance of algae in the presence rather than in the absence of macrophytes (Figure 5C and Figure 5D). These modeling results imply that the phytoplankton-dominated state is more stable compared to the phytoplankton-macrophyte state (for further details refer to the supplement). Qualitatively, this outcome matches the observations from our experiments and suggests that differences in the nature of resource limitation between macrophytes and phytoplankton may be a possible explanation. *Myriophyllum* macrophytes may have been able to consume initial nutrients faster than microalgae due to high initial (and further increasing) plant biomass, while plankton shading was not sufficient to reduce macrophyte growth rates. However, other processes such as the production of allelochemicals (Hilt and Gross 2008;

Nakai et al. 2012), modification of the light environment, or harboring more zooplankton grazers are alternative hypotheses that might also influence these changes in variance, although they potentially act on different timescales.

As expected, we found that macrophytes had strong effects on the dynamics of whole ecosystem metabolism. Differences in productivity were most pronounced in early summer, where mesocosms with macrophytes were significantly more productive than macrophyte free mesocosms (Figure 5, t1 and t3). However, this difference disappeared when the phytoplankton bloom occurred during the second measurement period (Figure 5, t2). This suggests that at intermediate densities, phytoplankton can increase productivity of aquatic ecosystems and match rates of primary production of macrophytes (Figure S4). Higher productivity of ecosystems with macrophytes was also reflected in P:R ratio, which is on average slightly higher for those mesocosms in t3 and t4 (Sep 5th - Oct 9th). In t2 (Aug 7th - Aug 27th) there is a tendency for higher P:R in mesocosms without macrophytes, probably due to very high phytoplankton biomass. These findings suggest that macrophytes might make shallow lake ecosystems more productive across the seasonal succession of ecosystem metabolism (Madsen and Adams 1988; Blindow et al. 2006; Brothers et al. 2013). These dynamics require additional investigation, especially in the context of successive phytoplankton blooms and their effects on the macrophyte community.

Another significant but often overlooked effect of macrophytes is their influence on dissolved organic matter. From the beginning of t2 (August 8th), fDOM measurements in mesocosms with macrophytes were nearly twice as high as in mesocosms without macrophytes. Furthermore, while total DOC concentrations were similar in both treatments, measurements from the scanning spectrophotometer showed consistently lower SSRs, indicating the presence of DOC compounds with higher molecular weight. The buildup and decay of macrophyte

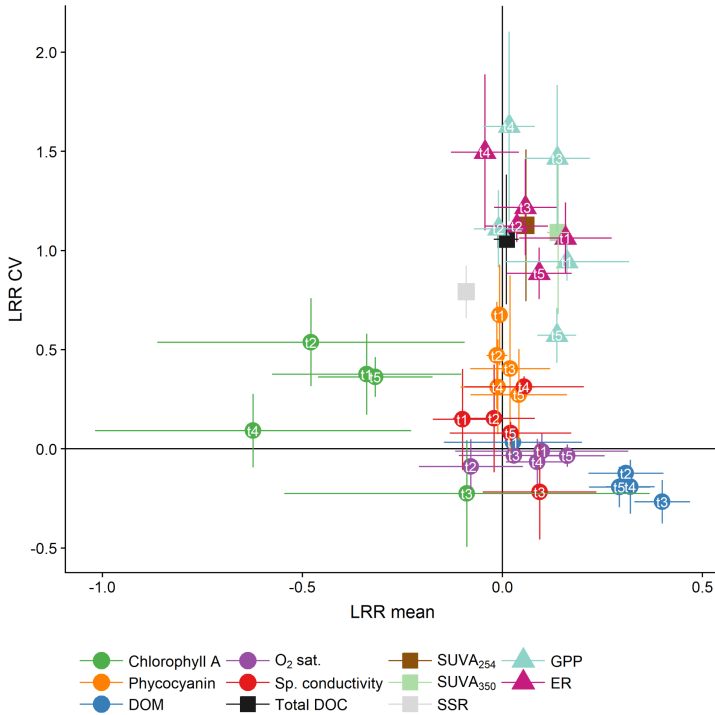


FIGURE 6: Average log response ratios (LRR) for macrophyte presence on mean and CV. Effect sizes were calculated differently for each data type: high frequency (●), metabolism (▲), or DOC point measurements (■) – for details refer to the methods section. Each point shows the average (mean \pm se) macrophyte LRR across all tank pairs (N=4, Figure 1) and in all measurement periods (t1-t5, except for the DOC point measurements, where all 10 measurements were used to calculate LRR for mean and CV).

detritus could explain the low SSR ratios at similar total DOC levels, particularly since much of the initial *Chara* biomass contributed to decomposition rather than taking root, and then decayed over the course of the experiment (Table S1). However, *Myriophyllum* biomass also increased substantially, and could have added high MW compounds into the mesocosms. It is also possible that production rates of DOC were similar in M+ and M- treatments (as the total DOC was similar), but that material originating from macrophytes has a higher MW, and is more difficult to break down by bacteria and (Bolan et al. 2011; Reitsema et al. 2018). Such changes in DOC composition might reflect differences in the balance of production and decomposition rates of different photosynthetic compounds, such as low MW sugars that are a byproduct of recent photosynthetic activity (Carpenter and Lodge 1986; Bolan et al. 2011; Reitsema et al. 2018). Regardless of the specific mechanisms of production and decomposition, macrophytes had a strong effect on pools of fDOM and DOC which culminated in higher photoreactivity, as indicated by lower SSR and higher SUVA on both measured wavelengths (Figure S3).

Using a common macrophyte assemblage, our experiment shows that communities of submerged plants can affect mean and variance of a wide range of biotic and abiotic ecosystem properties and processes over a relatively short amount of time (Figure 6). Some of the effects we found, most notably the negative relationship of macrophytes and phytoplankton densities and the positive relationship of macrophytes and fDOM, have been previously observed and are relatively well described. Other findings, like elevated variability of both phytoplankton pigments in the presence of macrophytes, were unexpected, and indicate that the relationships of macrophytes with other species can be very dynamic. Our results demonstrate that high frequency data are useful for investigating the temporal dynamics of interactions macrophytes and phytoplankton. Future experiments targeting shallow lake ecosystems should also encompass measurements

in high resolution, e.g. to detect the potential outcome of interactions among different trophic levels (e.g. between macrophytes, zooplankton and fish) or quantify the response to perturbations (e.g. nutrients or temperature). Our example highlights how complex and temporally variable interactions around foundation species can be, and underscores the need for further research that investigates biotic and abiotic components of these networks of interactions in detail.

Acknowledgements

We thank Gilles Antoniazza, Emil Birnstiel, Laetitia Catalano, Daniel Steiner, Jaime M. Anaya-Rojas and Marek Svitok for major contributions to mesocosm set-up, maintenance, and sampling. Patrick Kathriner, and Beat Kienholz provided lab facilities and infrastructure support. The Eawag Directorate provided financial support for RJB. MDL was funded by the center for Adaptation to a changing Environment (ACE) at ETH Zürich.

Supplement

Methods

Data treatment

Prior to the statistical analysis we removed incomplete days at the beginning and end of each time series, when we moved the sondes. After this, each time series had 864 data points (15 min interval = 96 data points per day = 9 days) for t1-3 and 576 data points (= 6 days) for t4 and t5. In a second step, we removed outliers by detecting residuals of the detrended data that were outside 2.5 times the interquartile range. We then removed the corresponding values from the original data. Finally, we used sliding windows with a size of 96 time points (= 1 day) to calculate time series of mean and cv with n=768 data points for t1-t3 and n=480 data points for t4 and t5.

A simple model of competition for light and nutrients between macrophytes and phytoplankton

We simulated dynamics of macrophytes and phytoplankton following a model by Scheffer et al (2003) that implicitly accounts for competition for light and nutrients. In the model, growth of macrophytes M and of phytoplankton P is determined by a gain and a loss term following:

$$\begin{aligned} \frac{dP}{dt} &= r_P \frac{n}{n+h_P} \frac{1}{1+\alpha_P P} P - l_P P + \sigma \varepsilon_P(t) \quad (\text{eq1a}) \\ \frac{dM}{dt} &= r_M \frac{1}{1+\alpha_M M+b_P P} M - l_M M + \sigma \varepsilon_M(t) \quad (\text{eq1b}) \end{aligned}$$

Phytoplankton grows with a maximum growth rate r_P that is limited by nutrients n in a saturating function with half-saturation constant h_P . Limitation of phytoplankton growth by macrophytes comes through nutrient availability given by eq2:

$$n = \frac{N}{1+q_M M+q_P P} \quad (\text{eq2})$$

Where N is the total amount of nitrogen in the system and nutrients decrease in a nonlinear way depending on the biomass of macrophytes and phytoplankton. Parameters q_M and q_P determine the strength of the response in decreasing nutrients per biomass increase in macrophytes and phytoplankton respectively. Phytoplankton growth is also limited by light due to self-shading scaled by α_P where $1/\alpha_P$ is the biomass of phytoplankton that makes the maximum growth rate equal to half. Loss is determined by loss rate l_P . In a similar way, macrophyte maximum growth rate r_M is limited only due to competition for light. In that case, light limitation is driven by self-shading through parameter a_M and due to shading by phytoplankton by parameter b . Loss is determined by loss rate l_M . We used parameters so that both macrophytes and phytoplankton are equivalent ($r_P=r_M=0.5$, $\alpha_P=\alpha_M=0.01$, $l_P=l_M=0.05$), but only differ between parameters that determine the asymmetry in light and nutrient limitation between macrophytes and phytoplankton. Nutrient limitation by macrophytes is tuned by setting $q_M = 0.075$ and $q_P = 0.005$, with $h_P = 0.2$, whereas light limitation by phytoplankton is tuned by setting $b(= 0.02)$ to values bigger than α_M . Lastly, we set $N=3.2$ that is a total nutrient level value for which the model can give rise to 2 alternative states, one with both macrophytes and phytoplankton present and the other with phytoplankton and no macrophytes. We simulated model dynamics at these two contrasting states in the presence of environmental stochasticity $\epsilon_P(t)$, $\epsilon_M(t)$ (iid and different for macrophytes and phytoplankton) with strength σ ($=0.5$). We produced 200 simulated sets of 1000 points length for each of the two states using the same sequence of stochastic realizations for both states. In that way, differences in the recorded standard deviation and coefficient of variation were only due to the stability of the two states and independent of the stochasticity.

All simulations were performed in MATLAB R2016b (Mathworks) using Grind v2:

www.sparcs-center.org/resources/dynamical-modeling-tools.html.

Equilibria and eigenvalues were estimated numerically, stochastic equations were solved with Euler-Murayama integration using a 0.01 step.

References

1. Scheffer, M. et al. Floating plant dominance as a stable state. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4040–4045 (2003).

Figures

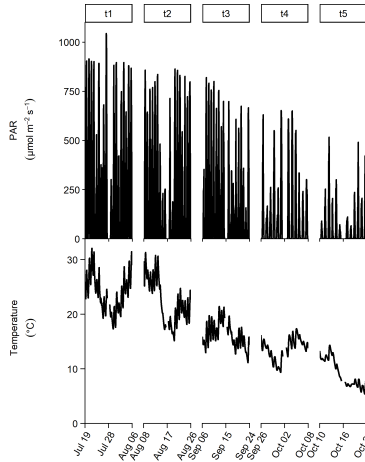


FIGURE S1: Abiotic conditions during experiment: Temperature was measured inside each mesocosm with a sensor that was installed on the sondes (Table 2: no significant difference among tanks). PAR was measured outside the mesocosms with a sensor (Li-Cor) that was installed at the center of the side at water level height.

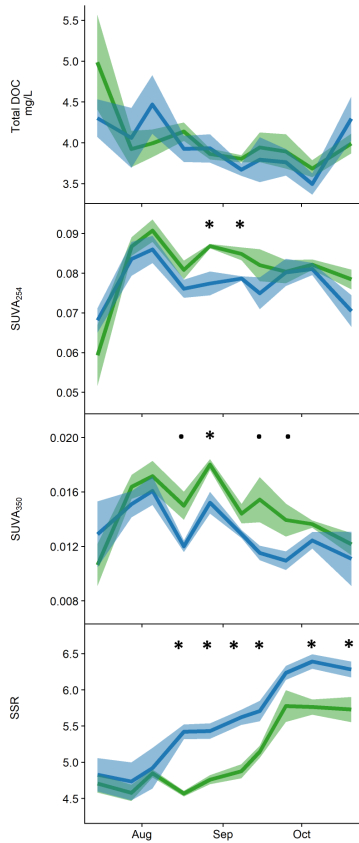


FIGURE S2: Point measurements of dissolved nutrients (top = phosphate, bottom = nitrite and nitrate). We only added nutrients at the beginning of the experiment; increasing nutrient concentrations therefore underlie natural dynamics (e.g. increase due to rain and decomposition, decrease due to uptake by plants).

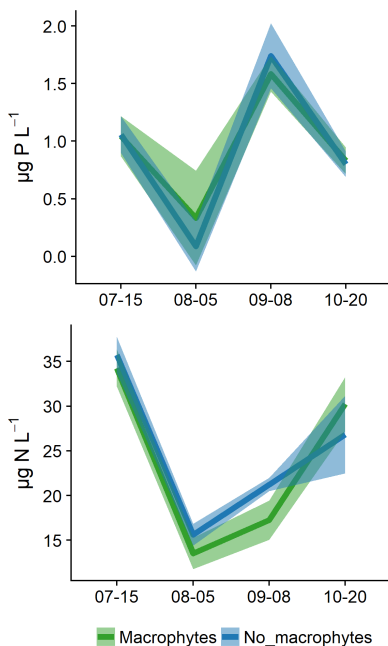


FIGURE S3: Point measurements of different DOC components: total DOC, specific UV absorbance (SUVA: smaller values = higher UV transmission) at 254 and 350 nm, and the ratio of spectral slopes (SSR; smaller values = higher molecular weight) at 275-295 and 350-400 nm. We used separate t-tests to test for differences in DOC components at each date (n per treatment level = 4). Significant differences ($p < 0.05$) are indicated by asterisks, trends ($p < 0.1$) are indicated by dots. All p-values are reported in [Table 2](#).

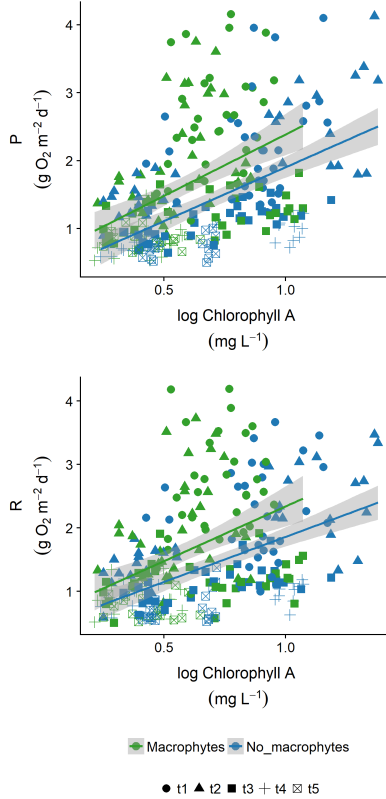


FIGURE S4: Metabolic rates as a function of Chlorophyll A concentration (top = gross primary productivity [P], bottom = respiration [R]).

Tables

Phase of experiment	Macrophyte treatment	Species	N	Dry weight in g (mean \pm SD)
Start	M+	<i>Chara</i>	10	165.1 \pm 21.65
		<i>Myriophyllum</i>		2.84 \pm 0.54
	M-	<i>Chara</i>	NA	NA
		<i>Myriophyllum</i>		NA
End	M+	<i>Chara</i>	4	5.08 \pm 7.6
		<i>Myriophyllum</i>		8.45 \pm 1.6
		Filamentous algae		8.33 \pm 10.54
	M-	<i>Chara</i>	4	0.27 \pm 0.54
		<i>Myriophyllum</i>		0.56 \pm 1.12
		Filamentous algae		3.21 \pm 5.46

TABLE S1: Plant biomass at the beginning and end of the mesocosm experiment. We used 10 aliquots (out of 18) to measure initial biomass in the macrophyte treatments. At the end of the experiment we collected all plant material of the entire benthic substrate. In the M- treatment both *Chara* and *Myriophyllum* were growing from the sediment, as well as a species of prostrate filamentous algae (which was growing in tanks of the M+ treatment as well).

Chapter 3

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

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Abstract

Variation in survival during juvenile development is a fundamental source of fitness variation in natural populations. During early life stages organisms can show plastic responses to the environment, which may dramatically affect the development of the phenotype and also determine fitness at later life stages. However, constraints and fitness costs of developmental plasticity that accrue during juvenile life stages are often poorly understood. In this study we i) quantified the degree of developmental plasticity in response to diet composition in juvenile isopods (*Asellus aquaticus*), and ii) investigated whether the resulting developmental trajectories of growth and pigmentation affected survival of juveniles until maturity. In a laboratory experiment we reared over 1000 individuals from 29 families in two different diet-environments (low/high protein concentration) and quantified developmental trajectories of body size and pigmentation for every individual over 12 weeks. We found that increased dietary protein had strong positive effects on the rate of pigmentation, but led to increased mortality under certain growth and pigmentation rates. This resulted in alternative “survival landscapes” of juvenile isopods that are determined by diet. Using *A.aquaticus* as a model, we show that environmental effects on development may constrain juvenile organisms in the range of developmental trajectories they can explore without suffering fitness consequences.

Introduction

Fitness variation among individuals in a population can emerge from variation in juvenile survival, which contributes to differential life-time reproductive success (Stearns 1992; Reznick 2013). This source of fitness variation is shaped by the genes underlying development and their interaction with the developmental environment

(West-Eberhard 2003; Naguib et al. 2017; Bonduriansky and Day 2018). Intra-population variation in developmental phenotypes arises from both genetic variation and phenotypic plasticity (West-Eberhard 2005; Pigliucci et al. 2006; Uller 2008), with the latter describing how individuals can produce different phenotypes over a range of environments. Developmental trajectories can have myriad effects on individual fitness. For example, faster or slower individual growth rates depending on available resources, may covary with the age at maturity, while variation in age at maturity and potential reproductive life-span may drive variation in life-time reproductive success (Stearns 1992). In addition, developmental trajectories can decrease survival, for example, because of internal resource competition (West-Eberhard 2003) inside organisms during the expression of multiple traits due to nutritional constraints (Walker et al. 2005; Lee et al. 2008; Lee et al. 2013). As a result, adapting a life-history strategy that maximises long term reproductive success can be a strategy to deal with constraints during early development. (Burgess and Marshall 2014; Vrтіlek and Reichard 2015; Nettle and Bateson 2015) However, little is known about how alternative developmental trajectories in response to constraints affect the survival of juveniles during development and before reproduction occurs (West-Eberhard 2005; Pigliucci et al. 2006; Uller 2008), which limits our understanding of life history evolution.

Developmental plasticity is ubiquitous in animals (Jablonka et al. 2005; West-Eberhard 2005; Pigliucci 2007) and there are many different ways by which environments can act on the developing phenotype. Either through specific events or prolonged exposure, the environment can cue developmental switches, serial or cascading points of decisions (Pfennig 1990; Ostrowski et al. 2002; West-Eberhard 2003), which regulate development at all life history stages (Naguib et al. 2017), and sometimes can even affect subsequent generations (Burton and Metcalfe 2014). Resource quality and quantity often have

large effects on the development of morphological, physiological, and behavioral traits of individuals. Throughout their ontogeny, organisms need to balance the allocation of acquired resources to maintenance, growth, and reproduction (Stearns 1992; West-Eberhard 2005; Naguib et al. 2017). Particularly during early life periods, developmental trajectories might be more susceptible to outside effects on internal resource- or energy-allocation (Metcalf and Monaghan 2001). This is due to high investment in somatic growth and accompanying trade-offs with the development of other traits on the one hand (West-Eberhard 2003), and effects of developmental switches that can cascade to later life stages on the other hand (Pfennig 1990; West-Eberhard 2003; Fusco and Minelli 2010).

Strong effects on the early life period and development can also stem from parental effects; i.e. where parental phenotypes affect offspring phenotypes via mechanisms other than the transmission of alleles (Mousseau and Fox 1998; Qvarnström and Price 2001; Uller 2008). Parental effects can span across generations (Uller 2008), and may be driven by parental behavior (e.g. mate or habitat choice - maternal and paternal), condition (e.g. lipid reserves - mainly maternal) or allocation to reproduction (e.g. yolk production or egg size - maternal) (Mousseau and Fox 1998). Condition and life history traits of the parents can also have strong effects on the number of offspring. For example, clutch size is usually positively correlated with female body size and can vary substantially within populations (Mousseau and Fox 1998). As clutch size increases, egg size, and consequently, the size of hatchlings, typically decreases. Small body sizes at hatching can affect the survival at juvenile stage, but also the traits that are expressed later in life history (Sutcliffe et al. 1981; Stearns 1992; Nieceza and Metcalfe 1997). For example, juveniles that have relatively small body size after birth can show increased growth rates throughout development (Metcalf and Monaghan 2001).

Detritivores are a useful model system to study environmental effects on developmental trajectories and life history. Many invertebrate detritivores experience a wide range of variation in diet and nutritional quality throughout their development (Rietsma et al. 1982; Friberg and Jacobsen 1994; Bloor 2011), and there is some evidence that diet breadth of detritivores has evolved in response to detrital resource quality (Belgrano et al. 2005; Adey and Loveland 2007). For example, depending on mobility (Gjerløv et al. 2003), dispersal ability (Palmer et al. 1996), and the magnitude of costs associated to mobility (Bonte et al. 2012) detritivorous invertebrates may be restricted to utilize only the locally available food resources, which may result in variation in development time and consequent life history trait expression (Marcus et al. 1978; Sutcliffe et al. 1981; Smock and Harlowe 1983; Verberk et al. 2008). One such example the detritivorous freshwater isopod *Asellus aquaticus*, which exhibits diet based plasticity in growth depending on different food items (Marcus et al. 1978), and in the development of pigmentation, depending on dietary protein content (Lürig et al. 2019). Isopod pigmentation is an ecologically relevant trait (Figure 1 and known to increase with the darkness of the habitat background to reduce risk from visual predation via crypsis (Hargeby et al. 2005; Eroukhmanoff et al. 2009a).

However, pigmentation in *A. aquaticus* is biosynthesized from the amino acid tryptophan and increases in correlation with somatic growth from juvenile to adult stages (Needham 1970; Oetinger and Nickol 1982): right after birth, all isopods completely lack pigmentation, which is built up progressively with age. Therefore, pigmentation of adult isopods can strongly depend on protein and amino acid composition of the diet that is available during juvenile development (Marcus et al. 1978; Lürig et al. 2019). Using the freshwater isopod *A. aquaticus* (Figure 1), we investigated how developmental trajectories towards different adult phenotypes are affected by different dietary environments of juveniles (concentration of proteins

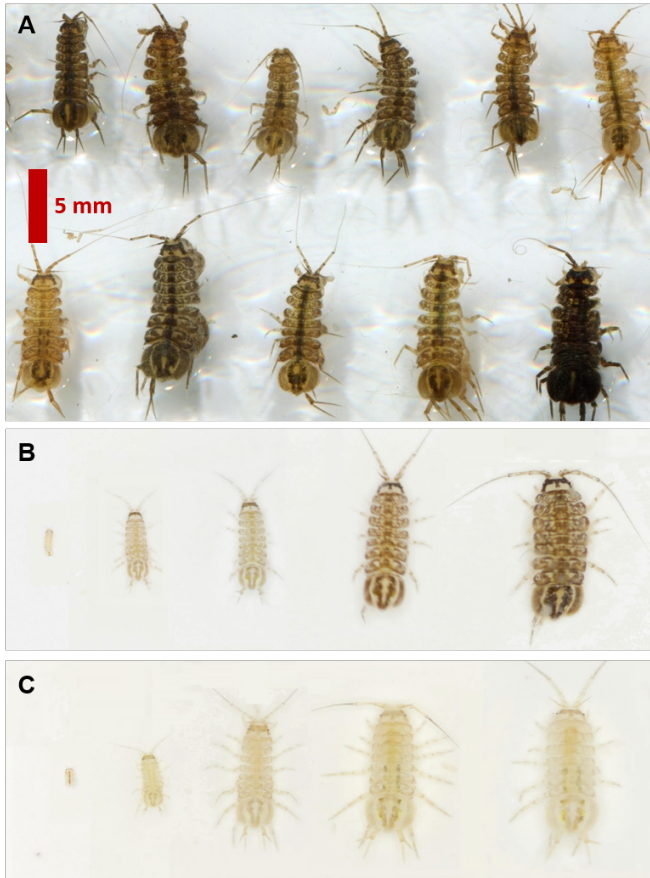


FIGURE 1: Phenotypic variation in pigmentation in the freshwater isopod *Asellus aquaticus*. A) A representative sample of isopods collected in macrophyte beds near Kastanienbaum, Lake Lucerne. Exemplary developmental trajectories of growth and pigmentation in *A. aquaticus* under B) low protein and C) high protein diet. Scale is for all three panels.

and tryptophan). The experimental design allowed us to investigate i) the extent of developmental plasticity in growth and pigmentation caused by dietary resources, ii) how both developmental rates are affected by the parental phenotypes and initial conditions right after birth, and iii) how variation in developmental rates may result in variation in juvenile survival. Here, we explore how developmental rates of growth and pigmentation depends on diet, and other life history traits such as size at birth and parental phenotype. Overall, our study shows that the correlated development of multiple traits can depend on the dietary environment of juveniles, and may have fitness consequences.

Materials and methods

Study system

The freshwater isopod *Asellus aquaticus* is common in benthic communities across Europe and parts of Asia (Sworobowicz et al. 2015). The small crustaceans (mature animals measure 4-15 mm, Figure 1, [Arakelova 2001]) are found in many different microhabitats, like beds of macrophytes (e.g. *Chara tomentosa* and *Elodea canadensis*), *Phragmites australis* (reed), or bare sand (Marcus et al. 1978; Hargeby et al. 2004; Hargeby et al. 2005). Because *A. aquaticus* is a detritivore and has a broad feeding niche (Marcus et al. 1978; Hargeby et al. 2004; Bohmann 2005), it is considered to play a significant role in freshwater food webs (Jeppesen et al. 1998; Calizza et al. 2013). There is some evidence that variation in dietary composition can affect rates of growth and pigmentation in *A. aquaticus* (Marcus et al. 1978; Lürig et al. 2019). This may be related to the elemental composition of detritus, which is considered to be high-quality when C:N and C:P ratios are low (Elser et al. 2000; Adey and Loveland 2007). This is indicated by a multitude of

positive effects of N-rich fungal diet on the life history of *A. aquaticus*, (Marcus et al. 1978; Rossi and Fano 1979; Rossi 1985; Graca et al. 1993).

There may be additional dietary requirements in the context of pigmentation: the major integumental pigment is a type of ommochrome (xanthommatin), which is synthesized by many arthropods in chromatophore cells that are located in the hypodermis, underneath the exoskeleton (Linzen 1974; Needham 1974b). Ommochromes are an important group of pigments in arthropods, where they occur as screening pigments in eyes, metabolic byproducts, or are deposited as colour giving granules inside the exoskeleton (Linzen 1974; Shamim et al. 2014). The bio-synthetic pathway of all ommochromes starts with the essential amino acid tryptophan, which is metabolized over four steps into xanthommatin (Linzen 1974; Shamim et al. 2014). Therefore, organisms that generate this pigment have to take up tryptophan with their diet. Furthermore, the synthesis and deposition of xanthommatin is a “one-way” process: the granules inside the hypodermis are unaffected by moulting and remain with the animal (Linzen 1974). Consequently, there have been no reports of pigment excretions or demetabolization.

Common garden experiment

Contrasts and food preparation

Using a common garden experiment, we wanted to test the extent of developmental plasticity of growth and pigmentation in *A. aquaticus* in response to diet composition. To do so, we exposed 1047 juvenile isopods from 29 families after their birth to different dietary contrasts and measured growth, pigmentation and survival of each individual over the course of 12 weeks. Half of all juveniles from each family were randomly assigned to either low or high protein diet (full-sib / split family). For the eight families with the highest number of offspring (40-60 juveniles), we added a tryptophan treatment. Juveniles

from these eight families (out of 29 total) were randomly assigned to one of the four possible diet treatment combinations. In other words, 29 families experienced high and low protein diet, and eight of these were also subjected to tryptophan presence and absence in a factorial design. For the high- nutrient diet, we used 80% dry yeast (*Saccharomyces cerevisiae*) and 20% potato starch that was autoclaved together with agar and filtered lake water into a paste that was dried and cut into pellets (dry weight 1.2 ± 0.1 g, n=10). The low- nutrient 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.

Isopod collection and mating

On April 25th 2017 we we picked up bushes of *Chara tomentosa* from the bottom of Lake Lucerne (47°00'06.8"N 8°20'02.7"E) and brought them to the shore. There we washed *A. aquaticus* out of the plant material within a big container that was filled with lake water. This was repeated several times until we poured the content through a 0.5 mm sieve. We then brought the retained substrate, which consisted of smaller plant material and invertebrates, to the laboratory, and transferred it to an aquarium (40 x 50 x 80 cm, 160 L). The aquarium contained filtered lake water (changed every two weeks), and was maintained at 20 °C with a 16:8 light:dark cycle and an aeration stone. From the aquarium, we collected mating isopods over the course of the following two weeks (April 25th to May 9th), and put them into a separate container (PE, 50ml, maintained under same conditions as the aquarium), which contained lake water and a piece of inoculated black alder leaf. We opened each container every second day and checked whether the male had released the female from the precopula, and whether the eggs inside the female marsupium were fertilized (indicated by colour change from white to beige/yellow). After precopula was complete and eggs were fertilized, we preserved the males by

freezing them, and let the female continue to breed inside the container. Following fertilization, juveniles typically needed two to three weeks until they hatch from the eggs into the marsupium. Starting after 10 days we checked every day to see if juveniles hatched, and if so, preserved the females by freezing it and transferring the juveniles to a separate container with just lake water.

Experimental setup and procedure

We used juvenile isopods from a total of 29 successful matings. Because not all juveniles from these families hatched at the same time, we started the common garden experiment in three blocks. That way we ensured that not more than three days passed between the time of hatching and the start of the experiment. 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 two diet types (or four in case of families with more than 40 juveniles where we applied the tryptophan contrast). We placed the jars inside racks that were arranged randomly inside a big container filled with water, to buffer against fluctuations in temperature. The setup was maintained 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 bucket containing lake water, and from there onto a flat tray containing lake water underneath a camera mounted on a camera stand. This was necessary to introduce as little water as possible from the tubes onto the tray. 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 five times (initial picture and four time points) until the experiment was terminated due to high mortality in the high protein diet (Figure 3C).

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 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 recently developed python package *phenotype* (Lürig 2018). Using thresholding algorithms and segmentation to locate isopods in the image, the algorithm extracts the phenotypic information from the pixels marking the animal (dorsal region of isopod torso = carapace, excluding legs and antennae). The grayscale values from these pixels were averaged and converted to a pigmentation scale from 0 (grayscale value of 255) to 1 (grayscale value of 0). Body size was measured as carapace length, excluding legs and antennae. A similar technique has been used in a previous publication, where results from *phenotype* were not different from measurements of the same images using ImageJ (linear correlation between methods: 0.97, $p = 0.0291$ [Lürig et al. 2019]).

Statistical analyses

Life history (mating, clutch size, phenotypes after birth)

We analyzed the effect of parental traits and clutch size on juvenile phenotypes by conducting a path analysis using Bayesian multilevel modeling with the package *brms* (Bürkner 2018). In addition to data from this study, we included data from other published (Lürig et al. 2019) and unpublished experiments conducted in 2016 and 2017 to

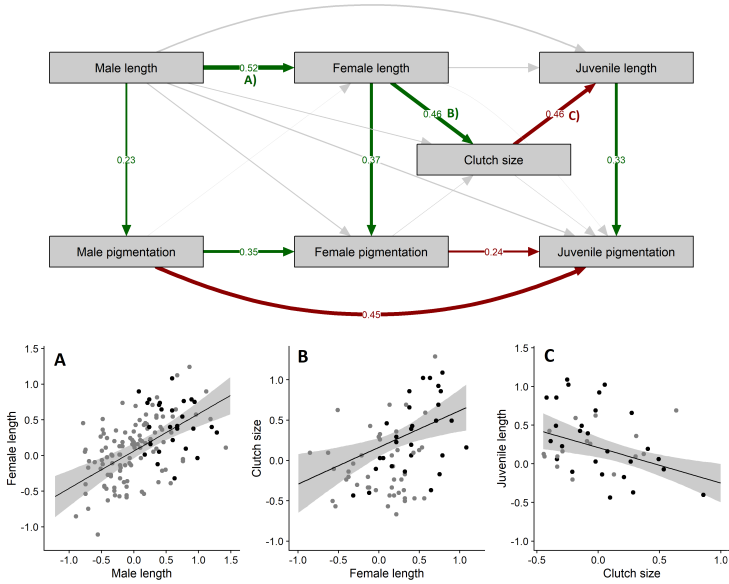


FIGURE 2: Path analysis (Bayesian multilevel modelling) of early life history in *A. aquaticus*: green arrows depict positive, red negative relationships. Gray arrows indicate the posterior overlapping with zero. In addition to data from this study, we included data from other published (Lürig et al. 2019) and unpublished experiments conducted in 2016 and 2017 to provide a broader basis for analysis. We extracted select estimates for different causal relationships (male length \sim female length [A], female length \sim clutch size [B], clutch size \sim juvenile length [C]). These panels contain data from this (black points) and from other studies (gray points), and the model estimates (solid line) with confidence interval (gray ribbon). Details on the path analysis are given in the supplement.

provide a broader basis for analysis. In these studies, we made isopod-crosses and allowed them to give birth using the same procedure described here. In a single model, we implemented four hierarchical levels that were linked through grouping structure (family ID), which allowed us to disentangle the effects of parental phenotypes and clutch size on juvenile phenotypes. From the model we extracted select estimates for different causal relationships (male length \sim female length, female length \sim clutch size, clutch size \sim juvenile length [Figure 2A-C]). Details on model specifications and parameters can be found in the supplementary material in Table S1.

Common garden experiment

We tested for effects of diet protein content and tryptophan supplement on developmental rates of body size and pigmentation and, as well as survival over the course of the experiment using a series of generalized additive mixed models (GAMM), using the *gamm* function in the package *mgcv* (Wood 2011). We fit separate models for body size and pigmentation (log transformed, m1 and m2, respectively Table 1), with time separated by diet contrast as the fixed effect and a thin plate spline term with time in weeks. Furthermore, we fit a GAMM 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 (m3, Table 1). All three models contained nested random terms for family and individual, and used diet as a parametric component in the spline terms. In a further step, we tested for effects of diet composition and of juvenile phenotypes right after birth on growth and pigmentation rates and survival by performing a path analysis using Bayesian multilevel modeling (Bürkner 2018). In a single model, we implemented three hierarchical levels, and included family as the grouping term, allowing us to estimate relative effect sizes of developmental rates and starting conditions on lifespan under all diet treatment contrasts. Details on

model specifications and parameters can be found in the supplementary material and in [Table S1](#). We applied both types of analysis in a complementary fashion: with separate additive models, we accounted for the non-linearity in developmental rates, and with the path analysis we were able to disentangle complex causal interactions in juvenile life history.

To test for interactions between growth and pigmentation on survival, we also applied a more complex multivariate additive model. To do so we first converted measurements of body size and pigmentation we collected until the middle of the experiment in week six (vertical line in [Figure 3](#)) to a single linear slope per individual isopod (hereafter growth and pigmentation rate, respectively). We chose to calculate slopes from this time frame, because pigmentation and growth increased linearly to this point, and isopods were still alive in sufficient numbers. We then implemented an additive model (m4) with the *gam* function from *mgcv*, using lifespan (in weeks) as the dependent variable, single thin plate spline terms for growth and pigmentation rate, and a tensor smooth product term to test for the interaction ([Table 1](#)). The model included family as a random effect, and the spline and tensor term included diet as a parametric component.

General remarks

In the additive models, all continuous numerical fixed effects were included as both linear and nonlinear terms. Main effects of nonlinear terms were included as thin plate regression splines, interactive effects between nonlinear terms were included as tensor smooth products (Wood 2011). Both, regression splines and tensor products were fixed at the lowest number of knots possible (= three knots) for all models. We tested for significance of random effects in all additive models by performing likelihood ratio tests on models with and without the respective random term. In the path analysis, effects were regarded as significant when the 95% credible intervals of effect size did

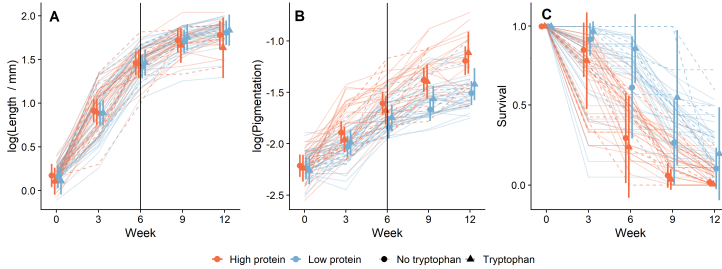


FIGURE 3: Family specific developmental trajectories for growth (A), pigmentation (B) and survival (C). Each line shows the family level average of each parameter at the given time point. Solid lines indicate only protein manipulation (orange = high protein, blue = low protein content), dashed lines indicate averages for the part of the families that were reared under tryptophan supplement. Details on model statistics are given in [Table 1](#).

not overlap with zero. All continuous variables were transformed to have mean 0 and a standard deviation of 0.5. Each path model was initialized with a normal prior with mean 0 and standard deviation 1, and ran for at least 10,000 MCMC iterations with four chains. We verified that all chains converged using the estimated potential scale reduction statistic R_{hat} (Carpenter et al. 2017).

Results

Mating, clutch size, and phenotypes after birth

Our path analysis confirmed previous reports for size assortative mating in *A. aquaticus*, reflected in a strong positive relationship of male and female length ([Figure 2A](#)). As expected, we also found that isopod clutch size strongly depends on female body size ([Figure 2B](#)), and that body size at birth of the juveniles depends on clutch size ([Figure](#)

Response variable	Fixed effect	df	F	P value	Smooth term	Smooth function	knots	edf	F	P value	Random effect	df	Chisq	P value
log(length)	Diet	1	4.644	0.031	High protein - T	tprs	3	1.998	4739.251	>0.001	Individual	1	89.921	>0.001
	Tryptophan	1	3.434	0.064	High protein + T	tprs	3	1.989	603.079	>0.001	Family	1	495.42	>0.001
	Diet x tryptophan	1	2.202	0.138	Low protein - T	tprs	3	1.999	7036.516	>0.001	Block	1	199.2	>0.001
log(Pigmentation)	Diet	1	221.957	<0.001	High protein - T	tprs	3	1.995	1196.426	>0.001	Individual	1	61.161	>0.001
	Tryptophan	1	2.735	0.098	High protein + T	tprs	3	1	271.881	>0.001	Family	1	541.72	>0.001
	Diet x tryptophan	1	7.003	0.008	Low protein - T	tprs	3	1.867	1179.348	>0.001	Block	1	111.84	>0.001
Survival	Diet	1	37.109	<0.001	High protein - T	tprs	3	1.968	342.591	>0.001	Individual	1	3318.9	>0.001
	Tryptophan	1	2.721	0.099	High protein + T	tprs	3	1.509	51.396	>0.001	Family	1	384.21	>0.001
	Diet x tryptophan	1	7.71	0.006	Low protein - T	tprs	3	1.95	324.69	>0.001	Block	1	644.95	>0.001
Juvenile survival	Food	1	106.397	<0.001	High protein x growth rate	tprs	3	1.964	14.856	>0.001	Family Block	1	23.466	0.217
					Low protein x growth rate	tprs	3	1.936	4.39	0.014				
					High protein x pigmentation rate	tprs	3	1	23.212	>0.001				
					Low protein x pigmentation rate	tprs	3	1.977	6.501	0.002				
					High protein x growth rate x pigmentation rate	tp	3	3.21	7.755	>0.001				
					Low protein x growth rate x pigmentation rate	tp	3	1	1.187	0.276				

TABLE 1: Statistical results of generalized additive models. Models m1 - m3 tested for an effect of protein content on growth, pigmentation and survival, m4 tested for interactive effects of growth, pigmentation and protein content on survival of isopods. Reported are results for linear (*Fixed effect*) and nonlinear (*Smooth term*) part of the model (tprs = thin plate regression spline, tp = tensor product). Significance of Random effects what tested with a likelihood ratio test.

2C). Additionally, as previously observed in various field surveys and experiments, pigmentation and body size are strongly correlated, but more so for females and juveniles than for males, which is likely linked to the presence of different size structures in each group. Finally, we observed a surprisingly strong effect of male pigmentation on juvenile pigmentation - a phenomenon we currently can't explain biologically.

Common garden experiment

Diet protein content significantly affected the development of body size and pigmentation, as well as survival during the experiment. However, while growth rate was only weakly affected by protein content, and not at all by the tryptophan supplement (m1, Table 1), rates of pigmentation differed strongly between both diet treatments, as indicated by the path analysis and m2 (Table 1): pigmentation rates

were lowest when juveniles were reared under low protein diet and in the absence of the tryptophan supplement. On the other hand, the tryptophan supplement resulted in slightly higher pigmentation rates under low protein, but not under high protein diet. This was indicated by a significant interactive effect of diet and tryptophan in m2 (Figure 3, Table 1) and in the path analysis (Figure 4). For both, growth and pigmentation rates, there was considerable family level variation, as indicated by the respective random effect (Table 1). Furthermore, the survival of juvenile isopods during the experiment also depended strongly on both diet and tryptophan supplement: there was significantly higher survival under low protein diet, which was further increased when tryptophan was supplemented, however, only under low protein diet (m3, Table 1). Furthermore, the path analysis revealed that length and pigmentation of juveniles at the start of the experiment (i.e. within three days after hatching from the marsupium) negatively affected the developmental rates of these traits.

Higher concurrent rates of growth and pigmentation had a negative impact on survival, as indicated by the interaction term in the path analysis (Figure 4D). A more comprehensive analysis of this effect in a multivariate additive framework, where we analyzed diet specific relationships between both developmental rates (m4, Figure 5, Table 1), revealed two distinct “survival surfaces”: under low protein diet, a single, high survival peak existed at moderate growth and pigmentation rates. Survival under high protein was overall lower and varied non-linearly across a wide range of both developmental rates, as indicated by a significant nonlinear interaction of diet and rates under high protein, but not under low protein (Figure 5, Table 1).

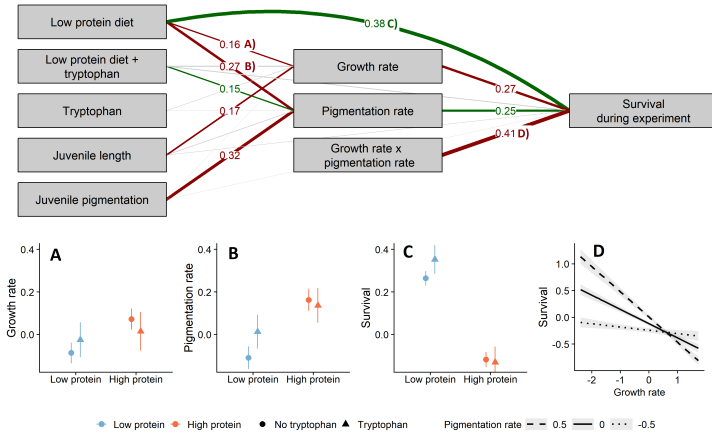


FIGURE 4: Path analysis using Bayesian multilevel modeling to investigate the effects of diet protein content and tryptophan manipulation, as well as juvenile traits at the start of the experiment, on growth and pigmentation rates, as well as survival during the 12 week experiment. Significant effects are indicated by coloured arrows (green = positive, red = negative), effect sizes are indicated by number on arrows. Panels illustrate the effects of the factorial manipulation of protein content and tryptophan on growth, pigmentation and survival rates (panels A, B and C, respectively), as well as an interactive effect of growth and pigmentation rates on survival, independent of diet manipulation (panel D). Details on the path analysis are given in the supplementary material.

Discussion

Our experiment provides evidence for phenotypic plasticity in pigmentation and, to a much lesser extent, also in growth of *A. aquaticus*, which confirms results from our previous study (Lürig et al. 2019). We found consistently higher pigmentation rates across all families for siblings reared under elevated protein content, but this coincided with increased mortality. As expected, we found that higher clutch sizes led to smaller body size at birth, but overall higher growth rates of these families. The effects of parental phenotypes on the development of juveniles likely contributed to the strong family level variation in growth and pigmentation rates and influenced fitness variation. Below we assess differences in plasticity between growth and pigmentation rates, and discuss how they affected fitness in our experiment. We also discuss whether development of pigmentation can be a way to metabolize toxic substances as an alternative hypothesis for the functional basis of pigmentation in *A. aquaticus*, which builds on the tryptophan-ommochrome developmental complex in insects and may be relevant for developmental processes in other arthropod species that use this type of pigment.

Effects of diet on developmental rates

Our manipulation of both protein content and the supplement of tryptophan led to small differences in juvenile growth rates, but large effects on pigmentation rates (Figure 3, Figure 4). The rate of body size increase we measured are comparable to previously measured isopod growth on naturally occurring food items (Murphy and Learner 2006). This suggests that caloric content and nutritional composition of the pellets that we fed ad libitum were sufficient to sustain high growth rates of isopods, similar to previously observed growth rates on natural food items (Marcus et al. 1978). Previously, variation in growth

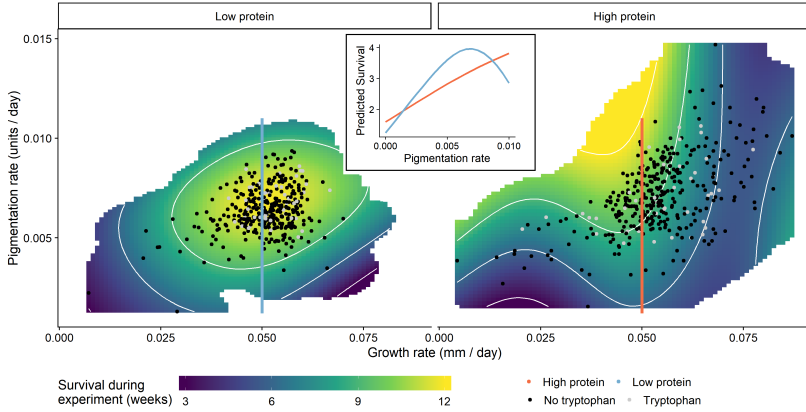


FIGURE 5: Survival landscapes based on individual growth and pigmentation rates. Each point denotes an individual isopod (black = only protein contrast, gray = tryptophan supplement). Diet specific surfaces are model estimates from a generalized additive mixed model (GAMM), with survival during experiment as the dependent and diet specific growth and pigmentation rates between start and week six as the independent variable (see Table 1 for details [m4]). 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.

rates of *A. aquaticus* has been attributed to differences in the concentration of bacteria and fungi, which are deemed important sources of nutrition for *A. aquaticus* (Graca et al. 1993). Another study on a sister species (*Asellus forbesi*, [Smock and Harlowe 1983]) found elevated growth rates with increasing on protein content. Likely, the low protein diet we provided still contained enough protein for near natural growth rates of isopods. In contrast to growth, rates of pigmentation were strongly affected by diet protein concentration: under high protein diet, juvenile isopods from a majority of families (22 out of 29, Figure S1) showed greatly increased rates of pigmentation, and also higher pigmentation at the end of the experiment. This is in agreement with our previous study (Lürig et al. 2019) and provides evidence for phenotypic plasticity of pigmentation during juvenile development, which moreover is irreversible for mature isopods (Hargeby et al. 2004).

The mechanism behind developmental plasticity of pigmentation could result from an internal resource-competition antagonism during the development of multiple traits (West-Eberhard 2003). Somatic growth, the correlated growth of thoracic and other tissues during early ontogeny and before reaching maximum body size is one main dimension of resource allocation in animals, next to physiological maintenance or reproduction (Stearns 1992; Mousseau and Fox 1998; Reznick 2013). However, depending on the resources available during early ontogeny, development of secondary characteristics like ornaments, weapons, or appendages can vary in comparison to body size, due to the necessity to develop fully sized body parts and organs to ensure their functionality (Emlen 1997; Bonduriansky and Day 2003; Frankino et al. 2005; Kodric-Brown et al. 2006). It is possible that during early ontogeny of *A. aquaticus*, resource allocation to growth is prioritized over the development of isopod pigmentation when protein availability is limited.

Our supplement of tryptophan to both high and low protein diets

also showed significant positive effects on pigmentation rates, but only under low protein diet: there pigmentation rates were higher when tryptophan was supplemented (Figure 3, Figure 4). Similar observations have been made for cabbage butterflies (*Pieris brassicae*), which showed increased wing pigmentation when reared under a tryptophan supplement (Kayser 1979). This essential amino acid is necessary to form the main pigment, xanthommatin, which is produced in the integument of various arthropods (Linzen 1974; Needham 1974a; Shamim et al. 2014). Thus, organisms can produce xanthommatin only when their diet contains sufficient amounts of tryptophan. If this has a fitness benefit, organisms may try to take up protein rich diets that contain tryptophan (Arganda Sara et al. 2017), or take up the amino acids directly (Katayama et al. 2016). In our experiment, the yeast strain we used to manipulate protein content, *Saccharomyces cerevisiae*, is known to contain tryptophan (Miozzari et al. 1978) and therefore, the high protein diet may have provided up to four times as much tryptophan as the low protein diet (80 % vs 20% dissolved yeast). Therefore, we hypothesize that faster development of pigmentation we observed under high protein content is explained by higher levels of tryptophan. Future work should target the physiological mechanism of alternative trajectories for pigmentation in *A. aquaticus*: on the one hand, the chromophoric system itself could be affected as a whole, i.e. in the abundance of chromatophores in the skin and their genetic basis (Protas et al. 2011); on the other hand, the amount of pigmentation that is produced inside the chromatophores may depend on the dietary environment during development.

Interactive effects of diet and developmental rates on survival

We found strong effects of developmental rates and protein content on survival of isopods during the experiment (Figure 3, Figure 4 and Figure 5). Independent of growth and pigmentation rates, lower protein

content had a positive effect on survival: the chance of isopods reared on this diet to be alive after six weeks was ~75 %, and after 12 weeks ~25%, compared to 35% and 10 % under high protein diet, respectively (Figure 2C). Moreover, across both diets, we found high growth and pigmentation rates resulted in lower survival, as indicated by the significant interaction in our path analysis (Figure 4). Both factors, dietary protein content and the interaction between developmental rates, had a similar effect on survival (Figure 4), but likely have a different mechanistic basis. Elevated dietary protein content has been shown to reduce survival in many other study systems (Piper et al. 2011; Fontana and Partridge 2015; Le Couteur et al. 2016), which is thought to be caused by costs of protein-digestion (Halton and Hu 2004) and potentially harmful breakdown products (Wright 1995). Moreover, a specific composition of the gut microbial community may be required to digest certain protein structures (Madsen et al. 2017). In our experiment, we do not know if juvenile isopods could acquire the necessary bacteria to facilitate the breakdown of proteins. However, decreased survival under high developmental rates may be the cost of internal resource competition antagonisms: isopods may not be able to develop pigmentation at high rates while maintaining high growth rates, without high resource levels (West-Eberhard 2003) or without suffering a fitness cost (Metcalf and Monaghan 2003; Lee et al. 2013).

Our test for diet specific interactions between both developmental rates (3-way interaction) in a multivariate additive framework (Figure 1, m4) confirmed the results from the separate tests, i.e. the univariate GAMMs and the path analysis: elevated protein content caused lower survival across nearly all families. However, the multivariate method allowed us to draw more nuanced conclusions (Figure 5): under low protein diet, survival was constrained around single peak with small variance at medium growth and pigmentation rate, whereas under high protein diet, growth and pigmentation rates

with high relative survival were more dispersed, with a tendency for clustering around intermediate growth rates. Similar regions of high fitness under a specific life-history trajectory have been observed for *Drosophila* (Piper et al. 2011; Lee et al. 2013), and a range of other organisms (Le Couteur et al. 2016). This suggests that under low protein diets, albeit higher survival, isopods are constrained in which developmental trajectories they can implement, whereas elevated protein intake allows isopods to explore a wider range of developmental trajectories, of which only few have high fitness. This could either be due to the aforementioned consequences of increased protein uptake, or it could be related to physiological stress from accelerated rates of development (McCarthy I. D. et al. 1994; Tarry-Adkins et al. 2009; Lee et al. 2013). It is known that the distribution of essential resources in nature can affect the dispersal of organisms (Bonte and Dahirel 2016) and also drive evolutionary patterns (Badyaev et al. 2019), but less is known about how dependencies on external resources affect developmental patterns. Our study suggests that for isopods the development of pigmentation, but also survival can depend strongly on the availability of proteins during early life stages.

The tryptophan-ommochrome complex

In our experiment, we supplemented tryptophan to both levels of protein manipulation to test whether it would affect the development of pigmentation in isopods. Xanthommatin pigment bio-synthesis and the requirement for tryptophan has been well described from early on (Needham and Brunet 1957; Linzen 1974), so the increased rates of pigmentation under low protein diet with tryptophan supplement were expected. However, although tryptophan is an important amino acid that is known to regulate pigmentation and eye vision in many animals, increased levels can be toxic and have detrimental effects (Linzen 1974; Figon and Casas 2018). Elevated toxicity has also been shown for other free amino acids that are supplemented or the result

of protein digestion (Dussutour A. and Simpson S. J. 2012; Le Couteur et al. 2016; Arganda Sara et al. 2017). Therefore we were surprised to find slightly increased survival of isopods reared under low protein diet with tryptophan supplement (Figure 2, Table 1). This interactive effect of protein content and tryptophan supplement was only significant in the GAMM (m3, Table 1), and not in the path analysis, because of its nonlinear shape (more pronounced difference during week six and nine, Figure 3). To our knowledge, elevated levels of tryptophan have been reported to have only negative effects the survival of developing organisms (Linzen 1974; Figon and Casas 2018). In juvenile *A. aquaticus*, elevated levels of tryptophan may trigger increased rates of pigmentation during development, which may be a way to alleviate toxicity.

In previous work, crypsis, i.e. the matching of body coloration with background, has been thought to be an important ecological basis of pigmentation in *A. aquaticus* (Hargeby et al. 2004; Hargeby et al. 2005). An alternative hypothesis to background matching could be that isopod pigmentation results from ingesting tryptophan rich substrates. Because isopods are detritivores, they often rely on the food sources they can acquire from their local microhabitat (Adey and Loveland 2007). Certain macrophytes contain tryptophan in relatively high levels (Muztar et al. 1978), but the breakdown of proteins containing tryptophan and their digestions may result in toxicity (Linzen 1974; Arganda Sara et al. 2017). 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 an adaptive trait in arthropods (Linzen 1974). This does not exclude the possibility for crypsis of pigmentation, but we need a better understanding of the associated costs of acquiring and using tryptophan to synthesize xanthommatin in natural environments. There could, for example,

be mismatches in microhabitats where the background is dark, but tryptophan is not available in sufficient amounts (Muztar et al. 1978). Regardless of the functional basis of the trait, the availability of tryptophan (and proteins containing tryptophan) can strongly affect the development of isopod phenotypes and likely is a major source of phenotypic variation of *A. aquaticus* in nature.

In summary, our study provides evidence for plasticity in the development of pigmentation and body size in *A. aquaticus* depending on the protein and tryptophan content of the diet provided to juveniles. In nature, variation in the protein content of the substrates that form microhabitats for isopods, i.e. detritus of macrophytes and leaves, epiphytes, or sediment (Muztar et al. 1978; Graca et al. 1993; Adey and Loveland 2007), could lead to the observed variation in pigmentation patterns (Hargeby et al. 2004; Hargeby et al. 2005). The observed lower survival of juveniles under high protein, but increased survival under low protein with tryptophan supplement requires further investigation. This could be addressed by manipulating the presence and absence of amino acids, rather than bulk protein (Arganda Sara et al. 2017). Future efforts should also be directed at the functional basis for the existence of pigmentation in *A. aquaticus*: pigmentation could be the consequence of protein breakdown and tryptophan uptake and serve as an inert deposit for toxicants (Linzen 1974; Needham 1974a), but this does not exclude the possibility for other benefits, e.g. crypsis (Hargeby et al. 2004) or UV protection (Tollrian and Heibl 2004; Emaresi et al. 2014).

Supplement

Figures

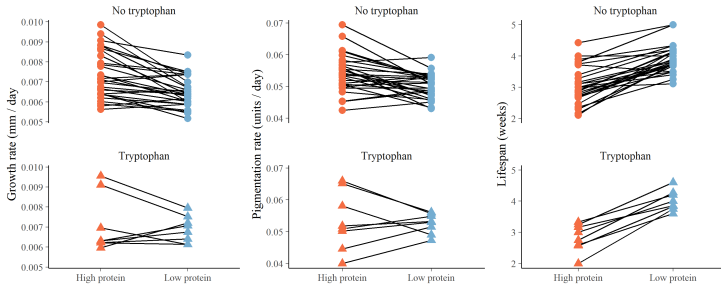


FIGURE S1: Family-specific reaction norms for growth, pigmentation, and survival (lifespan during experiment) for high and low protein diet, as well as with and without tryptophan supplement.

Tables

Model	Response variable	Fixed effect	Estimate	Estimate error	Lower 95% CI	Upper 95% CI
Path analysis 1	Male pigmentation	Intercept	0.179	0.062	0.062	0.3
	Female length	Intercept	0.056	0.056	-0.055	0.165
	Female pigmentation	Intercept	0.087	0.053	-0.017	0.189
	Clutch size	Intercept	0.149	0.061	0.029	0.269
	Juvenile length	Intercept	0.134	0.066	0.009	0.266
	Juvenile pigmentation	Intercept	0.197	0.053	0.093	0.3
	Male pigmentation	Male length	0.225	0.109	0.007	0.439
	Female length	Male length	0.521	0.097	0.33	0.712
	Female length	Male pigmentation	0.027	0.111	-0.193	0.247
	Female pigmentation	Female length	0.372	0.125	0.125	0.616
	Female pigmentation	Male pigmentation	0.353	0.102	0.155	0.551
	Female pigmentation	Male length	-0.125	0.109	-0.34	0.09
	Clutch size	Male length	0.11	0.122	-0.13	0.346
	Clutch size	Female length	0.456	0.149	0.156	0.748
	Clutch size	Female pigmentation	-0.05	0.14	-0.318	0.22
	Juvenile length	Clutch size	-0.46	0.15	-0.759	-0.166
	Juvenile length	Female length	0.122	0.173	-0.217	0.472
	Juvenile length	Male length	-0.204	0.143	-0.487	0.064
	Juvenile pigmentation	Juvenile length	0.334	0.096	0.145	0.519
	Juvenile pigmentation	Clutch size	-0.052	0.112	-0.276	0.175
	Juvenile pigmentation	Female pigmentation	-0.244	0.12	-0.475	-0.013
	Juvenile pigmentation	Male pigmentation	-0.452	0.102	-0.651	-0.252
	Juvenile pigmentation	Female length	0.001	0.127	-0.25	0.254
Juvenile pigmentation	Male length	-0.151	0.099	-0.349	0.046	
Path analysis 2	Pigmentation rate	Intercept	0.129	0.026	0.078	0.178
	Growth rate	Intercept	0.07	0.027	0.015	0.122
	Lifespan	Intercept	-0.142	0.024	-0.19	-0.095
	Pigmentation rate	FoodLowprotein	-0.267	0.037	-0.341	-0.198
	Pigmentation rate	TryptophanTryptophan	-0.017	0.08	-0.174	0.139
	Pigmentation rate	Juvenile pigmentation	-0.331	0.039	-0.406	-0.256
	Pigmentation rate	Juvenile length	0.159	0.039	0.084	0.234
	Pigmentation rate	FoodLowprotein:TryptophanTryptophan	0.125	0.106	0.02	0.337
	Growth rate	FoodLowprotein	-0.153	0.038	-0.225	-0.078
	Growth rate	TryptophanTryptophan	0.018	0.083	-0.147	0.178
	Growth rate	Juvenile pigmentation	-0.055	0.042	-0.137	0.025
	Growth rate	Juvenile length	-0.032	0.042	-0.115	0.051
	Growth rate	FoodLowprotein:TryptophanTryptophan	0.073	0.11	-0.145	0.291
	Lifespan	FoodLowprotein	0.38	0.034	0.313	0.446
	Lifespan	TryptophanTryptophan	-0.038	0.069	-0.173	0.096
	Lifespan	Growth rate	-0.289	0.038	-0.363	-0.218
	Lifespan	Pigmentation rate	0.257	0.04	0.177	0.335
Lifespan	Juvenile pigmentation	0.022	0.036	-0.05	0.093	
Lifespan	Juvenile length	-0.021	0.037	-0.094	0.051	
Lifespan	FoodLowprotein:TryptophanTryptophan	0.106	0.095	-0.077	0.293	
Lifespan	Growth rate:PigmentationXrate	-0.409	0.043	-0.494	-0.322	

TABLE S1: Statistical results of path analyses (Bayesian multilevel modeling) on mating and early life history of isopods (Figure 2, Path analysis 1), and the effect of diet on the development of body size and pigmentation of isopods (Figure 4, Path analysis 2). Directionality of effect size is given by sign, effects where the 95% credible interval does not overlap 0 are indicated in bold.

Chapter 4

Non-additive species interactions govern the response of aquatic ecosystems to nutrient perturbation

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Abstract

There is increasing interest in how interactions between species can influence the response of ecosystems to perturbation. Nutrient perturbations are a threat to the resilience of aquatic ecosystems worldwide and there is growing evidence for both gradual and sudden shifts in ecosystem dynamics. Multiple species, including macrophytes, benthic and pelagic grazers, and phytoplankton, are thought to mediate ecosystem responses to eutrophication, partly because of positive and negative interactions among them. However, almost nothing is known about singular and synergistic key species effects on lake ecosystems in the face of eutrophication. Here, we monitored artificial freshwater ponds in high resolution to experimentally test how a disturbance scenario characterized by multiple nutrient pulses affects pond ecosystem dynamics with the presence and absence of two important foundation species, namely the macrophyte *Myriophyllum spicatum* and the mussel *Dreissena polymorpha*. During the 20 month long experiment we found that the presence of foundation species strongly affected the response of multiple ecosystems properties to nutrient perturbation. When alone, macrophytes and mussels initially moderated the expected increase in phytoplankton abundances following nutrient additions. However, when both species were present, we observed the opposite effect, characterized by a dramatic increase in phytoplankton densities, which suggest a non-additive antagonistic interaction between macrophytes and mussels under elevated nutrient loading. Increases in phytoplankton abundances also coincided with correlated changes in several ecosystem parameters, including elevated concentrations of dissolved oxygen and ecosystem metabolic rates. Furthermore, the presence macrophytes was associated with high amounts of dissolved organic matter in the water column. Overall, our results demonstrate how interactions between key species can drastically change under

disturbance regimes, which, in the case of shallow lakes, challenges the notion of static species interaction networks.

Introduction

How organisms take up resources, grow, reproduce, or interact with competitors, pathogens, or consumers can be strongly affected by the presence of other species in an ecosystem (Stachowicz 2001; Olff et al. 2009; Kéfi et al. 2012). Interactions among species can also affect the functioning of ecosystems by regulating fluxes of energy and matter, ecosystem productivity and metabolism, or by mediating the response of ecosystems to perturbation (Loreau et al. 2001; Harmon et al. 2009; Chapin et al. 2011). Some species interactions are more important than others for ecosystem functioning (Angelini et al. 2011; Falkenberg et al. 2012), such that disturbing these interactions can have disproportionate impacts on ecosystems. In some instances, a disturbance to one species can have cascading effects on multiple ecosystem components (Ellison et al. 2005; Darling and Côté 2008), while in others, disturbance can change the occurrence and strength of species interactions causing non-additive, and surprising effects on ecosystems (Paine et al. 1998). Such complexity regarding the interplay between species interactions and environmental change make forecasting ecosystem responses to increasing anthropogenic disturbances particularly challenging (Petchey et al. 2015). In particular, it generates considerable uncertainty about how resilient ecosystems are to a given disturbance regime.

Eutrophication is a threat to the resilience of aquatic ecosystems worldwide (Smith et al. 1999; Smith 2003), and there is growing evidence for nutrient loading causing both gradual and sudden shifts in ecosystem dynamics, depending on the nature and strength of species interactions (Carpenter 2005). Multiple species, including macrophytes, benthic and pelagic grazers, and phytoplankton, are

thought to mediate ecosystem responses to nutrient perturbations, partly because of positive and negative interactions among them (Scheffer et al. 1993; Kéfi et al. 2016). In the network of species interactions in shallow lakes, for example, a key interaction is the competition between macrophytes with phytoplankton communities for dissolved nutrients and light (Scheffer et al. 1993; Ibelings et al. 2007). Assemblages of macrophytes are competitively dominant at low nutrient loading, and can persist at intermediate nutrient loading via a positive feedback between macrophyte growth and water transparency (Carpenter and Lodge 1986; Jeppesen et al. 1998). To keep phytoplankton abundances low, macrophytes can also produce allelopathic compounds, which can inhibit growth of phytoplankton populations. *Myriophyllum spicatum*, for example, has been shown to produce polyphenols and fatty acids that reduce growth of *Microcystis*, a cyanobacterium (Korner and Nicklisch 2002; Hilt and Gross 2008). In addition to these mechanisms macrophytes reduce overall particle re-suspension and nutrient recycling from sediments, which reduces the growth of phytoplankton and keeps turbidity low (Carpenter and Lodge 1986; Jeppesen et al. 1998). However, at high nutrient loading, increased phytoplankton growth decreases light availability for macrophytes, and can cause an ecosystem shift from a clear (macrophyte dominated) to a turbid (algae dominated) water state (Scheffer et al. 1993; Kéfi et al. 2016).

In shallow lake ecosystems, macrophytes, phytoplankton, grazers and other members of the community are strongly interacting with each other through various ecosystem compartments like dissolved nutrients, dissolved organic matter (DOM) or dissolved oxygen (O₂) (Scheffer et al. 1993; Kéfi et al. 2016; Olff et al. 2009). Therefore, external disturbances can reverberate through the network of biological interactions and simultaneously affect multiple ecosystem parameters. This can reflect both changes in mean and variance structures of the respective parameters, which can sometimes even foreshadow

a sudden or gradual change in ecosystem state (Carpenter et al. 2011; Scheffer et al. 2012; Gsell et al. 2016). To detect the dimensionality and speed of such changes, i.e. which ecosystem parameters change and how fast, automated sensor technology can be applied to monitor various biotic and abiotic ecosystem parameters. More recent studies have demonstrated how high resolution measurements can be used to detect changes in mean and variance patterns of various ecosystem metrics, for example, phytoplankton biomass and ecosystem metabolism (Carpenter et al. 2011; Batt et al. 2013; Nielsen et al. 2013). Metabolism is a fundamental ecosystem process largely driven by the benthic (i.e. macrophytes) and pelagic (i.e. phytoplankton) members of the autotrophic lake community, but can also be affected by DOM dynamics associated with the growth and decay of biomass (Catalán et al. 2014). Metabolic rates can be modeled with relatively high precision using repeated measurements of dissolved oxygen and water temperature (Staehr et al. 2010), which can be used to assess ecosystem resistance and resilience (Batt et al. 2013). However, still only little is known about how foundation species mediate effects of disturbances on mean and variance of ecosystem parameters like chlorophyll, DOM and metabolism, because factorial manipulations require a substantial array of high resolution sensors.

Here, we monitored freshwater ponds in high resolution to experimentally test how a disturbance scenario characterized by multiple nutrient pulses over two years affects pond ecosystem dynamics with the presence and absence of two important foundation species, namely macrophytes and mussels [Figure 1](#). We chose to manipulate macrophytes because of their aforementioned effects on transitions between alternative stable states in pond ecosystems. We chose to manipulate benthic grazers, specifically a Dreissenid mussel, because they can have significant impacts on aquatic ecosystems due to their high per capita filtrations rates and their greater persistence than planktonic grazers during periods of resource scarcity (Karatayev et

al. 2014b; Karatayev et al. 2014a; Strayer et al. 2019). The presence of Dreissenidae, for example, has been observed to coincide with dramatic changes in water clarity of lake ecosystems (Ibelings et al. 2007). *Dreissena* mussels can directly consume large amounts of phytoplankton, (Johengen et al. 1995; James et al. 1997), and have strong positive effects on nutrient cycling, re-mineralizing nutrients for phytoplankton and periphyton growth. Such effects can change the balance of pelagic and benthic production, and the biomass and composition of fish communities (Ibelings et al. 2007). Nutrient recycling, in combination with the ability of Dreissenidae to filter selectively, has also been associated with shifts in phytoplankton communities in eutrophic lake ecosystems: certain species of phytoplankton, e.g. *Cryptomonas*, have been found to be more palatable to *Dreissena* mussels than others, e.g. *Microcystis* and other cyanobacteria, which are then deposited as viable colonies in the form of pseudo-feces (Vanderploeg et al. 2001; Fishman et al. 2010; Bierman et al. 2005).

Only few studies have attempted to disentangle singular and synergistic effects of key species on ecosystem dynamics in response to changing environmental conditions (Stachowicz 2001; Angelini et al. 2011; Falkenberg et al. 2012). In shallow lake ecosystems, the presence of either macrophytes and *Dreissena* mussels have been linked to increased capacity to maintain a clear water state with low phytoplankton abundances (Jeppesen et al. 1998; Bierman et al. 2005; Ibelings et al. 2007). Current theory suggests that both species may facilitate the presence of each other (Figure 2): on one hand, some macrophytes, e.g. Characeae, provide habitat for *Dreissena* mussels, which require solid substrate to settle on (Ibelings et al. 2007; Karatayev et al. 2014b). On the other hand, by filtering particles, which decreases local turbidity, and the accumulation of dissolved nutrients, *Dreissena* mussels may improve environmental conditions for submerged macrophytes. Facilitation is common phenomenon in ecological communities (Stachowicz 2001; Angelini et al. 2011; Falkenberg et al. 2012), especially

for foundation species like macrophytes and mussels are considered to be. However, there is also potential for antagonistic interactions between macrophytes and *Dreissena* mussels that could unfold under nutrient perturbation scenarios. For example, the allelochemicals that macrophytes are known to produce under turbidity stress could be harmful to filter feeding organisms, which may reduce their filtration, growth or dispersal rates (Figure 1E, dashed arrow a). This may increase algal abundances and ultimately lead to levels of turbidity that are critical to the existence of submerged macrophytes. Mussels on the other hand may shift the composition of phytoplankton communities to species that are less affected by allelochemicals (Figure 1E, dashed arrow b), with negative consequences for submerged macrophytes.

When facing disturbance, species interactions can cause non-additive effects on ecosystem dynamics that are difficult to anticipate, and that may impair our ability to quantify resistance and resilience of ecosystems with a particular species configuration. In this context, little is known about how interactions between key species are affecting the functioning of ecosystems for a given disturbance regime. High resolution measurements of related ecosystem parameters like chlorophyll (phytoplankton abundance), DOM and metabolism could provide insight in how the presence and absence of foundation species affect resistance and resilience of shallow lake ecosystems to nutrient perturbation.

Here we performed experimental manipulations of presence and absence of the macrophyte *Myriophyllum spicatum* and the mussel *Dreissena polymorpha*, two important foundation species that are common in freshwater ecosystems worldwide. In factorial pond experiment we perturbed all ecosystems by progressively increasing the input of inorganic nutrients, and quantified the dynamics of several biotic and abiotic ecosystem parameters. The goal was to investigate how the presence and absence of two important foundation species affects the dynamics of a suite of ecosystem parameters during the

process of pond eutrophication (our disturbance regime). Specifically, we aimed at characterizing how the nature of interactions between the two species (additive vs. non-additive, synergistic vs. antagonistic)

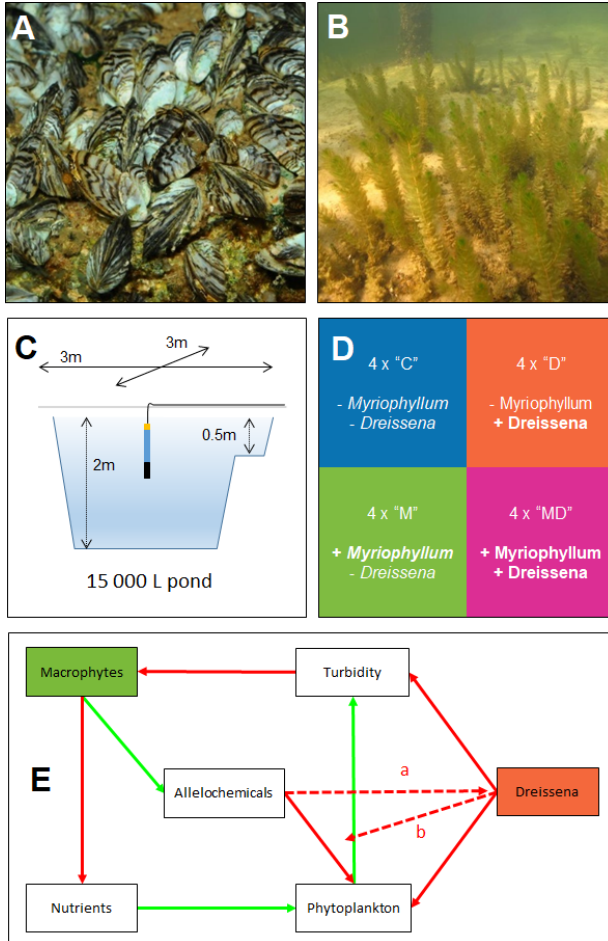


FIGURE 1: Factorial design and hypothesized interaction network of *Myriophyllum* macrophytes and *Dreissena* mussels. (caption continues on next page).

FIGURE 1: (continued) A) *Dreissena polymorpha*, “Zebra mussel” (Photo credit: N. Sloth). B) *Myriophyllum spicatum*, “Eurasian water millfoil” (Photo credit: P. Dynowski). C) Schematic of the experimental ponds: the ponds are approx 3 m in diameter and have a deep (2m) and a shallow end (0.5m), where we planted macrophytes and mussels. In the middle of each pond we placed a multiparameter sonde at 1 m depth to monitor ecosystems dynamics. D) factorial design (4 replicates per treatment combination) C=Control (no keystone species), D = *Dreissena* (mussels), M = *Myriophyllum* (macrophytes), MD= *Myriophyllum* + *Dreissena* (both keystone species present). E) Hypothesized interaction network of *Myriophyllum* macrophytes and *Dreissena* mussels thought to control phytoplankton biomass in the pond ecosystems. *Myriophyllum* can reduce phytoplankton abundances via competition for nutrients and by producing allelopathic substances that can inhibit phytoplankton growth, thus increasing water clarity and producing a positive feedback for itself. *Dreissena* mussels filter out macrophytes and other reduced particles, which reduces turbidity.

was affected by nutrient perturbation. Our results demonstrate how interactions between key species can drastically change under disturbance regimes, which, in the case of shallow lakes, challenges the notion of static, species interaction networks.

Materials and methods

Study design

In a 20 month long experiment, we manipulated the presence and absence of two keystone species: macrophytes (*Myriophyllum spicatum* [Figure 1A]; hereafter *Myriophyllum*) and a benthic grazer (*Dreissena*

polymorpha [Figure 1B]; hereafter *Dreissena*) in artificial pond ecosystems (15 000l). We used a fully factorial design with either both keystone species absent as control (C), *Myriophyllum* macrophytes (M), *Dreissena* mussels (D) or *Myriophyllum* and *Dreissena* together (MD). The ponds we used were made of fiberglass with a smooth surface (Figure 1C), had a rounded shape with approximately three meters diameter and a shallow (.5 m) and a deep (2 m) end. We perturbed all ponds by progressively increasing the input of inorganic nutrients, and measured the effect of presence or absence of both keystone species on several ecosystem parameters (Figure 1) in high frequency using automated multiparameter sondes. One year after the first nutrient addition, we perturbed all ponds again with a high nutrient loading, to test whether resistance and resilience patterns have changed between the disturbances. Each factorial treatment combination of eutrophied ponds was replicated four times ($4 \times 4 = 16$ ponds total).

Experimental procedure

The ponds were initially set up on May 6th 2016 by adding a 5 cm thick layer of gravel (2-4 mm) and filling them with water. The treatments were established on May 31st by distributing 40 shoots of *Myriophyllum*, each attached with a cable-tie to a small rock, among the shallow and deep ends of each pond designated to the M and MD treatment (Figure 1D). Each pond that was designated to the D and MD treatment received *Dreissena* specimen, distributed among the shallow and deep end. We ensured prior to the distribution of plant shoots and mussels that their size distributions were similar across all ponds of the respective treatment. To ensure that all ponds started with a similar overall amount of nutrients in the form of biomass, we added autoclaved mussels to the M, autoclaved macrophyte shoots to the D, and both autoclaved mussels and macrophyte shoots to the C ponds. In

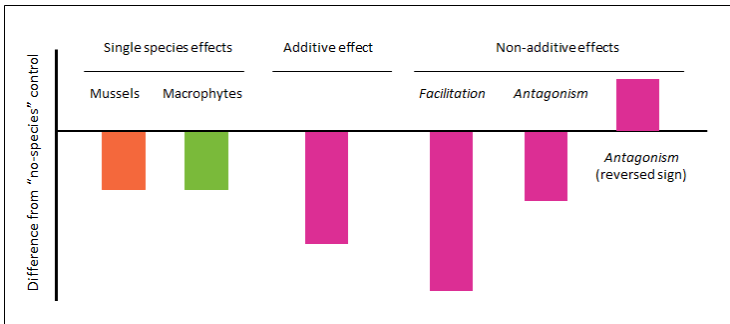


FIGURE 2: Potential types of interactions between mussels and macrophytes that may affect phytoplankton reduction in the ponds relative to control (no key species present): the presence of both species may result in an additive effect, which is the sum of the single species effect sizes, or a non-additive facilitative effect where the presence of either species increases the effect of the other. Non-additive antagonistic scenarios are also possible, where the presence of one species decreases the effect of the other, and vice versa.

May 2017 we added a similar amount of fresh and autoclaved *Myriophyllum* shoots to the respective ponds to ensure effective treatment contrasts.

After assembling the treatments and allowing the ponds to equilibrate, we started to load the ponds with nutrients on August 12th 2016. We progressively increased nutrient additions from 10 to 50 ug/L of P (with a double redfield ratio) over eight weeks until October 10th 2016, with two week intervals between additions. We perturbed all ponds a final time with a single addition of 50 ug/L of P on October 10th 2017. Using multiparameter sondes (EXO2, Xylem), installed in each eutrophied pond, we tracked high frequency changes (15 min intervals) in algal biomass and several other ecosystem metrics. Over the first winter period (Dec 1st 2016 - March 23rd 2017), we

did not monitor ecosystem dynamics due to ice cover in the ponds, followed by a sonde maintenance period. A second sonde maintenance period was implemented in the fall of 2017 (September 14th - October 3rd 2017). For simplicity, we consider three phases of the experiment: Phase 1 with the first five nutrient pulses (June - December 2016), Phase 2 without nutrient pulses (March 2017 - October 2017), and Phase 3 with the final nutrient pulse (October 2017 - February 2018).

Statistical analysis

Data treatment

Prior to all data analysis we first checked the data for obvious sensor anomalies and removed them (less than 1% of the data). We then performed an outlier analysis by excluding values higher than 3 times the median absolute deviation of all values in a sliding window of one day window size (15 min interval = 96 data points [Leys et al. 2013]). Using the purified data, we derived mean and coefficient of variation (hereafter CV) from all ecosystem parameters (Chlorophyll, Phycocyanin, dissolved organic matter [hereafter DOM], dissolved oxygen [hereafter O₂]). We did so in a two step process: we first aggregated the data from four to one data points per hour to improve data quality, and applied sliding windows with a window size of one week (7 x 24 = 168 data points) in a second step.

Effects of foundation species on ecosystem parameters

Using the data derived from the sliding windows, we tested for differences between treatments with single species (M and D - main effect) and multiple species (MD - interactive effect) and control (C). We used one linear model per hour (4 data points per treatment, 24 models per day) in which we tested for differences in mean, CV and AC of each

measured parameter. We report the results from all linear models directly in [Figure 3](#) and [Figure 4](#), where points that are colour coded by treatment indicate a significant difference of the respective treatment from the control. We calculated log response ratios (hereafter LRR) of all measured parameters by dividing each data point from the sliding window data set of the mean for M, D, or MD by the control, and then calculating the natural logarithm for these ratios. In addition, we calculated the predicted purely additive response of *Myriophyllum* and *Dreissena* as the sum of effect sizes for M and D. The interaction between the presence of *Myriophyllum* and *Dreissena* was considered non-additive, when the interaction term of the linear model from the sliding windows was significant (colour coded in [Figure 7](#)).

Ecosystem metabolism

We calculated gross primary productivity, net primary productivity, and respiration (hereafter GPP, NEP and R, respectively) of each pond ecosystem using the formulas in [Staehr et al. 2010](#) on time series of DO and Temperature collected by the sondes, as well as wind speed at 10 m from a nearby weather station operated by Meteo Swiss (Dübendorf, Giessen). Because the ponds were over saturated with respect to O_2 , we included rates of change in O_2 in the formulas as the coefficient of a linear model of hourly averages of O_2 concentrations between 13:00 and 17:00 for the day and 01:00 ad 05:00 for the night, where gas exchange dynamics in the ponds were considered to have equilibrated. Using the metabolism data we calculated mean, CV and AC of all three metabolism parameters by applying a sliding window with the size of 7 days. We then tested for differences between treatments with single species (M and D - main effect) and multiple species (MD - interactive effect) and control (C) using one linear model per day. We report the results from the linear models directly in [Figure 5](#) and [Figure 6](#) as colour coded points that indicate significant difference in metabolic rates of M, D or MD from C.

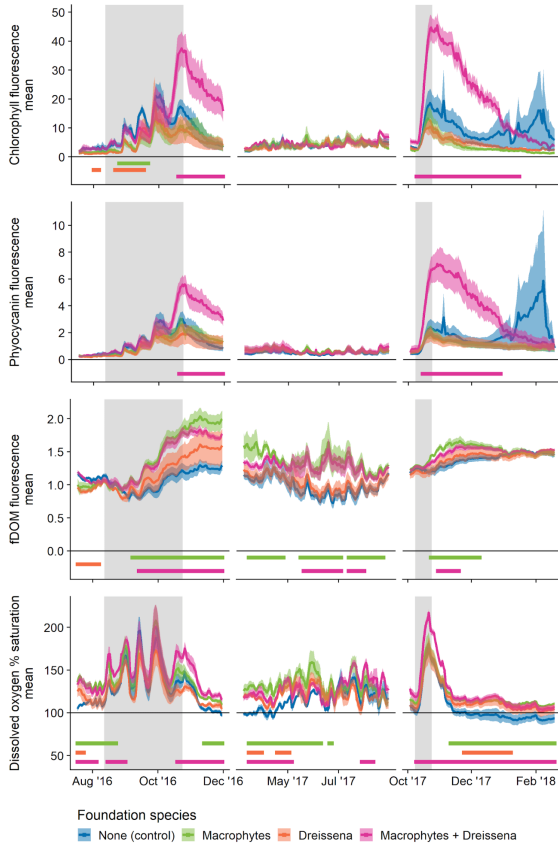


FIGURE 3: Effect of foundation species on mean of ecosystem parameters. The solid line indicates the average of all four ponds per treatment per hour (mean \pm SE). The gray shading indicates the disturbance phases, the coloured bars underneath the time series indicate whether a treatment was significantly different from the control (one linear model per hour).

Results

Effects of foundation species on mean ecosystem parameters

The presence and absence of *Myriophyllum* macrophytes and *Dreissena* mussels affected a wide range of ecosystem parameters. During the first nutrient addition, ponds with both *Myriophyllum* or *Dreissena* alone had lower chlorophyll fluorescence, i.e. lower algal biomass than ponds with neither species, consistent with their anticipated negative effects on the phytoplankton community (Figure 1, Figure 3). However, following both disturbance periods, the co-occurrence of these species had strong non-additive antagonistic effects on algae abundance, illustrated by their positive effects on mean chlorophyll and phycocyanin fluorescence (Figure 3, Figure 7). Furthermore, after the first disturbance period, and throughout the remainder of the experiment, the presence of *Myriophyllum* increased the concentration of DOM in the ecosystems, independent of *Dreissena* presence (i.e. in M and MD treatments, Figure 3). The presence of *Myriophyllum* and *Dreissena*, either alone or in combination, positively affected O₂ saturation throughout most of the experiment, however, not during most of the perturbation periods: each nutrient addition dramatically increased O₂ saturation to levels (between 150 and 200 %) that were not significantly different across all species contrasts.

Effects of foundation species on variance of ecosystem parameters

We found only small weak effects of *Myriophyllum* and *Dreissena* presence on variance patterns (CV [Figure 4]). Overall, we found strong increases in CV across all treatment combinations and ecosystem parameters immediately after the nutrient additions, which reflects the sudden changes in the mean to the disturbances. Prior to the first nutrient

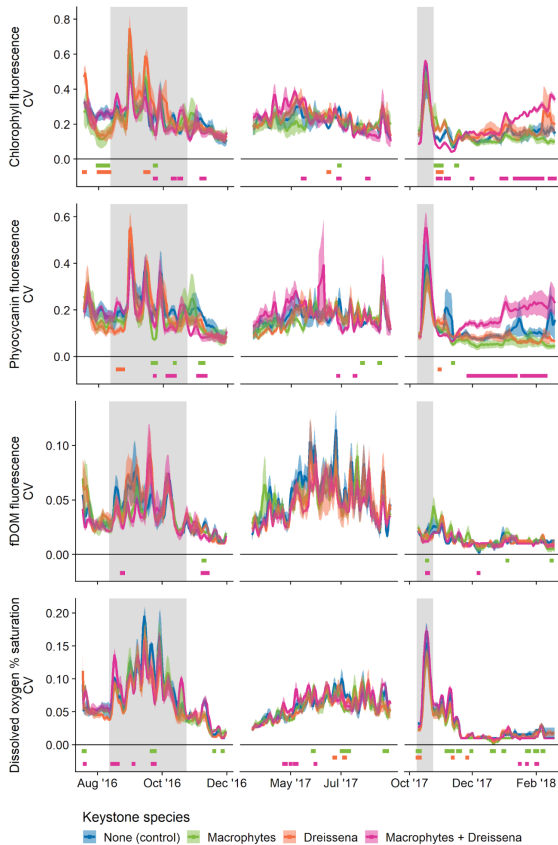


FIGURE 4: Effect of foundation species on variance (CV) of ecosystem parameters. The solid line indicates the average of all four ponds per treatment. The gray shading indicates the disturbance phases, the colored bars underneath the time series indicate whether a treatment was significantly different from the control (one linear model per hour).

additions, the pond ecosystems with either *Myriophyllum* or *Dreissena* alone were less variable in chlorophyll fluorescence. After the second nutrient pulse, ecosystem where both species were present variance of chlorophyll and phycocyanin fluorescence were significantly higher than when species were alone, or absent. There were almost no effects of foundation species presence or absence on variance of DOM fluorescence. There were indications of *Myriophyllum* presence affecting CV of O₂ saturation, however, only to weak effect and with variable sign.

Ecosystem metabolism

Gross and net ecosystem primary productivity, as well as ecosystem respiration was strongly affected by nutrient perturbation and seasonal dynamics, but less so by the presence or absence of foundation species (Figure 5). Each nutrient addition lead to correlated increases of GPP, NEP and Respiration, which reverted within days after the maximum was reached. During each of these peaks, there were only little differences across all species contrasts and all metabolism metrics. During spring 2017, at the beginning of the second phase, all pond ecosystems containing *Myriophyllum* or *Dreissena* had lower NEP and higher R than ecosystems devoid of foundation species. We found a similar pattern towards the end of the experiment, after the second nutrient addition in phase three, where both GPP and NEP were lower and R higher when foundation species were present. Overall, there were only weak effects on variance patterns of ecosystem metabolism Figure 6: there was a tendency for MD ponds to have higher CV of GPP and NEP than ponds without any foundation species, especially around the second perturbation phase. Interestingly, nutrient perturbation led to increasing CV of GPP and NEP, but not R, which had a relatively static CV of approx 0.8 throughout the entire experiment.

Discussion

Perturbation of the pond ecosystems with nutrients evoked strong responses in all ponds, which were dependent on the presence of foundation species and, in some cases, their interactions. As expected, both nutrient pulses lead to strong increases in phytoplankton abundances across all species contrasts, which, at first, was mediated by the presence of either macrophytes or mussels in the single species treatment. However, when both *Myriophyllum* and *Dreissena* were present within a pond, nutrient additions lead to a contrasting pattern: phytoplankton biomass in these ponds increased stronger than in the presence of a single species or when none of the two species were present. These patterns suggest strong non-additive interactions between macrophytes and mussels that affected phytoplankton biomass during and following the disturbance periods.

Mediation of phytoplankton blooms under increased nutrient loading by either macrophytes or mussels alone was expected, and is in agreement with a large body of previous theoretical and empirical work (Nes et al. 2007; Iacarella et al. 2018; Yamamichi et al. 2018). Macrophytes can keep phytoplankton biomass in the water column at lower levels compared to ecosystems that lack macrophytes. Such control of phytoplankton biomass by macrophytes is often linked to their competitive relationship with phytoplankton for nutrients and light (Scheffer et al. 1993) or the production of allelopathic substances that can inhibit phytoplankton growth (Korner and Nicklisch 2002; Hilt and Gross 2008), especially of some cyanobacteria (Nakai et al. 2001; Nakai et al. 2012). However, these mechanisms are only effective below the “critical turbidity” threshold (Scheffer et al. 1993), above which light limitation prohibits macrophytes growth and can lead to macrophyte die off, which marks the transition to a turbid water state (Scheffer et al. 1993; Nes et al. 2007; Kéfi et al. 2016; Yamamichi et al. 2018). In our experiment, macrophytes died out

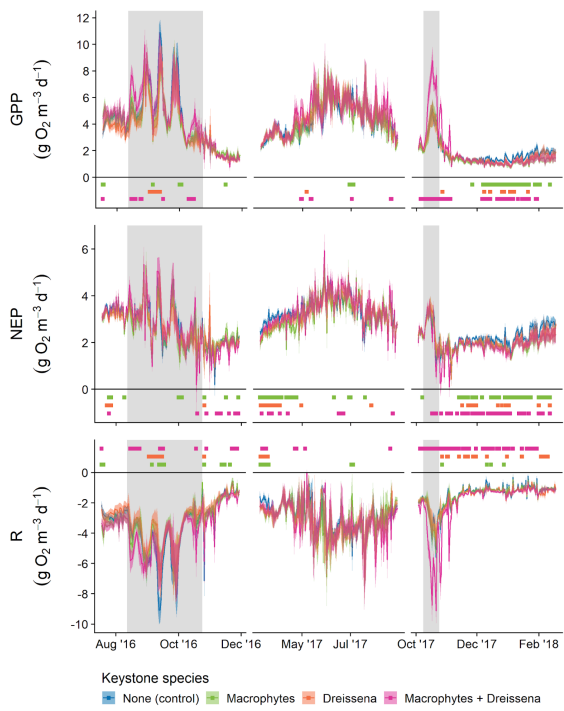


FIGURE 5: Effect of foundation species on mean of ecosystem metabolism. The solid line indicates the average of all four ponds per treatment per hour (mean \pm SE). The gray shading indicates the disturbance phases, the coloured bars underneath the time series indicate whether a treatment was significantly different from the control (one linear model per hour). All rates were calculated using Odum's diel oxygen technique (Staeher et al. 2010).

and did not re-establish after the final pulse of the first nutrient addition (October 10th 2016) until the following spring, which we confirmed by visual inspection of all ponds in March 2017. Therefore

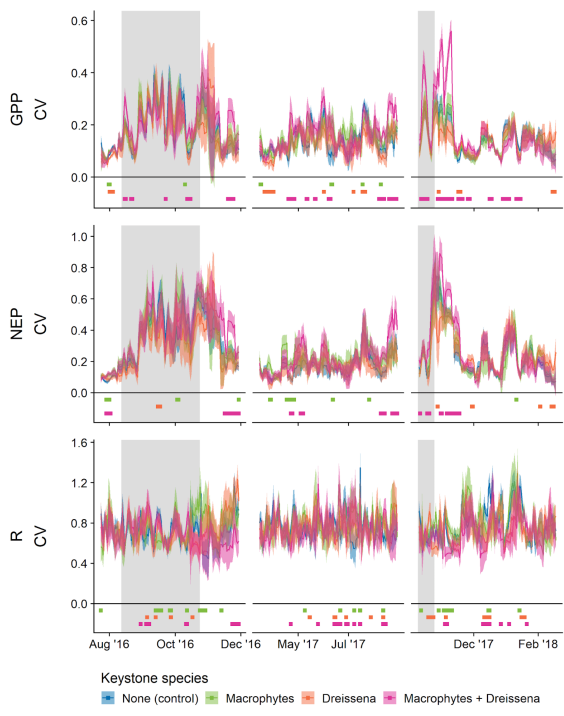


FIGURE 6: Effect of foundation species on variance (CV) of ecosystem metabolism. The solid line indicates the average of all four ponds per treatment per hour (mean \pm SE). The gray shading indicates the disturbance phases, the coloured bars underneath the time series indicate whether a treatment was significantly different from the control (one linear model per hour).

the observed differences between treatments with and without *Myriophyllum* macrophytes can only be explained by the legacy of their prior impact. Macrophytes also affected the dynamics of fDOM: in both M and MD treatments, fDOM more rapidly and to higher levels than in ponds without *Myriophyllum* (treatments C and D). This was

expected, as macrophytes are known to be a producer of a wide range of organic substances, including allelopathic chemicals (Reitsema et al. 2018; Catalán et al. 2014).

The presence of *Dreissena* mussels alone lead to the expected mediation of phytoplankton biomass, relative to the control without foundation species during parts of the first, and, by tendency, also throughout the second nutrient addition. Filter feeding organisms like *Dreissena* mussels can remove large quantities of algae and suspended materials from the water column, which can help stabilizing aquatic ecosystems in a clear water state, even when the nutrient input is high (Gulati et al. 2008; McLaughlan and Aldridge 2013). In this context, *Dreissena* mussels have higher persistence than macrophytes, because they are not limited by increasing turbidity like macrophytes. Indeed, *Dreissena* mussels, in contrast to *Myriophyllum*, survived the disturbance periods and the winter 2016/2017, such that their population wide grazing rate may have increased over time. It has been shown that population growth of mussels can be very high in eutrophied lakes (Karatayev et al. 2014a; Strayer et al. 2019), if sufficient amounts of hard substrate are available (Ibelings et al. 2007; Fishman et al. 2010). Then Dreissenidae can not only affect water clarity, and also nutrient cycling but also directly lead to shifts in the composition of the phytoplankton community towards a higher proportion, in some cases dominance, of cyanobacteria like *Microcystis* (Vanderploeg et al. 2001; Fishman et al. 2010; Bierman et al. 2005). *Dreissena* mussels can selectively reject particles as pseudo-feces that bypass the digestive tract, thus releasing less palatable particles like cyanobacteria back to the environment (Vanderploeg et al. 2001). If this loosely consolidated substrate contains viable cyanobacteria they are then re-suspended to the water column, while other phytoplankton species are absorbed by the mussel.

A shift in the phytoplankton community may also have been the driver behind the observed divergence in overall phytoplankton

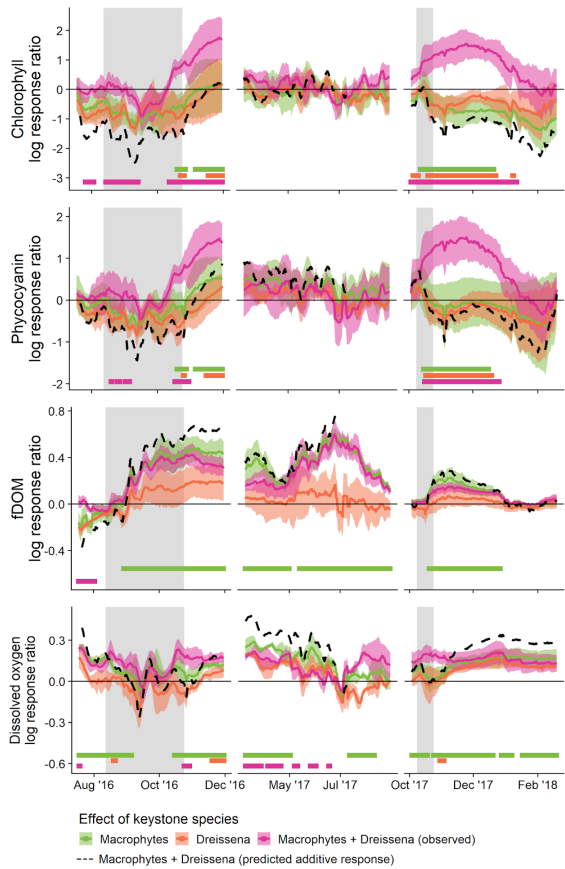


FIGURE 7: (for caption see next page)

FIGURE 7: (*continued*) Non-additivity of species interactions (refer to [Figure 2](#) for details on terminology). The solid line indicates the average effect size of all four ponds per treatment per hour (mean \pm SE). The gray shading indicates the disturbance phases, the coloured bars underneath the time series indicate whether a single-species effect was significant (for macrophytes and mussels), or whether it was non-additive (for macrophytes + mussels). The dashed line indicates the predicted additive response based on macrophytes or mussels alone.

biomass between MD and all other treatments, which was characterized by a dramatic increase in both phytoplankton pigments when both *Myriophyllum* and *Dreissena* were present. Contrary to what previous work suggests, the presence of *Myriophyllum* or *Dreissena* alone did not significantly affect the phytoplankton community. However, a dramatic shift towards cyanobacteria occurred when both macrophytes and mussels were present: a sister project to our study determined phytoplankton community composition from pond water samples taken at regular intervals, and found that increases in the small cyanobacterium *Synechococcus* sp. lead to a phytoplankton community dominated by cyanobacteria when both *Myriophyllum* and *Dreissena* were present in a pond (Narwani et al. 2019). Similar dynamics were reproduced in an additional experiment, where (Narwani et al. 2019) tested how the presence of allelochemicals (“*Myriophyllum*-tea”) or *Dreissena*, alone and in combination affected the relative concentration of two species of microalgae that were most dominant in the pond ecosystems (*Lagerheimia* sp. and *Synechococcus* sp.). Similar to the dynamics observed in the pond experiment, *Synechococcus* sp. increased in abundance relative to *Lagerheimia* sp. when both *Dreissena* and allelochemicals were present. This suggests that a relative growth rate advantage in the presence of both foundation

species, while other taxa in the community experienced stronger negative effects, may have contributed to the shift of phytoplankton communities toward cyanobacteria, resulting in an overall increase in phytoplankton biomass.

We found strong effects of nutrient disturbances on the dynamics of whole ecosystem metabolism: following the nutrient additions, GPP, NEP and R increased dramatically, despite an overall negative trend during the first phase that was driven by seasonal dynamics, i.e. decreasing ambient temperature and light. Seasonal dynamics appear to also have been the main driver of metabolic dynamics during the second phase, which was characterized by an increase of all rates until the middle of June, followed by a decrease until the final nutrient addition at the beginning of phase 3 in October. During phase 1 and 2 there were only sporadic signs of effects of foundation species on metabolic rates, which, except increased NEP and R after the winter at the beginning of phase 2, did not follow a consistent pattern. The lack of a clear effect of macrophytes or mussels on metabolic parameters during these periods may be explained by potentially similar biomass relationships between foundation species and phytoplankton. Previous work has shown that chlorophyll A can be an important determinant of metabolism in lakes that can be stronger than the signal coming from the presence of other species in the ecosystem (Coloso et al. 2011; Honti et al. 2016; Istvánovics and Honti 2018). In our experiment, the coincidence of high algae abundances and elevated metabolic rates indicates that phytoplankton, if at bloom, may superimpose the signal of metabolic rates of foundation species. However, following the final nutrient addition, all ecosystems containing foundation species showed consistently lower GPP and NEP, but higher R. This could be explained two patterns occurring in parallel: on the one hand, chlorophyll A concentration in the control ponds without foundation species continued to increase throughout the winter 2017/2018, whereas DOM fluorescence was decreasing in all ponds, except the

control ecosystems. Thus, higher productivity from phytoplankton and higher respiration from DOM breakdown may be responsible for the observed divergence in metabolic patterns towards the end of the experiment.

Multiple lines of evidence suggest that non-additive interactions between *Myriophyllum* and *Dreissena* strongly affected ecosystem dynamics in ponds experiencing progressive nutrient perturbations [Figure 7](#). This was especially visible in the phytoplankton communities: the presence of both macrophytes and *Dreissena* lead to a higher algae biomass relative to control, instead of a decrease when only one species was present in the ponds. This demonstrates how a non-additive, antagonistic interaction between two foundation species can have dramatic effects on the ecosystem, by providing an opportunity for a third species, in this case a cyanobacterium, to dominate the community. Ecological synergies following ecosystem perturbation are a known, but not well researched phenomenon (Suttle et al. [2007](#); Darling and Côté [2008](#); Thompson et al. [2018](#)). In some cases it may be difficult to uncover the effects that non-additive species interactions have on ecosystems: in our experiment, the phytoplankton biomass decreased again after we ceased the nutrient additions. However, after perturbing the ecosystems a year later with a single strong pulse of nutrients, effect was even stronger than during the first addition, indicating that non-additive species interactions can have legacy effects on ecosystems.

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Concluding remarks

All species interact with other species one way or the other, thus reciprocally affecting their own and the interaction partner's ecology (Bruno et al. 2003; Goudard and Loreau 2008; Olff et al. 2009). It is safe to assume that during their lifetime most species interact with more than one species in their effort to grow, maintain themselves and reproduce. On the one hand, the number and types of interactions (competition, predation, etc.) define a species' ecological niche (Olff et al. 2009). On the other hand, effects of interactions on fitness can give information on how strongly species interactions are subject to evolutionary change (Thompson 1999). The importance of species interactions can change throughout an organism's lifetime: for example, consumer resource species interactions may be more important during early development when an organism needs to invest in growth than later in life, after reproduction (Metcalf and Monaghan 2001; Plaistow et al. 2004). Furthermore, some species may interact with other species in multiple dimensions (Angelini et al. 2011): for example, many plants are not only habitat forming, but also produce oxygen, provide shelter and food (Carpenter and Lodge 1986; Jeppesen et al. 1998). Given this complexity, experimental studies manipulating the presence and absence of species can be a useful tool to study their interactions from different ecological and evolutionary perspectives. In this thesis I used a series of experiments to investigate the role of species interactions across a range of ecological contexts: from phenotypes to ecosystems.

Dimensionality of species interactions

In the mesocosm experiment I conducted in chapter 1, I manipulated the presence and absence of macrophytes and fish, two species thought to interactively shape the phenotype of benthic isopods. Previous field studies have suggested that visual predation along a gradient of macrophytes with different background colour is responsible for divergent patterns of pigmentation in isopods (Hargeby et al. 2004; Hargeby et al. 2005). The mesocosm project from chapter 1, essentially an experimental test of this hypothesis, showed that macrophytes can modify predation pressures (higher isopod densities in the presence of macrophytes). However, the experiment did not provide evidence for phenotypic divergence in isopod pigmentation driven by the interaction of macrophytes and fish. Instead, the presence of macrophytes led to increased isopod pigmentation independent of fish density. One explanation for this could be that mesocosms with macrophytes provided a different suite of resources than mesocosms without macrophytes. In nature, macrophytes and detritus can vary strongly in their composition, for example, in elemental ratios, amino acids and proteins, or fatty acids (Muztar et al. 1978; Adey and Loveland 2007). Indeed, results from the accompanying lab experiment indicate that variation in dietary protein can strongly affect pigmentation in natural populations of isopods. Overall, these findings suggest that in nature, macrophytes could be both, *modifiers* of natural selection stemming from visual predation, and *agents* of natural selection, by altering local resource environments. In nature, such a duality in macrophytes-fish-isopod interactions may be hard to detect, because it plays out on different timescales: through seasonal biomass buildup and decay, it takes macrophytes several years to alter the local resource environments for detritivores, whereas modifications of background colour and structure may happen continuously.

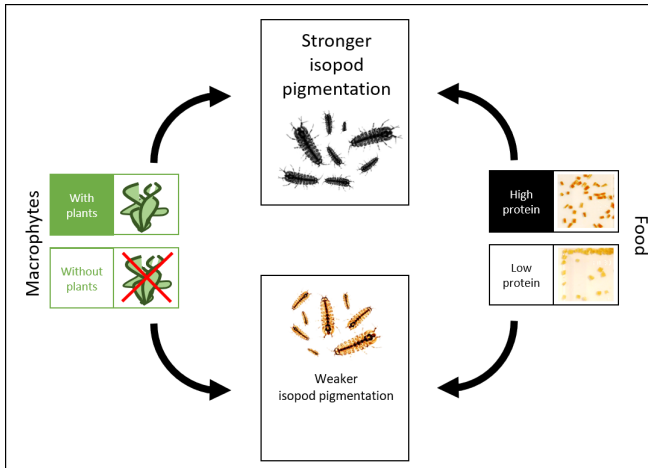


FIGURE 1: (Divergent pigmentation in *Asellus aquaticus* can be driven either by different backgrounds (crypsis; [Hargeby et al. 2004; Hargeby et al. 2005]) or different food items (developmental plasticity; [Chapter1, Chapter3]). Future experimental work should be directed at disentangling these two agents using a factorial manipulation.

In Chapter 2 and 4 I investigated the role of macrophytes as foundation species and how they affect temporal stability and resistance and resilience of aquatic ecosystems to disturbance. Just like in chapter 1, I found that macrophytes engaged in a suite of strong interactions with other species, in this case phytoplankton communities and benthic grazers, and thereby modified biotic and abiotic ecosystem properties. In agreement with the theoretical expectations (Scheffer et al. 1993; Hilt and Gross 2008; Kéfi et al. 2016), the mesocosm and the pond experiment provided evidence for the hypothesized reduction of phytoplankton in the presence of macrophytes during the undisturbed phase. This likely stemmed from competitive interactions between macrophytes for nutrients and

light, and, as indicated by a side experiment (Narwani et al. 2019), the production of allelochemicals by *Myriophyllum*, which reduced phytoplankton growth. Interestingly, and unexpectedly, the presence of macrophytes increased variance in phytoplankton biomass. So far almost no theory exists about how interactions with macrophytes can affect temporal variability of phytoplankton communities (Scheffer et al. 2003; Ives and Carpenter 2007). A simple mathematical model we implemented in chapter 2 indicates that differences in the abilities of macrophytes and phytoplankton to compete for nutrients and light may be a potential driver: by assuming that phytoplankton and macrophytes compete for both light and nutrients, we found that the presence of macrophytes kept phytoplankton densities low, whereas in the absence of macrophytes, the system reaches a high phytoplankton density state. Taken together with our empirical data, the modeling results imply that the phytoplankton-dominated state is more stable compared to the phytoplankton-macrophyte state, but clearly more empirical work is needed to investigate this phenomenon.

With the high frequency sensor network I was able to detect a range of other effects of macrophytes on the ecosystem: measurements of dissolved oxygen and temperature allowed me to calculate rates of ecosystem metabolism, which tended to be higher in the presence of macrophytes. Through metabolic activity, macrophytes may affect the capacity of aquatic ecosystems to maintain oxic conditions when facing high productivity, and subsequent breakdown of phytoplankton biomass, which often shifts ecosystems to anoxic conditions (Giorgio and Peters 1994; Nielsen et al. 2013). Metabolism is an understudied component of how species interaction networks affect shallow lake ecosystems, and only with the advent of high frequency sensor techniques can we gain significant insight into this complex process (Batt et al. 2013; Nielsen et al. 2013; Honti et al. 2016). For example, pools of dissolved organic matter (DOM)

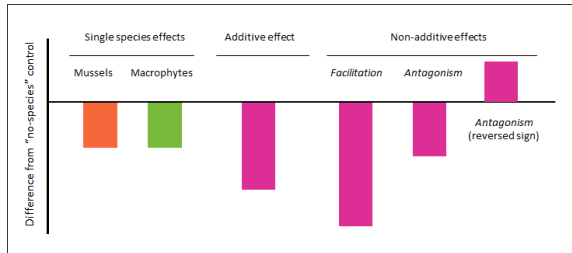


FIGURE 2: (Species interactions can be additive or non-additive (reproduced from chapter 4). In the pond experiment we observed non-additive antagonistic effects with reversed sign when mussels and macrophytes were present in the same pond: when alone, both species led to a reduction of phytoplankton biomass relative to the control, but when together, phytoplankton biomass increased relative to the control without either species.

can strongly affect ecosystem metabolism through degradation by bacteria (Hanson et al. 2003; Hansen et al. 2016). As indicated by high frequency fluorescence measurements, the presence of macrophytes lead to greatly increased DOM concentration in both mesocosms and ponds, which may provide a link to elevated rates of respiration we found in these ecosystems. Taken together, these experiments have shown that macrophytes can influence a suite of ecosystem properties and processes via antagonistic interactions with phytoplankton, through increased metabolic rates, and by modifying pools of DOM. Future work needs to be directed at the mechanistic basis of these interactions.

Ecological surprises

Chapter 4 provided experimental evidence for non-additive effects of species interactions on pond ecosystems during perturbation with

nutrients. When both *Myriophyllum* macrophytes and *Dreissena* mussels were present, nutrient additions lead to a dramatic increase in phytoplankton biomass compared to the control without either key species, whereas the presence of a single species reduced phytoplankton biomass relative to the control. A possible explanation for this may lie in separate interactions between *Myriophyllum* and *Dreissena* with the phytoplankton community that created an ecological opportunity for a bloom of cyanobacteria: the production of allelochemicals by *Myriophyllum* that can inhibit phytoplankton growth, and selective feeding by *Dreissena* that can increase cyanobacteria densities. In the same pond setup, Narwani et al. 2019 found that phytoplankton community shifted towards dominance of the cyanobacterium *Synechococcus* sp. when both *Myriophyllum* and *Dreissena* were present. Follow up laboratory trials revealed that the presence of allelochemicals (“*Myriophyllum*-tea”) or *Dreissena* alone did not affect the relative concentration of two species of microalgae that were most dominant in the pond ecosystems (*Lagerheimia* sp. and *Synechococcus* sp. Narwani et al. 2019]). However, when together, allelochemicals and *Dreissena* lead to increased *Synechococcus* sp. abundance relative to *Lagerheimia* sp.. This suggests that a relative growth rate advantage in the presence of both foundation species may have contributed to the shift of phytoplankton communities toward cyanobacteria, resulting in an overall strong increase in phytoplankton biomass.

Similar ecological synergies have been observed in the context of multiple stressors (Darling and Côté 2008), where the combination of single stressors lead to responses that were more often non-additive (facilitative or antagonistic) than purely additive. Robert Paine also referred to non-additivity as “ecological surprises”, because they are difficult to predict based on the singular effects. It seems that a mechanistic understanding is required to model interactions that go beyond

a pure additive effects. In our pond experiment we provide a species-specific biological mechanism that may help explain the observed increase in phytoplankton biomass, which brought the ponds with both macrophytes and mussels to the brink of a turbid water state. In other systems, non-additive effects are related to dual nutrient enrichment which affects autotroph productivity (Allgeier et al. 2011) or shading (Falkenberg et al. 2012). Foundation species may be particularly prone to antagonistic (or facilitative (Kéfi et al. 2016)) non-additive effects due to their central position in ecosystems and the complex interaction structures they have with other species and ecosystem compartments (Angelini et al. 2011; Ellison et al. 2005; Ellison 2019).

Species interactions as evolutionary dependencies

In chapter 3 I manipulated protein content and the concentration of the essential amino acid tryptophan in a laboratory experiment, where I reared isopods from right after birth until the age of 12 weeks (typical age of maturity in nature (Arakelova 2001)). In this experiment I wanted to investigate variation and extent of phenotypic plasticity in body size and pigmentation in isopods in response to diet composition, and test for effects of developmental trajectories on survival of juvenile isopods until maturity. Across all families, elevated dietary protein increased developmental rates of pigmentation, but decreased survival. This is in agreement with previous work, which has shown that elevated dietary protein content can reduce survival (Piper et al. 2011; Fontana and Partridge 2015; Le Couteur et al. 2016), which is generally associated with costs of protein-digestion (Halton and Hu 2004) and potentially harmful breakdown products (Wright

1995). I also supplemented tryptophan to both levels of protein manipulation to test whether it would affect the development of pigmentation in isopods. The supplement of tryptophan to both high and low protein diets also showed significant positive effects on pigmentation rates, but only under low protein diet: there pigmentation rates were higher when tryptophan was supplemented. Similar observations have been made for cabbage butterflies (*Pieris brassicae*), which showed increased wing pigmentation when reared under a tryptophan supplement (Kayser 1979). Since the high protein diet was made from yeast, and likely also contains tryptophan, it is possible that faster development of pigmentation that I observed under high protein content is explained by tryptophan alone.

All animals rely on external compounds that they can not synthesize themselves (Ellers et al. 2012; Badyaev et al. 2019) like proteins or vitamins that are needed for growth, maintenance and reproduction. Recent work has suggested that variation in the availability of external compounds can affect evolutionary patterns in animals (Badyaev et al. 2019; Starr et al. 2017). For example, enzymatic conversion of essential carotenoids to ornamentation in birds, for example, has been shown to be subject of multiple evolutionary cycles of gains and losses of internal, physiological control (Badyaev et al. 2019). In this context, the tryptophan - xanthommatin pathway in many arthropod taxa provides a promising avenue of further research. Xanthommatin is an ommochrome pigment that is synthesized based on the essential amino acid tryptophan, produced exclusively by plants and microorganisms (Miozzari et al. 1978; Shamim et al. 2014), and is responsible for cuticular and eye pigmentation in many insects and crustaceans (Linzen 1974; Needham 1974a). For eyes, the cause of evolving this pathway is clear: it is being used as a screening pigment to regulate the amount of light that is being absorbed by the receptors (Linzen 1974). For body pigmentation however, there are several possibilities

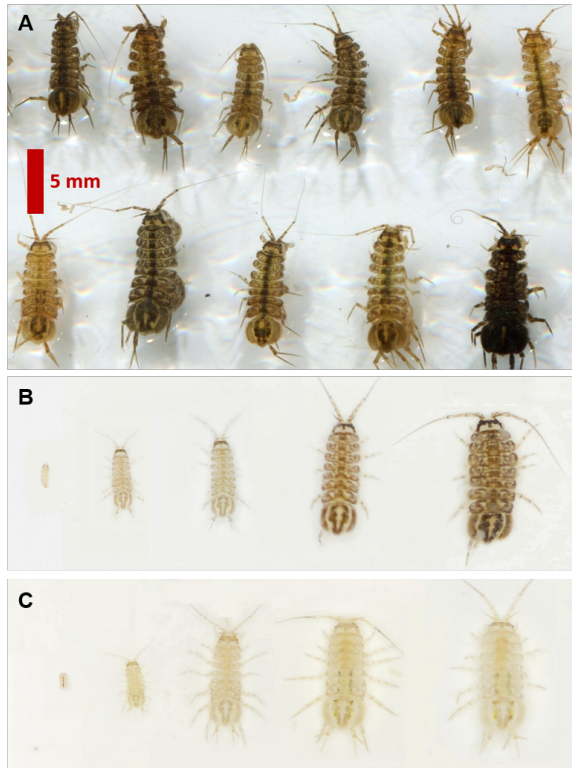


FIGURE 3: (Phenotypic variation in *Asellus aquaticus* (reproduced from chapter 3). Manipulation of diet has allowed the creation of different "pigmentation-morphs" that by the original crypsis hypothesis (Hargeby et al. 2004; Hargeby et al. 2005) would require different backgrounds and predation. In my thesis found diet-dependent developmental plasticity to be a strong source of phenotypic variation in isopods.

that could explain why organisms have evolved this relatively complex pathway. On the one hand, it could simply be a way to create body coloration of ecological relevance, e.g. for crypsis or signaling (Kayser 1979). On the other hand, excess tryptophan inside the organism is toxic, and “local excretion”, i.e. the formation of inert pigment granules from soluble tryptophan, might be an adaptive trait in arthropods (Linzen 1974).

A. aquaticus also synthesizes xanthommatin for pigmentation, and thereby can occur as darker or lighter morphs (Needham 1970). In shallow lakes, isopod pigmentation was correlated with background colour of the habitat (Hargeby et al. 2004; Hargeby et al. 2005), and is thought to have evolved rapidly in response to visual predation along a gradient of different backgrounds (Hargeby et al. 2004; Er-oukhmanoff et al. 2009b). As I discussed above, and in chapters 1 and 3, pronounced developmental plasticity in pigmentation does not exclude the possibility for pigmentation to have evolved in response to selection from predation along a gradient of differently coloured backgrounds. However, an alternative hypothesis could be that these microhabitats simply vary in the amount of tryptophan that isopods can take up from detritus. Not all macrophytes produce tryptophan, but the ones that do vary strongly in their concentration (Muztar et al. 1978). If xanthommatin increases isopod fitness by making them less conspicuous under elevated visual predation pressure, this could lead to mismatches in microhabitats where the background is dark, but tryptophan is not available in sufficient amounts. Alternatively, pigmentation in isopods may be a way to bind excess tryptophan to avoid cell toxicity, either from the breakdown of proteins into amino acids or direct uptake (Linzen 1974; Arganda Sara et al. 2017). This does not exclude crypsis as the cause for pigmentation, but more research is needed on the costs of acquiring and using tryptophan to synthesize xanthommatin in natural environments.

Outlook: Can evolution of species interactions affect ecosystem dynamics?

In 1972, Robert May's seminal paper challenged the prevailing intuition that more speciose communities would be more stable over time (May 1972). This seemed paradoxical to many ecologists at the time, because in nature, many complex systems like coral reefs or tropical rain forests are stable over time. Later, Pimm 1984, and McCann et al. 1998, expanded May's original models, and found that the stability of ecosystems is governed by only a few strongly interacting species, but many weak interactions among species. This principle was reflected again in findings of the biodiversity effects on ecosystem functioning (BEF) studies during the late 1990s and early 2000s. There, works of Tilman and Downing 1996, Loreau et al. 2001, or Cardinale et al. 2006 showed that ecosystems containing a more diverse community had higher rates of biomass production and decomposition, nutrient recycling, and range of other ecosystem rates. However, it was immediately identified that some of these strong relationships are simply a product of a higher chance of diverse assemblages containing species that have a large effect on ecosystem function ("sampling effect"[Hillebrand and Matthiessen 2009; Cardinale et al. 2012]). These species can have disproportionate effects on the functioning, but also on the stability of ecosystems, through facilitative and antagonistic interactions with other species. For example. the works of (Scheffer et al. 1993) and Scheffer et al. 2015) have shown that interactions between a few foundation or keystone species (Ellison 2019) and the rest of the community can increase the stability of shallow lake ecosystems against perturbation with nutrients.

The above mentioned classic schools of thought acknowledge that species interactions can structure populations and communities, affect ecosystem function, and determine resistance and resilience of ecosystems against external perturbation. Currently the disciplines

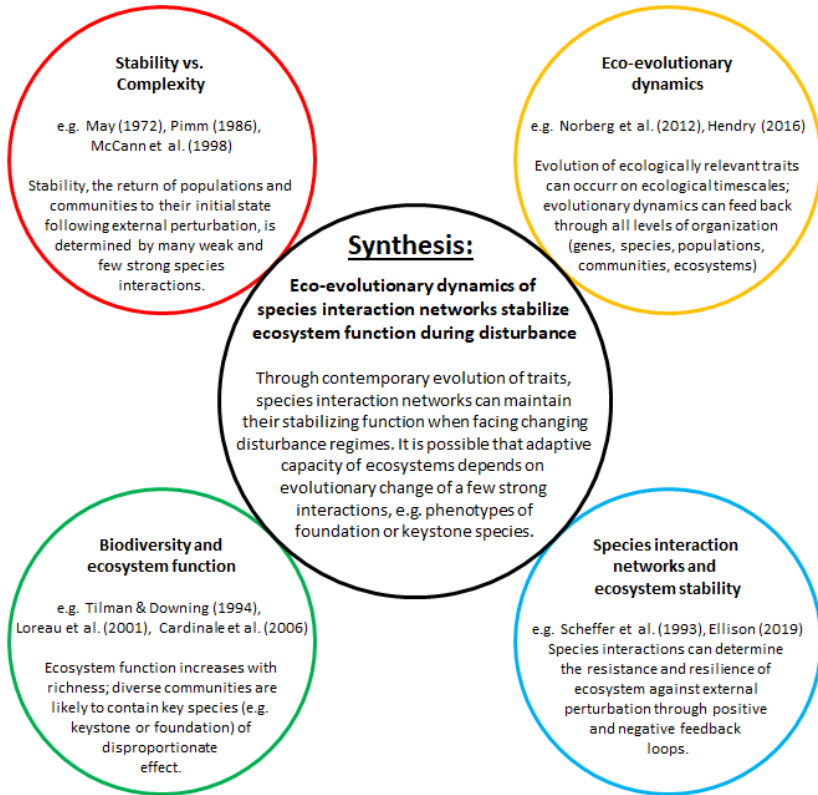


FIGURE 4: Further avenues of research and synthesis based on ideas that I discussed in the chapters of my thesis. The coloured circles depict ideas from distinct, classic schools of thought; the black circle at the center outlines a research programme that synthesizes these ideas in experiments similar to the ones I have conducted for my dissertation.

of ecological interactions and ecosystem science are only weakly integrated with the field of eco-evolutionary dynamics (eco-evo dynamics), which is an emerging school of thought at the intersection of ecology and evolution (Hairston et al. 2005; Fussmann et al. 2007). Eco-evo dynamics describe interactions between ecological and evolutionary processes that play out on contemporary time scales and on all levels of ecological organization (genotypes/phenotypes to ecosystems) (Hendry 2016). Given that the strength and direction of interactions are determined by the phenotypes of the participating species (Werner and Peacor 2003), rapid phenotypic evolution may be an important mechanism to stabilize interaction networks under disturbance or fluctuating environments. The evolution of species interactions is not a novel concept (Thompson 1999; Agrawal 2001), but only few experiments have been conducted to investigate how reciprocal evolutionary change of species interactions can affect ecosystem dynamics (Harmon et al. 2009; Fischer et al. 2014; Matthews et al. 2016). In my thesis, I have demonstrated how important macrophytes and mussels are for the functioning of aquatic ecosystems under disturbance regimes. Tracking phenotypic distributions of these key species through multiple generations, and monitoring ecosystem status under different disturbance regimes may provide insight on how the evolvability of species interactions can affect resistance and resilience of ecosystems to perturbation (Harmon et al. 2009; Fischer et al. 2014; Matthews et al. 2016).

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