The ecological dynamics of natural selection in a community context

Inauguraldissertation
der Philosophisch-naturwissenschaftlichen Fakultät
der Universität Bern

vorgelegt von

Marvin, Moosmann

von Egg, Zürich

Leiter der Arbeit:

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und
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General introduction

As humans and as scientists we can only marvel at the diversity of life that surrounds us. Curiosity about the mechanisms that underlie this diversity has motivated diverse biological disciplines and their approaches. Community ecologists study the dynamics of populations and the interactions among species to understand the organizing principles that determine the abundance, diversity and distribution of species. Evolutionary biologists seek to understand the origins of diversity by studying how the genetic and phenotypic make-up of populations changes from one generation to the next. It has long been recognized that the mechanisms that drive evolution and those that underlie the organization of populations and communities are inextricably linked (Darwin 1859, Lion 2018), and may represent "different perspectives on the same whole" (McPeek 2017). In the recent decades, the realization that evolutionary change can occur on timescales that are relevant for understanding ecological dynamics has led to an increasing integration between community ecology and evolutionary biology (Hairston *et al.* 2005; Haloin & Strauss 2008; Matthews *et al.* 2011; Schoener 2011; Govaert *et al.* 2021). This theoretical and empirical integration lays the foundation for understanding how community contexts affect phenotypic evolution (Schluter 2000; terHorst *et al.* 2018; Harmon *et al.* 2019), how evolutionary change affects the ecological dynamics of communities (Emerson & Gillespie 2008; Harmon *et al.* 2009; Des Roches *et al.* 2018; Reznick *et al.* 2019; Cropp & Norbury 2020), and how evolutionary and ecological dynamics can feed back on one another (Hendry 2016; Best *et al.* 2017; McPeek 2017; Lion 2018). Studying this interplay between ecological dynamics and evolutionary change in nature, however, is challenging. Some of the most substantial challenges to overcome involve understanding how complex interactions among species shape natural selection, and how some of the outcomes of evolution, such as phenotypic change, can shape selective environments (Wade & Kalisz 1990; Vellend 2010; Matthews *et al.* 2014; terHorst *et al.* 2018).

In this thesis I will address some outstanding issues regarding the interplay between evolutionary change and the ecological dynamics of species communities. For this purpose, I will use a combination of literature review and theory (chapter one), experimentation (chapter two), and comparative analyses of natural populations and ecosystems (chapters three and four). I will address the questions of how the community context can drive phenotypic variation (chapter one and four), and how phenotypic divergence affects species-interactions and community structure (chapters two and three). For the empirical parts of this thesis (chapters two to four), I use the threespine stickleback (*Gasterosteus aculeatus* species complex, Linnaeus, 1758) and the communities they are embedded in as a focal study system.

The ecological dynamics of natural selection

Evolutionary mechanisms such as natural selection, drift and gene flow are increasingly incorporated into our thinking about ecological dynamics (Lion 2018), however, understanding the ecological causes and consequences of evolutionary outcomes remains a challenge for evolutionary ecology (MacColl 2011). Evolution by natural selection results from the population dynamics of genetically diverse populations, where groups of individuals carrying some genotype have higher growth rates (i.e. fitness) than individuals carrying other genotypes (Lion 2018). This differential fitness between genotypes depends on how genetic variation translates into phenotypic variation (i.e. the heritability of the trait), and on how phenotypic variation conveys ecological performance and, ultimately, fitness, in a given environmental context (Arnold 1983). A rich body of theory has laid the foundation for predicting phenotypic outcomes of natural selection (Lande & Arnold 1983), linking the ecological dynamics of population and communities to trait changes (McPeek 2017), and describing how ecological and evolutionary dynamics can reciprocally interact (Lion 2018). However, while these models help us understand *how* traits evolve in ecological contexts, they do not inform us about *why* they evolve (Wade & Kalisz 1990). Addressing the latter question requires identifying the environmental causes of fitness variation, understanding the ecological mechanisms by which they affect survival and reproduction, and tracking how they change over time (Wade & Kalisz 1990; MacColl 2011).

1) What are the relevant agents of selection in a community context, how do they interact, and how do they affect fitness?

Correlations between phenotypes and fitness are not intrinsic to traits, but are contingent on environmental conditions (Wade & Kalisz 1990; MacColl 2011). Identifying these conditions – often dubbed agents of natural selection – is central to understanding the causes and ecological dynamics of selection and, as such, the evolutionary trajectories of populations (Gibbs & Grant 1987; MacColl 2011). The communities within which populations are embedded are an important source of fitness variation (Haloin & Strauss 2008; terHorst *et al.* 2018; Harmon *et al.* 2019). A classic example for an ecological cause of natural selection is seed availability and its effects on bill size in the Galapagos finch *Geospiza fortis* (Boag & Grant 1981; Gibbs & Grant 1987; Hairston et al. 2005). During drought years the resources available to individuals consisted primarily of hard seeds. In this community context, individuals with large bills, which are better suited to crack open hard seeds, had a fitness advantage over individuals with small bills (Boag & Grant 1981). In years without droughts, however, more soft seeds became available, resulting in a reversal of the selection gradient, where small bills became more beneficial to fitness than large bills (Gibbs & Grant 1987). The evolutionary dynamics of bill size between years were driven by how bill morphology was causally related to biomechanical performance, and by how the availability and the traits of other species in the community (seed bearing plants) changed over time. This example illustrates, how a mechanistic understanding of natural selection requires an understanding of both the links between phenotypes and performance ("the performance gradients" (Arnold 1983)) and the ecological (community) contexts, within which these trait-performance relationships become relevant for fitness ("the fitness gradient" (Arnold 1983)) (Figure 1).

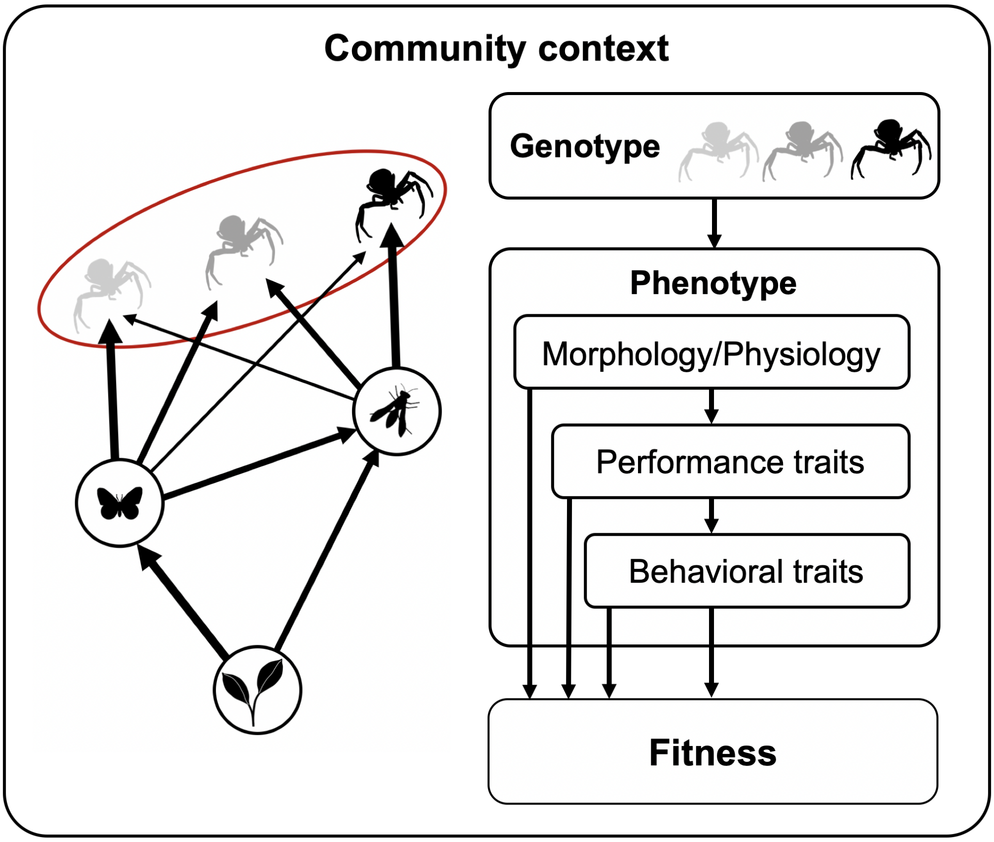

Figure 1: Understanding the ecological dynamics of natural selection in a community context requires understanding the complex ecological interactions that determine differential survival and reproduction in a focal population. Fitness variation arises from how the hierarchies of traits that determine the overall phenotype of individuals convey fitness in a given environment. Community contexts can not only determine how phenotypes link to fitness, but can also affect the links within this trait hierarchy. For example, environments can determine how genotypes translate into phenotypes (phenotypic plasticity) and affect behaviors that alter the links between morphology and performance.

In nature, populations are embedded in diverse physical and geochemical environments, and interact directly or indirectly with a myriad of other species. In such complex settings a suite of environmental factors can affect the survival and reproduction of individuals, which challenges our ability to identify the ecological causes of natural selection (MacColl 2011). Environmental variables with putative effects on fitness often covary in nature but may not equally contribute to fitness variation (Schluter 2000; MacColl 2011; Singkam & MacColl 2019). For example, the addition of predators to an ecosystem often reduces the densities of prey populations, alters the competitive interactions among prey species, and affects lower trophic levels via cascading ecological effects (Polis & Strong 1996; Chase *et al.* 2002). An evolutionary response to changing predation regimes may thus result from the effects of predators, competitors, conspecifics, resources, or combinations thereof (terHorst *et al.* 2018; Reznick *et al.* 2019; Singkam & MacColl 2019). In complex ecological contexts it is likely that several agents affect fitness simultaneously, and so it is hard to attribute any particular agent to the observed evolutionary response (Van Leeuwena *et al.* 2008; TerHorst *et al.* 2015; Czorlich *et al.* 2022). Such attribution is particularly difficult when selection is affected by indirect ecological interactions between species (Haloin & Strauss 2008; TerHorst *et al.* 2015; Czorlich *et al.* 2022), i.e. when selection imposed by one species (e.g. a consumer) onto another (e.g. a prey) is altered by the presence of a third species (e.g. a top predator), without the third species interacting with the focal populations (TerHorst *et al.* 2015). This complexity is further compounded by abiotic conditions that influence the nature and strength of species interactions (Sanford & Worth 2010).

Identifying the agents of selection is crucial for a mechanistic understanding of natural selection, however, the ecological complexities of natural systems often make it difficult to identify these causes from patterns of phenotypic variation in nature (e.g. phenotype-environment correlations (Schluter 2000)), or to predict evolutionary responses in complex contexts from more simple conditions (e.g. experiments). Approaches to identifying agents of selection include observations of spatiotemporal patterns of phenotypic variation or selection (Schluter 2000; Reznick *et al.* 2019; Czorlich *et al.* 2022), and experimental manipulations, where putative selective agents can be directly manipulated (Svensson & Sinervo 2000). Such approaches have their limitations, but collectively they provide important windows into the complex dynamics of natural selection and shape our perspective about ecological mechanisms underlying evolution.

2) How does phenotypic variation affect selective environments?

Rapid evolutionary change of organisms can affect ecological dynamics and, as such, may change selective environments (Hendry 2016). Understanding how organisms modify their environment can, therefore, improve our understanding of ecological and evolutionary dynamics in ecosystems, particularly if these modifications affect putative agents of selection, including those influenced by the community context (Matthews *et al.* 2011). Reciprocal dynamics between rapid evolutionary changes and ecological dynamics, are well supported in theory (McPeek 2017; Lion 2018; Gibert & Yeakel 2019; Cropp & Norbury 2020), and experiments have documented ecosystem-wide phenotypic effects of consumers in general, and changes in community structure in particular (Des Roches *et al.* 2018). In some cases, these modifications might feed back to affect trait distributions or trait expression of subsequent generations (Matthews *et al.* 2016; Best *et al.* 2017; Brunner *et al.* 2017). However, only a few studies have investigated how spatiotemporal phenotypic variation drives community structure in natural systems and have identified the underlying traits that govern such modifications ("ecosystem-effect traits" (Violle *et al.* 2007)) (Post *et al.* 2008; Post & Palkovacs 2009). In nature, the relative importance of phenotypic change/variation in driving community structure likely depends on the complex interplay between various abiotic and biotic factors (Polis & Strong 1996; Paquette *et al.* 2022). Hence, questions remain about when and how rapid trait evolution can meaningfully affect selective environments in nature.

A useful starting point to study the ecological effects of phenotypic change is to understand the ecological function of traits (Wainwright *et al.* 2007; Post *et al.* 2008; Post & Palkovacs 2009; Schmid *et al.* 2018). This can provide hypotheses about how phenotypic variation and trait change might alter the ecological interactions of focal populations. These hypotheses can then be experimentally tested in semi-natural ecosystems (e.g. mesocosm-ecosystems) or comparatively tested in nature by correlating phenotypic variation among independent communities with variation in community structure (Post *et al.* 2008). However, the causality of such phenotype-environment correlations cannot be unambiguously interpreted, and are most often interpreted with a particular causal structure in mind, namely that traits evolve in response to local environmental conditions. From this perspective, phenotype-environment correlations offer supportive, albeit not conclusive, evidence for local adaptation (Schluter 2000; Sanford & Worth 2010; Holding *et al.* 2018). However, if phenotypes shape environmental conditions, then the underlying causality of these correlations is reversed (i.e. phenotypes to environment, rather than environment to phenotypes). While experimental work has demonstrated phenotypic effects on environments, we lack observations of phenotypic effects in nature, and this would provide an important stepping stone towards understanding the role of evolutionary change in driving ecological dynamics.

Threespine stickleback as a study system

Threespine stickleback are small teleost fish that have a (near) circumpolar marine distribution in the northern Hemisphere (Fang *et al.* 2018). After the last glacial maximum, the ancestral marine species has independently colonized diverse freshwater habitats in North America and Eurasia, resulting in repeated adaptive diversification (McKinnon & Rundle 2002; Hendry *et al.* 2009). This diversification typically occurs along a lake-stream habitat axes, and, more rarely, along a benthic-limnetic axis within lakes (Schluter & McPhail 1992; Magurran 1994; McKinnon & Rundle 2002; Gow *et al.* 2008; Willacker *et al.* 2010; Østbye *et al.* 2016). The rapid adaptation to freshwater, and the subsequent diversification within freshwater habitats, as well as their suitability as laboratory animals, has made stickleback an important model species in ecology and evolutionary biology (Magurran 1994; Gibson 2005; Hendry *et al.* 2013), and an ideal system to study the interplay between ecological and evolutionary dynamics (Hendry *et al.* 2013).

Sticklebacks inhabit diverse aquatic habitats and sit at an intermediate position in aquatic food-webs. They prey upon a variety of invertebrates, ranging from small bodied zooplankton (copepods, cladocera, etc.), to relatively large bodied benthic invertebrates (e.g. isopoda, insect larvae, etc.) (Lucek *et al.* 2012; Bretzel *et al.* 2021). They are preyed upon by invertebrates, piscivorous fish, and birds (Vamosi & Schluter 2002; Leinonen *et al.* 2011; Zeller *et al.* 2012). Previous work has investigated the evolutionary dynamics of stickleback in response to various selective agents, including habitat (Schluter 1993; Bolnick & Lau 2008; McGee *et al.* 2013) and resource environments (Singkam & MacColl 2019). A body of experimental work has identified associations between in foraging traits, local feeding habitats and resource-use (Schluter 1993; Matthews *et al.* 2010; Lucek *et al.* 2013; McGee *et al.* 2013; Arnegard *et al.* 2014), and has related phenotypic variation in some of these traits to prey-specific foraging performance in different environmental contexts (Schluter 1993; Robinson 2000; Higham *et al.* 2017; Schmid *et al.* 2018). A separate body of work has investigated the effects of lineage divergence on ecosystem processes in semi-natural systems (mesocosms), including changes in community structure (Harmon *et al.* 2009; Rudman *et al.* 2015; Matthews *et al.* 2016; Best *et al.* 2017), and has related community changes to divergent phenotypes (i.e. putative ecosystem-effect traits) (Schmid *et al.* 2018). Combined, these previous studies lay the foundation to study the ecological dynamics of natural selection in a community context.

The Swiss stickleback system

In chapter two of this thesis, I focus on European stickleback, and specifically, on the stickleback populations of two large perialpine lakes - Lake Constance and Lake Geneva. Due to the glaciation history of the continent, the timing of freshwater colonization varies widely among European stickleback lineages: Populations in Western-European watersheds (e.g. Rhine, Rhône, Seine) likely established prior to the last glacial maximum, whereas North-eastern-European watersheds (i.e. the Baltic drainage) were colonized only after the retreat of the ice sheets ~12,000 years ago (Lucek *et al.* 2010; Marques *et al.* 2016, 2019). Recent range expansion and anthropogenic introductions has brought some of these lineages into secondary contact in perialpine lakes of the Swiss plateau, which was mostly uninhabited by stickleback until recent historical times (Lucek *et al.* 2010; Marques *et al.* 2016, 2019; Hudson *et al.* 2021b). Due to their divergent evolutionary histories, hybridization, and of rapid adaptive diversification in the newly colonized watersheds, Swiss stickleback populations are highly variable and differ in various ecological, physiological and morphological phenotypes (Berner *et al.* 2010; Lucek *et al.* 2013; Alexander *et al.* 2016; Best *et al.* 2017; Hudson *et al.* 2021a). This makes Swiss stickleback an interesting study system to investigate the ecological consequences of evolutionary divergence. Previous experimental work has investigated how both rapid and ancient lineage divergence can differentially affect semi-natural ecosystems (mesocosm), and how these ecological modifications influence the survival and growth of a subsequent generation (Matthews *et al.* 2016; Best *et al.* 2017). In this thesis (chapter two), I aim to identify the traits that underlie such ecosystem modifications, particularly those associated with shaping prey communities in littoral and pelagic environments.

The Greenlandic stickleback system

In chapters three and four I focus on lake and pond dwelling stickleback populations of Southern Greenland (Kujalleq municipality) (Figure 2A). During the last glacial maximum (ca. 22 kb BP) this study area was fully submerged, and became subject to vertical uplift following

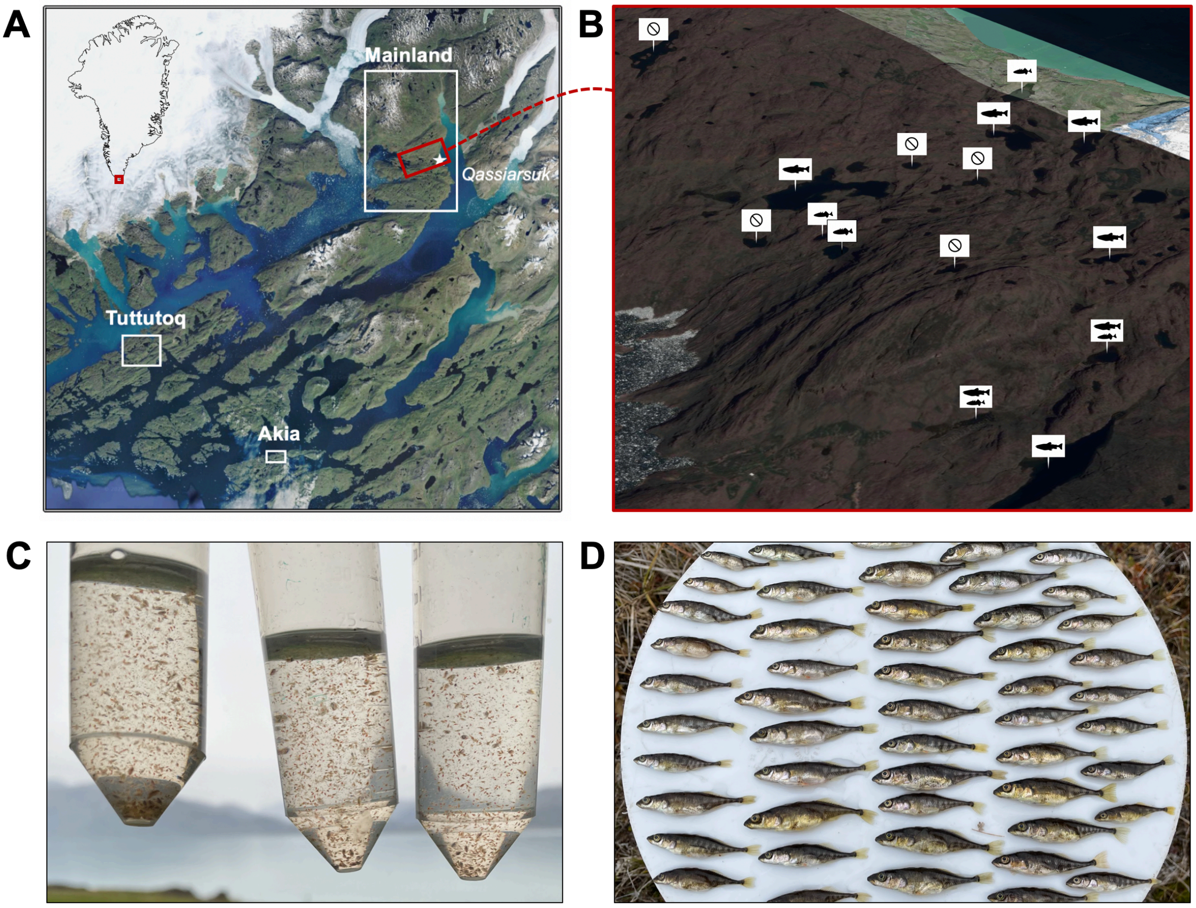

Figure 2: The Greenlandic study system is located on the Tunulliarfik fjord in the south of Greenland (A). The landscape is scattered with hundreds of independent freshwater lakes that contain only two fish species – arctic char and threespine stickleback – in a fully factorial design (B). This creates a highly replicated natural experiment, which has allowed us to investigate the phenotypic drivers of zooplankton community composition across the landscape (C; chapter three) and the effects of community context on the phenotypic variation of stickleback (D; chapter four).

deglaciation (Sparrenbom *et al.* 2013). The landmass emerged ~10 '000 years ago from the ocean (Sparrenbom *et al.* 2006, 2013), forming a landscape, where hundreds of lakes of varying size occur over small geographic scales. These lakes were subsequently colonized by two species of freshwater fish, threespine stickleback and the salmonid arctic char (*Salvelinus alpinus*). This resulted in a patchwork of lakes with either no fish, only char, only stickleback or both species (Bergersen 1996; Doenz *et al.* 2019) (Figure 2B). This natural setting provides interesting contrasts in food-web configurations across the landscape: comparatively simple invertebrate communities are preyed upon by stickleback and/or char. Where stickleback and char co-occur, large char additionally prey on stickleback (Røen 1994; Doenz *et al.* 2019).

While the natural history of Greenlandic stickleback is comparatively understudied (Liu *et al.* 2016), there is a body of work investigating the ecological impacts of stickleback (and char) on the invertebrate communities (Jeppesen *et al.* 2003, 2017; González-Bergonzoni *et al.* 2014), and the evolutionary consequence of this interaction for prey species (Oester *et al.* 2022). With the high replication of independent ecosystems on small spatial scale, the clear ecological contrasts between these ecosystems (e.g. the presence/absence of fish), and the simplicity of their species communities, these lakes are ideally suited for comparative analyses on how community contexts affects trait evolution (Oester *et al.* 2022), and how intraspecific phenotypic variation affects community dynamics.

Overall the combination of these study systems in Switzerland and Greenland afford opportunities to investigate the effects of evolutionary divergence on community dynamics, and to explore the ecological causes of natural selection arising from community contexts.

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Chapter 1

On the evolution of trophic position

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PERSPECTIVE

On the evolution of trophic position

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Abstract

The trophic structure of food webs is primarily determined by the variation in trophic position among species and individuals. Temporal dynamics of food web structure are central to our understanding of energy and nutrient fluxes in changing environments, but little is known about how evolutionary processes shape trophic position variation in natural populations. We propose that trophic position, whose expression depends on both environmental and genetic determinants of the diet variation in individual consumers, is a quantitative trait that can evolve via natural selection. Such evolution can occur either when trophic position is correlated with other heritable morphological and behavioural traits under selection, or when trophic position is a target of selection, which is possible if the fitness effects of prey items are heterogeneously distributed along food chains. Recognising trophic position as an evolving trait, whose expression depends on the food web context, provides an important conceptual link between behavioural foraging theory and food web dynamics, and a useful starting point for the integration of ecological and evolutionary studies of trophic position.

KEYWORDS
food webs, intraspecific variation, natural selection, phenotypic plasticity, trait evolution, trophic position

INTRODUCTION

Trophic position is a continuous measure of an organism's position in relation to the transfer of energy from the bottom to the top of a food web (Box 1) (Levine, 1980). The variation in trophic position among species, populations and individuals is a defining feature of food webs, and can strongly influence the dynamics and stability of natural ecosystems (Arim & Marquet, 2004; Ingram et al., 2009; McCann et al., 1998; Pimm, 1991; Post, 2002a; Rooney & McCann, 2012). Ecological research has focused on the causes of variation in trophic structure among food webs across ecosystems (Bell, 2007; Hatton, 2015; Potapov et al., 2019; Shurin et al., 2006), as well as variation in trophic position among and within the constituent species of food webs (McMeans et al., 2019; Tewfik et al., 2016). In addition, evolutionary research has investigated the origins of trophic novelty (Coll & Guershon, 2002; Cropp & Norbury, 2020; Denno & Fagan, 2003; Herrel et al., 2008) and the phylogenetic patterns of variation in trophic position among species (Burin et al., 2016; Ingram & Shurin, 2009; Muschick et al., 2012; Price et al., 2012). However, few studies have investigated how the ecological dynamics of natural selection can affect the evolution of consumer trophic position (Cropp & Norbury, 2020; Gibert & Yeakel, 2019).

Insights into the ecological causes and pace of trophic position evolution are evident from examples of

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Box 1 How to calculate trophic position

The uptake and transfer of energy is a central feature of ecosystems, and consumers often derive their energy through multiple, complex pathways (Polis & Strong, 1996). Contrary to the concept of trophic levels, in which simple trophic topologies (i.e. food chains) and discrete levels of energy processing are assumed, measures of trophic position account for complex food web configurations. Trophic position is the weighted mean path length (i.e. the number of trophic transitions) taken by energy from primary producers to consumers, and thus represents a continuous measure of an individual's function in the flow of energy through a food web (Levine, 1980). Specifically, the trophic position of a consumer can be calculated as:

$$TP_i = 1 + \sum_{j=1}^{n} p_{ij} TP_j \tag{1}$$

where TP_i is the trophic position of consumer i, TP_j is the trophic position of prey type j, and p_{ij} is the relative contribution of j to the diet of i (Adams et al., 1983). Thus, while TP_j is determined by the prey environment of a consumer, p_{ij} can be dependent on consumer properties. For example, Gibert and Yeakel (2019) model p_{ij} as a function of a consumer's type two functional response to different prey types, in which the consumption rate C_{ij} of prey j by predator i, depends on prey-specific abundances R_j, attack rates a_{ij}, and handling times h_{ij}:

$$C_{ij} = \frac{a_{ij} R_j}{1 + a_{ij} h_{ij} R_j} \tag{2}$$

Prey-specific attack rates and handling times can vary among predator individuals, and covary with (potentially heritable) traits in predators, such as body size (Cuthbert et al., 2020). Thus, (heritable) trait variation in a consumer population can introduce variation in functional responses to different prey types, which in turn will affect the diet composition of an individual. This will lead to variation in trophic position among consumers, provided that the prey types differ in trophic positions (Figure 1).

There is a long history of calculating trophic position in ecology, and early approaches relied on estimations based on dietary data (Vander Zanden & Rasmussen, 1996; Williams & Martinez, 2004), however, a broad application of this approach was limited due to the low availability of dietary data for most natural systems. Over the past few decades, isotope analysis has become an increasingly standard method to estimate trophic position and is based on the accumulation of the nitrogen isotope ^{15}N in consumer tissue relative to their resources (Post, 2002b). Such isotope-based approaches have made time-integrated trophic position data more readily available to both ecologists and evolutionary biologist and is widely used to quantify trophic dynamics and estimate the trophic structure of food webs, and the trophic position of individuals.

the adaptation of consumers to new habitats and from adaptive radiations. For example, five rodent lineages in the family Muridae independently increased their degree of carnivory after colonising the Indo-Australian Archipelago (Rowe et al., 2016), whereas lizards from a largely insectivorous ancestral population that were experimentally translocated to an island in the Adriatic Sea evolved morphological features consistent with greater herbivory within ~30 generations (Herrel et al., 2008; Wehrle et al., 2020). The rapid evolution of diet and resource use is also characteristic of many adaptive radiations of consumers, where there is both rapid emergence and sustained diversity of trophic position variation among closely related species (McGee et al., 2020; Muschick et al., 2012; Reding et al., 2009). Radiations of East African cichlid fishes, for example, harbour considerable trophic diversity both among and within species-rich guilds (e.g. omnivores, and predators), providing evidence for rapid evolution of trophic position of individual species, and rapid divergence among closely related species (Moser et al., 2018; Muschick et al., 2012). The rapid evolution of trophic position is also evident from other instances of ecological speciation of consumers (Arnegard et al., 2014; Matthews et al., 2010; Richards et al., 2021; Shi et al., 2021).

While considerable work has documented dramatic patterns of variation in trophic position among and within consumer species, much less is known about the ecological drivers underlying the expression and evolution of trophic position in food webs. The expression of an organism's heritable traits, such as body size (Bergland et al., 2008; Lafuente et al. 2018) and habitat choice (Rodriguez et al. 1992; Via & Hawthorne 2002), emerges from gene expression in a relevant environmental context. For trophic position of consumers, the food web is the relevant environmental context, and trophic

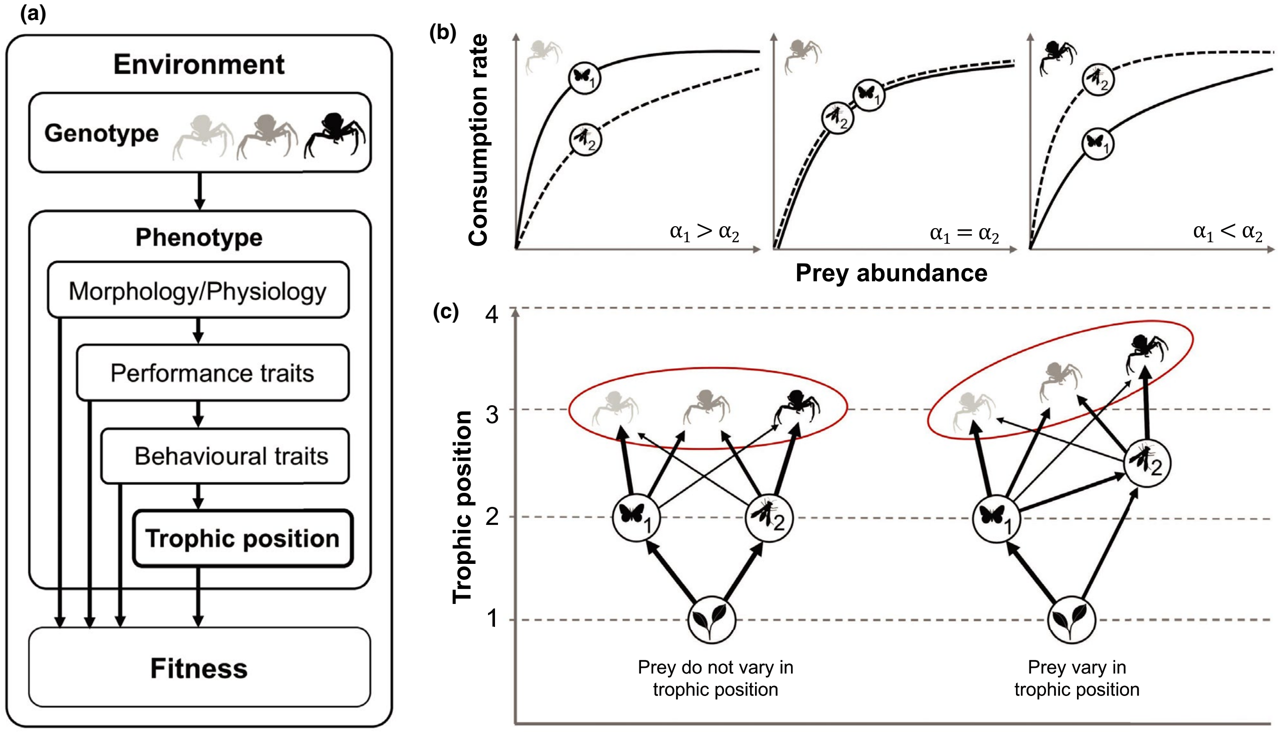


FIGURE 1 The food web context and consumer characteristics determine trophic position variation. (a) In a given environmental context, genetic differences between individuals can lead to phenotypic variation throughout the trait hierarchy that affects diet composition, which ultimately determines the trophic position of an individual. (b) For example, genotypes can differ in attack rates (α) on prey species (1 and 2), which affects their functional response (Box 1; Equation 2) and their diet composition (Gibert & Yeakel, 2019). (c) Such differences in diet (thickness of arrows) among genotypes leads to heritable variation in consumer trophic position when the prey species differ in trophic position (which is the case in the scenario on the right but not on the left)

position of consumers can evolve either when it is a direct target of selection or when it is correlated with other heritable traits under selection (Box 2). However, as we discuss below, the food web context of trophic position expression and evolution presents a challenge for (1) identifying the determinants of trophic position variation, (2) quantifying the heritability and genetic architecture of trophic position, (3) identifying putative mechanisms of the adaptive evolution of trophic position and (4) understanding the relevance of these mechanisms for explaining macroevolutionary patterns of trophic diversity in natural food webs.

DETERMINANTS OF TROPHIC POSITION VARIATION AMONG INDIVIDUALS

Trophic position is a quantifiable aspect of an individual organism that is expressed in a food web resulting from the sum of its trophic interactions (Box 1) (Levine, 1980). As a result, trophic position can change not only due to changes in an organism's diet (e.g. due to changes in foraging behaviour and/or performance) but also due to changes in the trophic position of dietary items. Thus, a challenge when identifying evolutionary shifts in trophic position is to distinguish variation in trophic position due to phenotypic change in the focal population from variation arising solely from changes in the underlying food web structure. The dependence of trophic position expression on other organisms is somewhat analogous to the context-dependency of expression for other evolving traits: social dominance and social network position require meaningful variation in social interactions (Moore et al., 2002; Wice & Saltz, 2021), and boldness requires meaningful variation in risk (Réale et al., 2007). In natural populations, there is substantial opportunity for diet variation among individuals (Bolnick et al., 2003) to translate into intrapopulation variation in the expression of trophic position (Vander Zanden et al., 2000). Such diet variation can result from differences in the abundance, diversity and trophic structure of prey (Gibb & Cunningham, 2011; Lei et al., 2019; van Rijssel et al., 2017; Tewfik et al., 2016), as well as from individual differences in the ability to capture prey with different trophic positions (Araújo et al., 2011; Bolnick et al., 2003). Trophic position can also change over the lifespan of individuals, often as a consequence of transitions in resource use among different life stages or with increasing body size (Werner & Gilliam, 1984). For example, in some Lepidopterans trophic position changes following metamorphosis: in Hawaiian *Eupithecia sp.*, insectivorous caterpillars transition to herbivorous imagos (Montgomery, 1983), while in *Calyptra sp.* ('vampire

Box 2 A formal description of selection and evolution of trophic position

We can formally describe the evolutionary change in trophic position (TP) by substituting in the mathematical definition of TP (Box 1, Equation 1) into Price's theorem (Frank, 2012; Price, 1970), which provides a complete description of evolutionary change in a trait, yielding

$$\begin{aligned} \Delta\overline{TP} &= cov\left(w, 1+\sum_{j=1}^{n} p_j TP_j\right) + E(w\Delta TP) \\ &= \sum_{j=1}^{n} cov(w, p_j TP_j) + E(w\Delta TP) \end{aligned} \tag{3}$$

where $\overline{TP}$ is the mean TP of the focal consumer population and w is individual relative fitness. The first term on the right-hand side of Equation 3 describes the covariance between relative fitness and TP. This term is a selection differential describing within-generation change in TP due to selection ($\Delta\overline{TP}_s$; Lande & Arnold, 1983). The second term in Equation 3, $E(w\Delta TP)$ describes transmission bias, or fidelity of transmission of TP from parents to offspring. Thus, this second term captures effects such as imperfect heritability and changes in prey trophic position, including independent environmental changes in prey TP (e.g. arising from the insertion mechanism).

We can expand the first selection differential term of Equation 3 (assuming no third moments; Bohrnstedt & Goldberg, 1969), to gain insight into the contributions to selection of TP,

$$\begin{aligned} &\sum_{j=1}^{n} cov(w, p_j TP_j) = \\ &\sum_{j=1}^{n} \left[\overline{p_j} cov(w, TP_j) + \overline{TP_j} cov(w, p_j)\right] \end{aligned} \tag{4}$$

Equation 4 indicates that two main sources contribute to the total selection of TP. The first term, $\sum_{j=1}^{n} \overline{p_j} cov(w,\ TP_j)$, describes the covariance between prey TP and consumer fitness, weighted by the proportional diet content of each prey type. This covariance term will be nonzero when prey j vary in their TP and this variation covaries with consumer fitness. The second term in Equation 4, $\sum_{j=1}^{n} \overline{TP_j} cov\left(w, p_j\right)$, fitness effects arising from variation in consumer diet content weighted by the average TP of the prey item.

Partitioning this selection differential illustrates that selection of TP can occur through independent contributions from the selection of prey TP and of diet content. These terms will reflect total selection; including that arising from selection for correlated traits even if diet or TP have no causal effects on fitness. To explore how causal effects on fitness contribute to total selection, we can define individual fitness explicitly,

$$w = \mu_w + \beta_{TP} TP + \sum_{j=1}^{n} \beta_{p,j} p_j + \epsilon \tag{5}$$

where a consumer's fitness is causally determined by both its TP ($\beta_{TP} TP$) and independently by its diet content ($\beta_{p,j} p_j$) (see also Lande & Arnold, 1983). Although we focus on selection on the actual traits (diet proportions), selection on linear combinations of traits (e.g. a PCA of diet content) can readily be transformed to such (Chong et al., 2018). Substituting this definition of fitness into Equation 4 and expanding yields

$$\begin{aligned} \Delta\overline{TP}_s = &\sum_{j=1}^{n} \beta_{TP}\left[\overline{p_j} cov\left(TP, TP_j\right) + \overline{TP_j} cov\left(TP, p_j\right)\right] \\ &+ \sum_{j=1}^{n}\sum_{k=1}^{n} \beta_{p,k}\left[\overline{p_j} cov\left(p_k, TP_j\right) + \overline{TP_j} cov\left(p_k, p_j\right)\right] \end{aligned} \tag{6}$$

Equation 6 illustrates that direct selection on TP (β_{TP}) and direct selection on diet content (β_p) can both contribute to the total selection of TP. In both cases, the effects are mediated by the phenotypic (co)variances

Box 2 Continued

between TP and diet content. Note that we can arrive at an analogous expression to Equation 6, but where covariances are genetic, by expanding $cov\left(w,\ a_{TP}\right)$, where $a_{TP} = [\mu_{p_j} a_{TP_j} + \mu_{TP_j} a_{p_j} + a_{TP_j} a_{p_j}]$ is the genetic value for individual consum*er TP, resulting in the breeders equation for TP.*

This mode*l of TP evolution yields several insights. First, it illustrates that consumer TP* can influence fitness and selection completely independently of variation in diet content if variation in trophic position within a prey type exists. If such within-prey variation does not exist, causal fitness effects of consumer TP (β_{TP}) can still exist and contribute to total selection on TP and its evolution, and these effects are mediated by variation in diet (e.g. see Figure 4), specifically the phenotypic covariance between diet proportions and consumer TP, $cov(TP, p_j)$. Direct selection on diet content, β_p, generates a selection of TP even if there is no causal relationship between TP and fitness, and this effect is mediated by the phenotypic variance in diet, $cov\left(p_k, p_j\right)$ (note that this is variance where k = j), and the covariance between prey TP and diet, $cov\left(p_k, TP_j\right)$.

Second, this model illustrates that selection on TP can readily be measured and partitioned empirically. When fitness measures or proxies are available, total selection cov(w, TP) can be calculated and the parameters of Equation 5 estimated in a multiple regression (Lande & Arnold, 1983).

Although selection on TP may occur regardless of how variation in TP arises, TP must be transmitted from parents to offspring (measured at the same life stage) for evolutionary change in TP to occur. Although we may expect diet content to often be heritable (see main text), our model also illustrates that transmission fidelity of within-prey TP (TP_j), when it occurs, will also be important for the evolution of TP.

moths') herbivorous larva transition to blood feeding adults (Hill et al., 2010). In *Micropterus salmoides* (largemouth bass) trophic position increases as individuals grow larger, allowing them to switch from feeding on invertebrates to fish (Post, 2003). In this case, even within an age cohort of largemouth bass (i.e. young-of-the-year), individuals that hatched earlier in the season with higher growth rates were the first to transition to piscivory. While such ontogenetic niche shifts are common, studies rarely focus at the individual level on the developmental reaction norms of trophic position, so it is often unknown whether the trophic position trajectories over an individual's lifespan are heritable and how they might evolve, or not, over time.

In addition to arising from diet variation among individuals, trophic position can also change in response to changes in the underlying structure of trophic interactions in the food web (Figures 1c, 2). This can occur when determinants of food web structure, such as ecosystem size and productivity (Post, 2002; Post et al., 2000), are independent of a focal consumer's diet. For example, non-prey species of a focal consumer could be added or removed from a food web, causing a change in the trophic position of the focal consumer without any change in its diet (insertion mechanism; Figure 2) (Post & Takimoto, 2007). A similar effect could arise from altered trophic interactions among prey species in the food chain leading up to the focal consumer (Post & Takimoto, 2007). Such changes in trophic position might be prevalent in natural systems, however, they do not represent evolutionary change in trophic position because they are entirely driven by the external environment and can occur without any genetic or phenotypic changes in the focal population (e.g. if trophic structure changes but diet does not).

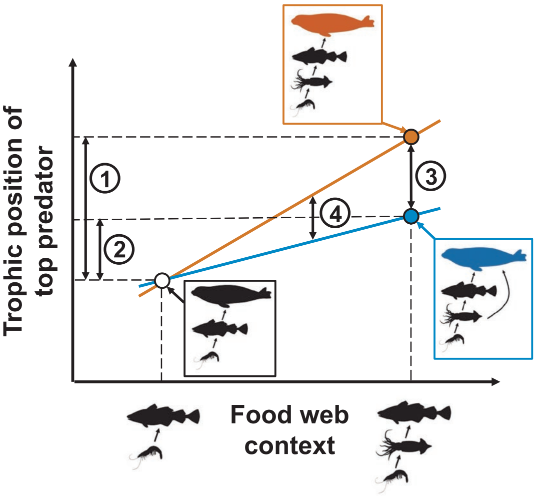


FIGURE 2 Shifts in the trophic position of a focal species (e.g. a seal in a marine food web) can result from multiple mechanisms. When there is a new food web context (e.g. the addition of squid to the food chain) the trophic position expression of a focal consumer can change either (1) without a diet change (see 'insertion mechanism' described in main text) or (2) with a diet change. Evolutionary change in trophic position can occur either (3) when there is heritable change in the mean trophic position of the population within a given food web context or (4) when there is a change across generations in the population distribution of norms of reaction for trophic position of different genotypes (i.e. orange and blue lines) and a variable food web context (i.e. variation in squid occurrence over time or space)

As we discuss further below, the more relevant sources of variation to understand the evolution of trophic position arise from the determinants of individual variation in diet, and the extent to which variation in the trophic position of dietary items can explain fitness variation within consumer populations.

The variation in trophic position relevant to understanding its evolution is that which arises from heritable phenotypic differences among individuals. There is growing evidence that variation in trophic position within populations can be correlated with heritable traits that are associated with foraging performance, such as body size, aspects of the foraging apparatus and behaviours (Cucherousset et al., 2011; Dumont et al., 2016; Matthews et al., 2010; McCarthy et al., 2004; Musseau et al., 2020; Post, 2003; Wagner et al., 2009). For instance, Matthews et al. (2010) showed a correlation between trophic position and gill raker morphology in *Gasterosteus aculeatus* (threespine stickleback)—a heritable trait that is relevant for foraging performance in the pelagic habitat of lakes (Robinson, 2000). Heritable behavioural traits, such as boldness, activity levels or prey selectivity, could also prove to be important determinants of trophic position variation. For example, intraspecific variation in the predatory seabird *Stercorarius skua* (great skua) likely arises from dietary specialisation (fish vs. seabirds), which in turn affects the levels of contamination with biomagnifying pollutants (Leat et al., 2019). However, previous reviews of such behavioural traits highlight the ongoing challenge of quantifying both their heritability and their role as determinants of individual diet variation (Araújo et al., 2011; Bengston et al., 2018; Sih & Bell, 2008).

Trophic position evolves within a hierarchy of heritable traits that collectively influence an individual's fitness in a given food web. Variation in survival and reproduction directly result from individual performance differences in an environmentally relevant context, such as foraging, avoiding predators and finding mates (Arnold, 1983; Garland & Losos, 1994). Underlying this variation are 'performance traits', such as prey capture rate, locomotor speed and olfactory perception that constrain which behaviour an animal can express in a given environment (Garland & Losos, 1994). Performance traits, in turn, are determined by multiple underlying morphological and physiological traits (Arnold, 1983). This morphology/physiology → performance → behaviour → fitness paradigm is a useful starting point for thinking about how selection acts on ecologically relevant traits, such as trophic position (Figure 1a) (Bolnick & Araújo, 2011). Trophic position is at a high-level in the trait hierarchy, arising from dietary differences among individuals that result from variation in behaviour, performance, physiology and morphology (Bolnick et al., 2003). As such, trophic position might integrate some of the fitness effects of multiple subordinate traits, as we discuss below.

HERITABILITY OF TROPHIC POSITION

Intraspecific variation in consumer trophic position likely emerges from both environmental and genetic determinants, though our understanding of the latter is relatively poor. Environmental variation can affect the availability and accessibility of resources, along with the distribution of trophic positions among prey (Gibb & Cunningham, 2011; Lei et al., 2019; van Rijssel et al., 2017; Tewfik et al., 2016). For example, the trophic position of *Ocypode quadrata* (ghost crabs) varies in relation to the width of their beach habitat, which determines the composition and trophic structure of their prey communities (Tewfik et al., 2016). Alternatively, variation in trophic position within a population may arise from phenotypic differences in resource exploitation that have underlying genetic causes (Figure 1). Heritability is crucial for traits to evolve by natural selection, and a variety of approaches exist to help understand how genetic and environmental factors interactively shape trait variation (Lynch et al., 1998). While these approaches have not been used to explicitly address the heritability of trophic position or its reaction norms, studies on the heritability and genetic basis of dietary variation, such as the degree of omnivory (Dumont et al., 2016), predation (Konczal et al., 2016), cannibalism (Wagner et al., 1999) or dietary specialisation (Richards et al., 2021) suggest that trophic position likely has underlying genetic causes. For instance, both predation rate and diet preference (animal vs. plant diet) of the omnivorous insect *Campylomma verbasci* (mullein bug) exhibit heritable variation, and jointly affect the expression of omnivory by individuals (i.e. trophic positions are between 2 and 3) (Dumont et al., 2016, 2017).

Despite our lack of understanding about the genetic basis of trophic position, its position in the trait hierarchy means it will likely be correlated with other heritable traits. In simple terms, if trophic position is largely determined by the expression of a heritable trait, then variation in trophic position would also have underlying genetic causes via mediated pleiotropy (Solovieff et al., 2013). There are some trivial situations, for example, when the potential for phenotypic variation is low (Figure 1c), where we would not expect to find heritability. We would expect limited heritability in populations where individuals feed on either few diet items (i.e. species with highly specialised diets), or many diet items with limited variation in trophic position (e.g. strict herbivores). Importantly, we expect limited heritability when the environmental conditions do not provide an opportunity for the expression of trophic position variation among individuals. The expected amount of heritability in trophic position will also depend on the underlying structure of the trait hierarchy. High-level traits, such as life history and behaviour, generally have low heritability (Mousseau & Roff, 1987) due to their proximity to fitness and cumulative environmental contributions to their expression (Price & Schluter, 1991). Therefore, as an increasing number of traits contribute to trophic position, we might expect the contribution of environmental sources of variation to increase and heritability to decrease.

As with other heritable traits whose expression varies with the environment, trophic position can be understood in the context of phenotypic plasticity, with the norms of reaction of trophic position measured in relation to variation in food web configuration (Figure 2). For example, Dumont et al. (2017) exposed multiple families of *Campylomma verbasci* to two different resource settings (diets of either only animals, or animals and pollen), and found that the time spent foraging on animals (i.e. a behavioural trait associated with trophic position) was heritable in both settings. Furthermore, families differed in their phenotypic response to the environmental treatments, such that some remained largely carnivorous irrespective of the resource environment, while others adopted a more herbivorous strategy when pollen was available (Dumont et al., 2017).

It is increasingly recognised that phenotypic plasticity may facilitate the emergence of morphological and dietary novelty (Sommer, 2020). For example, tadpoles of the genus *Spea* (western spadefoot toads) express plastically induced alternative morphs (omnivores vs. carnivores), depending on the presence of large animal prey in the environment (e.g. other tadpoles, fairy shrimp), whereas most closely related anurans produce only omnivorous tadpoles (Levis et al., 2018). These morphological and behavioural innovations in *Spea* that go along with an increase in trophic position, likely arose from the selection on ancestral, non-adaptive variation in reaction norms (refinement) between alternative resource environments that is still present in contemporary populations of closely related species (Levis et al., 2018). While the carnivorous morph is environmentally induced in most *Spea* species, some populations of *S.bombifrons* that live in sympatry with another *Spea* species produce carnivorous tadpoles irrespective of the environment (Levis et al., 2017). In these populations, genetic assimilation has likely led to the loss of environmentally induced variation in gene expression and the emergence of environment-independent novelty in morphology (Levis et al., 2017), and ultimately, to the evolution of trophic position within and among species (Levis et al., 2018).

EVOLUTION OF TROPHIC POSITION BY NATURAL SELECTION: DIRECT AND INDIRECT MECHANISMS

The environmental causes of fitness variation (agents of selection) can vary in consistent ways along food chains, and thereby generate covariation between trophic position and fitness (i.e. selection) (Box 2). Identifying these general patterns in how selective environments vary along food chains can help us predict whether shifts in trophic position result either directly from a covariance between trophic position and fitness, or indirectly from covariances between trophic position and other traits under selection (Box 2; Equation 6) (Price & Langen, 1992). With increasing height in the food chain, for example, we might expect: (1) a decrease in the overall biomass and thus of available energy (Arim et al., 2007), (2) a change in the strength of species interactions (e.g. competition) (Cropp & Norbury, 2020), (3) an increase or decrease in food quality (Denno & Fagan, 2003) and (4) an increase in the concentration of toxicants (Vander Zanden & Rasmussen, 1996). We explore each of these in turn.

Biomass is often unequally distributed across food webs and typically decreases with increasing trophic position (Hatton et al., 2015; Trebilco et al., 2013), although inverted trophic pyramids do exist (Woodson et al., 2018). As a result, trophic structure may constrain the upper trophic position limit of consumers, due to energy limitations on the maintenance of viable predator populations (Arim et al., 2007). However, the distribution of biomass along food chains can also impact fitness variation among individuals within consumer populations, and this relationship can change over time. For example, the evolutionary assembly of trophic structure in Lake Victoria, occurring over the past 15 000 years, has culminated in an incredibly trophically diverse food web of over 500 cichlid fish species (McGee et al., 2020; Seehausen, 2015). The evolution of predatory cichlid species probably occurred only after considerable population expansion and diversification of more generalist cichlids at lower trophic positions (Seehausen, 2015). At some point over the course of the radiation, omnivorous populations probably experienced positive selection for trophic position, potentially when there was sufficient fish biomass to favour more predatory individuals (Harmon et al., 2019).

The distribution of competitors along food chains can impose strong selective gradients that may influence the likelihood of trophic position evolution. In some cases, competition could promote the evolution of a higher consumer trophic position, for example, if it becomes more beneficial to prey upon a competitor than to compete for the common resource (Cropp & Norbury, 2020). In other cases, competition may cause the evolution of lower trophic positions of consumers. For example, the invasion of centrarchids (*Micropterus dolomieu* and *Ambloplites rupestris*) to lakes inhabited by *Salvelinus namaycush* (lake trout) has led to steep declines in the trophic position of *S. namaycush*, as they shift from a more piscivorous to planktivorous diet (Vander Zanden et al., 1999). In this particular example, the shift was likely due to plasticity, but such competitive interactions might cause selection against lake trout individuals that were less proficient at planktivory (e.g. due to morphological constraints).

Resource quality can vary in predictable ways between adjacent trophic levels, setting up the possibility for adaptive evolution of consumer trophic position. For arthropod consumers, food quality (in terms of nitrogen content) differs between plants (poor quality) and herbivorous prey (high quality), and further increases with the trophic position of animal prey (Denno & Fagan, 2003). This distribution of nitrogen along the food chain may have fitness implications for consumers such as the jumping spider *Portia fimbriata*, which have better survival when feeding on higher trophic level prey (Denno & Fagan, 2003; Li & Jackson, 1997). As another food quality example, many of the phytosterols that are essential for the reproduction of the lady beetle *Coccinella septempunctata* are produced only by plants but not aphids. Therefore, lady beetles with a trophic position of 3 (foraging exclusively on aphids), would have lower fitness than those feeding as omnivores (i.e. trophic position <3) (Ugine et al., 2019).

Directional selection gradients for trophic position could result from biomagnification of organochlorine pollutants in food webs (Kiriluk et al., 1995; Vander Zanden & Rasmussen, 1996). If there is a positive correlation between the trophic position of organisms in a food web and pollutant concentration in tissues, then individual predators might face fitness costs when feeding on organisms at higher trophic levels (Leat et al., 2019). This could lead to negative selection gradients for trophic position within such predator populations. Indeed, for *Larus hyperboreus* (glaucous gulls), a top predator in arctic food webs, trophic position is positively correlated with pollution load (Sagerup et al., 2002), which, in turn, is negatively correlated with reproductive success and adult survival (Bustnes et al., 2003).

In addition to the food chain distribution of individual selective agents, covariation among multiple selective agents along food chains can also affect trophic position evolution. For example, the distribution of resource quality along food chains can covary negatively with the distribution of resource biomass (Fagan et al., 2002). Because both resource biomass and quality are potential agents of selection on the trophic position of consumers, their covariation can lead to consumers trading off between foraging on high-quality and rare resources rather than low-quality and abundant resources. Such a trade-off has been proposed to explain the evolution of omnivory and intraguild predation in arthropods (Denno & Fagan, 2003). More generally, selection gradients for trophic position can emerge from the interaction of multiple, putatively independent, agents of selection (e.g. resource biomass and quality, pollutants, etc.), and from how such agents of selection are distributed across food chains (Figure 3).

In addition to the direct causal relationships between trophic position and fitness mentioned above, trophic position can also evolve as a correlated response to selection on genetically correlated traits (Box 2; Equation 6; second term). Genetic correlations between phenotypes can arise when a locus independently affects two phenotypes (biological pleiotropy) or when one heritable phenotype is causal for another phenotype (mediated pleiotropy) (Solovieff et al., 2013). It is well known that selection acting on one trait can affect genetically correlated traits, leading to an evolutionary response of traits that are unrelated to fitness (Price & Langen, 1992). For instance, artificial selection on four different behavioural traits underlying predation behaviour (consumption rate, conversion efficiency, dispersal and olfactory attraction to prey) of predatory mites (*Phytoseiulus persimilis*), led to correlated responses among these traits, and revealed genetic correlations in phenotypes related to predation (Nachappa et al., 2010). However, in this example the trophic position would not be affected, since the consumer is a specialist, (i.e. the evolution of predation behaviour is not affecting diet composition). Yet, similar heritable variation exists in consumers with diverse prey spectra (Dumont et al., 2017), and selection on phenotypes in the trait hierarchy might cause the evolution of trophic position, without it having direct fitness consequences (Dumont et al., 2018).

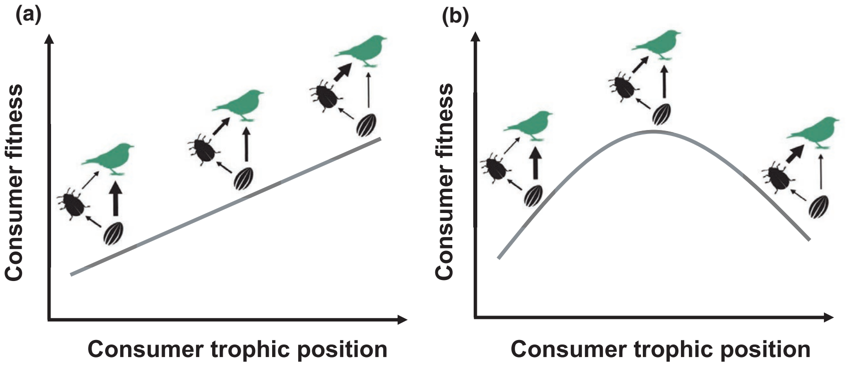


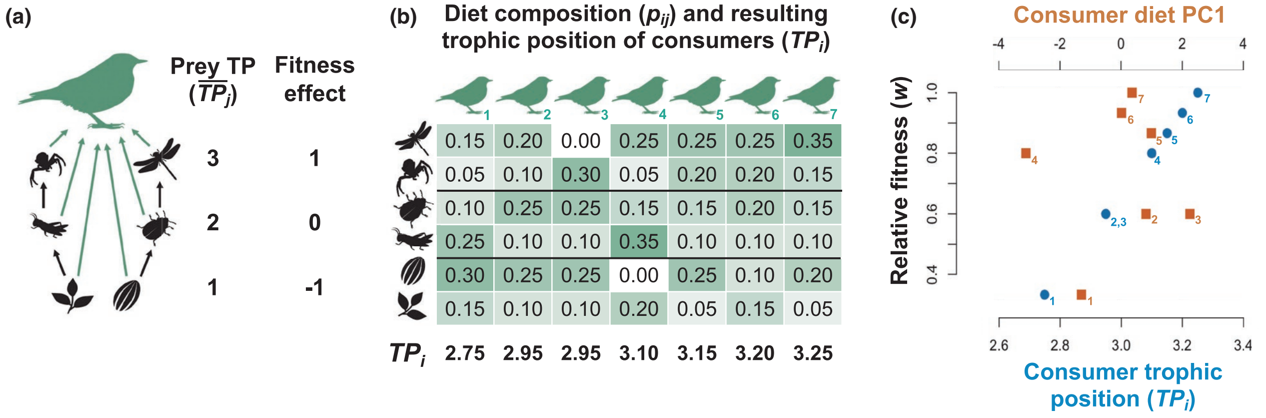
FIGURE 3 Trophic position can become associated with fitness variation when the fitness effects of the resources are structured along the food chain. (a) In a population of omnivores (i.e. individual birds ranging between trophic position 2 and 3) feeding on a higher trophic position can be positively correlated with fitness, for example, because animals are generally higher quality food than plants. (b) However, foraging entirely on animals might come with a fitness cost if they are less abundant than plants. This might create a trade-off between foraging on abundant resources (usually plants) and food quality (usually animals), creating selection gradients with an optimal trophic position for individuals with intermediate levels of animal prey in their diet

A prevailing challenge in evolutionary ecology is to determine the ecological mechanisms underpinning trait evolution (Henshaw et al., 2020; MacColl, 2011; Wade & Kalisz, 1990), and the same challenge holds for understanding the evolution of trophic position by natural selection. In a study of *Gasterosteus aculeatus* populations, Bolnick and Araújo (2011) found covariation among trophic position, foraging traits (gill raker morphology), and individual growth rate (a proxy for fitness). They used path analysis to partition the fitness effects of diet (trophic position and habitat use; estimated using isotopes analyses) and morphology, and found that diet explained fitness variation better than morphology, and that the apparent correlation between foraging morphology and fitness arises indirectly from a correlation between morphology on diet. In one (but not all) of the lakes in this study, individuals foraging at a lower trophic position diet attained higher growth rate, and trophic position explained more of the variation between individuals in growth rate than did habitat use (Bolnick & Araújo, 2011). Thus, in some situations trophic position can be under selection due to its effects on fitness, however, the ecological mechanisms that promote such situations are largely unknown.

DISCUSSION

Phylogenetic analyses of dietary preferences have revealed that macro-evolutionary shifts in both diet and trophic position are common in many clades, and often associated with eco-morphological variation among species (Burin et al., 2016; Ingram & Shurin, 2009; Muschick et al., 2012; Price et al., 2012; Román-Palacios et al., 2019; Shi et al., 2021). Such diversification is ultimately responsible for the emergence of trophic structure in ecosystems, and there is growing evidence that evolutionary processes play an important role in the emergence and structure of ecological networks in general (Segar et al., 2020), and food webs in particular (Gibert & Yeakel, 2019; Loeuille & Loreau, 2005; Segar et al., 2020). Trait differences among species arise from processes acting on intraspecific variation, and some study systems provide insight into how differences in trophic position between species can arise from genetic variation within populations (Levis et al., 2018; Richards et al., 2021). For instance, the increase in trophic position in *Spea* tadpoles likely arose from a refinement of ancestral variation in phenotypic plasticity in an omnivorous ancestral population, which led to the emergence of a novel polyphenism, and the subsequent fixation of carnivory (Levis et al., 2018). However, we know little about how ecological processes and natural selection jointly act on intraspecific variation in trophic position, and how this affects the evolutionary trajectories of populations, creating a gap in our understanding of how macroevolutionary patterns arise from microevolutionary processes.

So far, we have discussed the sources of intraspecific variation in trophic position and highlighted that variation among individuals is not only determined by the food web context but also by genetic factors underpinning associated fitness-relevant traits. We argue that trophic position, measured at the individual level, can be studied as a heritable quantitative trait that can evolve via natural selection (Box 2). However, whether it is generally useful to study trophic position as an evolving trait depends on its importance in explaining fitness variation relative to other causally linked traits, such as dietary variation (Figure 4). If the fitness of consumers is determined by the position of their prey in the food



	1	2	3	4	5	6	7
	0.15	0.20	0.00	0.25	0.25	0.25	0.35
	0.05	0.10	0.30	0.05	0.20	0.20	0.15
	0.10	0.25	0.25	0.15	0.15	0.20	0.15
	0.25	0.10	0.10	0.35	0.10	0.10	0.10
	0.30	0.25	0.25	0.00	0.25	0.10	0.20
	0.15	0.10	0.10	0.20	0.05	0.15	0.05
TP_i	2.75	2.95	2.95	3.10	3.15	3.20	3.25

FIGURE 4 Causal fitness effects of consumer trophic position (β_{TP}) can be mediated by dietary variation (Box 2). (a) Prey items may vary in trophic position $\overline{TP}_j$ and in their effect on consumer fitness, and these fitness effects can be structured along the food chain (e.g. when nutritional quality increases along the food chain). (b) Differences in the dietary composition among consumers then lead to variation in consumer trophic position (TP; Box 1; Equation 1), as well as variation in fitness resulting from the covariance between p_j and fitness (Box 2; Equation 4). When consumers forage on a variable diet (in terms of both trophic position and prey identity) the link between diet and trophic position can decouple (i.e. individual consumers can have the same trophic position but different diets; note birds 2 and 3). (c) If the trophic position of prey has casual effects on fitness, the trophic position of the consumer can explain fitness variation that is unexplained by diet composition (i.e. illustrated here by a principle component analysis of the diet - PC1)

web, rather than other aspects of the prey, then trophic position will be the target of selection and diet will change as a correlated response. Identifying the target of selection is especially important because consumers can have different diets but identical trophic positions (Vander Zanden et al., 1997). Such 'many-to-one' mapping presents a general challenge for understanding the evolutionary causes of phenotypic variation because it decouples selection on high-level traits from the selection on underlying traits. For example, organism can apply different morphological solutions to resolve selection on mechanical performance (e.g. locomotion), which weakens parallel morphological evolution (Muñoz, 2019). 'Many-to-one' mapping of diet to trophic position may lead to different dietary outcomes resulting from selection on trophic position (Figure 4).

Gathering evidence of trophic position evolution

The strong environmental dependence of the expression of trophic position poses some challenges for comparative and experimental studies of trophic evolution. In the wild, patterns of trophic position variation along environmental gradients (i.e. phenotype-environment correlations: Figure 5a) can help generate some hypotheses about the drivers of trophic position evolution. However, such comparative approaches need to account for ontogenetic sources of variation in trophic position, and, more generally, variation that arises from genetic and/or environmental causes, specifically food web configurations. This can be done, for example, with breeding experiments and quantitative genetic analyses (Lynch et al., 1998), but such approaches have not been explicitly applied to trophic position. Indeed, combining estimates of trophic position (e.g. from stable isotopes, or dietary studies) with pedigree data (Kruuk et al., 2008), either in a comparative or experimental context, could reveal new statistical insights into the genetic variation underlying trophic position. Additionally, as with any heritable phenotype, one could attempt to identify regions of the genome that are associated with trophic position variation. This could involve treating trophic position as a continuous trait within a QTL framework (quantitative trait loci; where laboratory crosses have the possibility to feed on a variety of prey with varying trophic position, and the subsequent variation in offspring trophic position can be linked to inherited allelic variation) or in a GWAS (genome-wide association studies; where individual markers along the genome are correlated with trophic position across a large number of individuals) (Bengston et al., 2018). In both of these cases the number and distribution of loci associated with trophic position, along with the variance in trophic position explained by these loci could be elucidated. While challenging for behavioural traits, such approaches might help identify the genetic basis of trophic position variation and its underlying traits (i.e. traits lower in the hierarchy), provided that such studies are applied in appropriate food web contexts (Arnegard et al., 2014; Bengston et al., 2018).

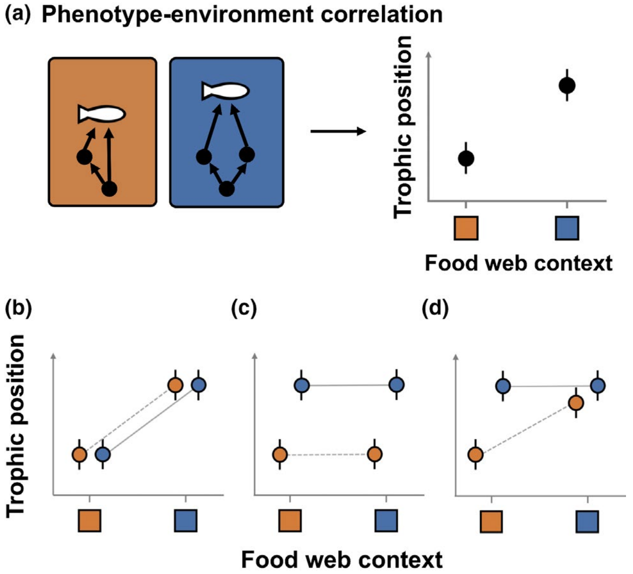


FIGURE 5 Hypothetical outcomes of a reciprocal transplant experiment on two populations with divergent trophic position: (a) From observational data, it is not possible to infer whether divergence in trophic position between two populations (fish) arises from different food web contexts or from genetic differences between the populations. Exposing individuals to the respective other food web context might reveal that (b) trophic position variation is entirely driven by the food web context (i.e. is fully plastic), (c) is entirely driven by the genetic identity of the individuals or (d) a combination of the two (i.e. genotype-by environment-interaction)

Common garden experiments are another useful approach to isolate the influence of the food web context and genetic background on trophic position variation. By establishing a common garden food web design, one can isolate the role of genetic variation in determining trophic position. Furthermore, by including more than one common environment (e.g. food webs with contrasting structure), potential interactions between genotypes and environments can be revealed (i.e. variation in phenotypic plasticity/non-parallel reaction norms). A general challenge with trophic position is that it is highly dependent on the food web context, and artificial experimental food webs might not provide the meaningful context for trophic position expression. Therefore, common garden experiments involving reciprocal transplants in the wild (Figure 5) could be applied to ensure a more meaningful food web context. This approach would have the additional benefit of providing information about the fitness relevance of trophic position variation.

Concluding remarks

The idea of an evolving trophic position is not new to evolutionary biology. The origin and maintenance of trophic diversity is a central theme in adaptive radiation research (Harmon et al., 2019; Muschick et al., 2012), and phylogenetic studies often focus on broad patterns of trophic position variation and transitions between trophic strategies across the tree of life (Burin et al., 2016; Price et al., 2012; Román-Palacios et al., 2019). Evolutionary ecologists often quantify variation in the trophic ecology of populations in contrasting environments (Zandonà et al., 2017), as well as in the context of micro- and macroevolutionary analyses (Arnegard et al., 2010; Ingram & Shurin, 2009; Wagner et al., 2009). At microevolutionary scales, models often describe shifts in resource use, including those that affect trophic position, and culminate in the emergence of multiple trophic levels (Cropp & Norbury, 2020; Ingram et al., 2009).

Here, we argue that viewing trophic position as a quantitative trait that can be correlated with fitness variation can provide novel insights into causal mechanisms of natural selection (i.e. how do traits evolve?). The robustness of such inferences inevitably depends on understanding the relationships between traits, fitness components and the environment (Henshaw et al., 2020). For example, classical evolutionary hypotheses linking intraspecific variation in morphology, diet and fitness, such as the niche variation hypothesis (Van Valen, 1965), are often not explicit about the trophic structure of the consumers' resources (Bolnick et al., 2007).

Models that examine the role evolutionary processes in structuring food webs typically involve selection on traits governing species interactions (e.g. via attack rates, body size, etc.) that ultimately lead to shifts in trophic position (Cropp & Norbury, 2020; Gibert & DeLong, 2017; Gibert & Yeakel, 2019; Loeuille & Loreau, 2005). However, they usually do not consider variation in trophic position as a potential source of fitness variation. In Box 2, we outline a standard evolutionary framework for studying trophic position of single consumer species that can also be expanded to understand coevolution of trophic position in complex food webs and other interactions. For example, when fitness functions of interacting species depend on evolving traits of each species, these interactions can be explicitly defined in the equations for fitness that are then used to expand Price's theorem to explore models of between-species coevolution (De Lisle et al. 2021). Such an integration of trophic position into the hierarchy of traits that contribute to fitness, might help discover new causal pathways connecting ecologically relevant traits with fitness, and improve our understanding about the evolutionary determinants of species interactions and food web structure.

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AUTHORSHIP

All authors conceived and formulated the ideas presented. MM and BM led the writing of the manuscript, SDL formulated the mathematical model in box 2, and all authors provided critical feedback.

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Chapter 2

The phenotypic determinants of diet variation between divergent lineages of threespine stickleback

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Abstract

Lineages with independent evolutionary histories often differ in both their morphology and diet. Experimental work has improved our understanding of the links between the biomechanics of morphological traits and foraging performance (trait-utility). However, because the expression of foraging-relevant traits and their utility can be highly context-specific, it is often unclear how dietary divergence arises from evolved phenotypic differences. Here, we explore the phenotypic causes of dietary divergence between two genetically and phenotypically divergent lineages of threespine stickleback (*Gasterosteus aculeatus*) with independent evolutionary histories of freshwater colonization and adaptation. First, using individuals from a line-cross breeding design, we conducted 150 common-garden foraging trials with a community of multiple prey species and performed morphological and behavioural analyses to test for prey-specific trait-utility. Second, we tested if the traits that explain variation in foraging performance among all individuals could also explain the dietary divergence between the lineages. Overall, we found evidence for the utility of several foraging traits, but these traits did not explain the observed dietary divergence between the lineages in common garden. This work suggests that evolved dietary divergence results not only from differences in morphology but also from divergence in behaviours that underlie prey capture success in species-rich prey communities.

Keywords: Trait utility, dietary divergence, foraging, trait evolution

Introduction

Lineages with independent evolutionary histories often show divergence in both morphology and resource-use (Grant et al. 1976; Herrel et al. 2008; Bassar et al. 2010; Muschick et al. 2012). It is typically assumed that dietary variation between individuals arises from phenotypic differences that affect their ability to capture different prey (Araújo et al. 2011), but the links connecting morphology, behaviour, and dietary divergence among populations are often unclear. The ecomorphological paradigm provides a mechanistic framework for understanding the linkages between morphology, performance, and fitness (Arnold 1983). In relation to foraging, both morphological and behavioural traits determine an organism's performance in a particular environmental context, where performance measures can include rates of prey encounter and attack, the kinematics of prey capture, prey processing and overall rate of consumption. Extensive experimental work, particularly involving biomechanics (Higham et al. 2021), has improved our understanding about the relationships between traits and foraging performance in vertebrates, such as fishes (Wainwright and Richard 1995; McGee et al. 2013; Muñoz 2019), birds (Parchman and Benkman 2002), mammals (Piras et al. 2013) and reptiles (Herrel et al. 2004). However, compared to morphology and performance traits, diet is more proximal to the outcomes of fitness relevant tasks (i.e. energy acquisition for survival and reproduction) (Storz et al. 2015), and we often have a poor understanding of how functional trait variation translates into realised diet variation in natural populations. This makes it challenging to understand the underlying ecological causes of evolutionary divergence of consumer diets, particularly if diet variation is complex in nature, and trait utility is inferred from experiments in simple environmental contexts.

Comparative studies can help us develop hypotheses about the functional links between morphology and diet, but are often unable to establish how morphological variation translates into feeding performance, diet variation, and ultimately fitness variation. This is largely because dietary variation in natural consumer populations is determined by both contemporary resource availability, and the phenotypes of individuals that are expressed in a given environmental context (Araújo et al. 2011). Dietary divergence between populations can result from multiple abiotic and biotic environmental factors, such as habitat structure, prey community composition, and individual interactions with predators and competitors (Araújo et al. 2011; McWilliam et al. 2013; Tewfik et al. 2016). Dietary divergence might correlate with functional trait divergence among populations, if either trait expression depends on resource conditions (Olsson et al. 2007; Levis et al. 2017) or if the traits determining diet in each population have underlying genetic causes (Bolnick et al. 2003). In the latter case, consumer populations might locally adapt to prey communities via, for example, evolution of functional traits in response to local resources (Grant et al. 1976; Herrel et al. 2008; Sanford and Worth 2010; Holding et al. 2018). As a result, testing whether dietary divergence between populations is due to evolved phenotypic differences, plastic trait expression or differences in the resource base, requires rearing and testing the dietary variation of individuals in foraging environments with common prey communities.

Common garden foraging experiments are frequently used to identify the underlying causes of dietary variation, and are ideally performed in an environmental context that is relevant for understanding the observed dietary variation in nature (Bassar et al. 2010; Arnegard et al. 2014). Such experiments can provide insights into the genetic basis of dietary differences. However, divergent populations typically differ in many heritable traits, which can covary within populations, making it challenging to specify links between functional traits and diets. Performing common garden experiments with phenotypically variable experimental populations, can increase the chance of detecting trait utility, and provide a better mechanistic understanding of functional trait-diet relationships (Arnegard et al. 2014). In this respect, a line-cross breeding scheme between ecologically divergent populations is a particularly useful way to generate relevant phenotypic variation in a consumer population (Lynch et al. 1998). This is because hybrids can express phenotypes that are either intermediate between parental lineages, or exceed the phenotypic range of their parents (i.e. transgressive segregation). Hybridization can also change the covariances among traits with putative utility (Holzman and Hulsey 2017). The insights gained from such individual level analyses of trait utility can then be useful for explaining patterns of dietary divergence observed between the parental populations in common garden.

Here, we experimentally test for dietary divergence between two lineages of European freshwater threespine stickleback *(Gasterosteus aculeatus* complex*)*, and explore how this divergence relates to variation in functional traits in a complex resource context (Figure 1). Marine sticklebacks have repeatedly established freshwater populations that have then adapted to local (resource) conditions (McKinnon and Rundle 2002). Due to the glaciation history of Europe, the timing of freshwater population establishment varies widely across the continent. Stickleback populations were likely established in the Western-European watersheds (e.g.

Rhine, Rhône, Seine) prior to the last glacial maximum, and in the North-eastern watersheds (i.e. the Baltic drainage) only after the retreat of the ice sheets ~12,000 years ago (Lucek et al. 2010; Marques et al. 2016, 2019). Recent range expansion and anthropogenic introductions have brought some of these lineages into secondary contact in the perialpine lakes of the Swiss plateau, providing a useful study system to explore ecological differences between lineages with independent evolutionary histories of freshwater adaptation (Lucek et al. 2010; Best et al. 2017; Anaya-Rojas et al. 2019; Hudson et al. 2021b).

Using a common garden feeding experiment, we aim at understanding the functional basis of dietary divergence between two European stickleback lineages that have independently invaded and adapted to freshwater habitats, and show a range of ecological and phenotypic differences in the wild. In a first step, we tested for the relationship between traits and prey-specific foraging performance across a phenotypically variable population derived from a line-cross breeding design. This gave us a general understanding of how traits relate to foraging performance in our experimental context, irrespective of the genetic background of individuals. In a second step, we characterised the dietary divergence between the lineages in common garden. We then tested whether the trait-performance relationships identified at the individual level, can explain evolved dietary divergence between the lineages in a complex environment.

Materials and Methods

Study system

In this study we focus on the stickleback populations of two large perialpine lakes; Lake Constance (Rhine drainage, surface area: 536 km², average depth: 90 m) and Lake Geneva (Rhône drainage, surface area: 580 km², average depth: 154.4m) (Figure 1A). Historical, ichthyological and genomic evidence suggest that both lakes were colonised only in recent historical times (mid 19th century) by two stickleback lineages that diverged 27-11 Kya ago (Fang et al. 2018, Hudson et al. 2021b) and have independently colonised freshwater. Lake Constance was colonised by fish that belong to a lineage of recent freshwater invaders (post-glacially ~17-5 Kya; (Fang et al. 2018, 2020)), originating from a river system that drains into the Baltic Sea (Lucek et al. 2010). On the opposite side of the Swiss plateau, Lake Geneva was colonised by fish that originated from the upper Rhône, and belong to an older, pre-glacial Atlantic freshwater lineage (Lucek et al. 2010; Marques et al. 2016, 2019). Both lakes also received smaller genetic contributions from the other lineages, including reciprocal gene flow between the Lake Constance and Lake Geneva lineages (Lucek et al. 2014a; Marques et al. 2016, 2019).

In accordance with their divergent evolutionary histories and ages of freshwater invasion, Lake Constance and Lake Geneva stickleback differ in various ecological, physiological and morphological phenotypes (Lucek et al. 2013; Alexander et al. 2016; Best et al. 2017, Hudson et al. 2021a). Most notably, Lake Constance harbours stickleback populations that live and

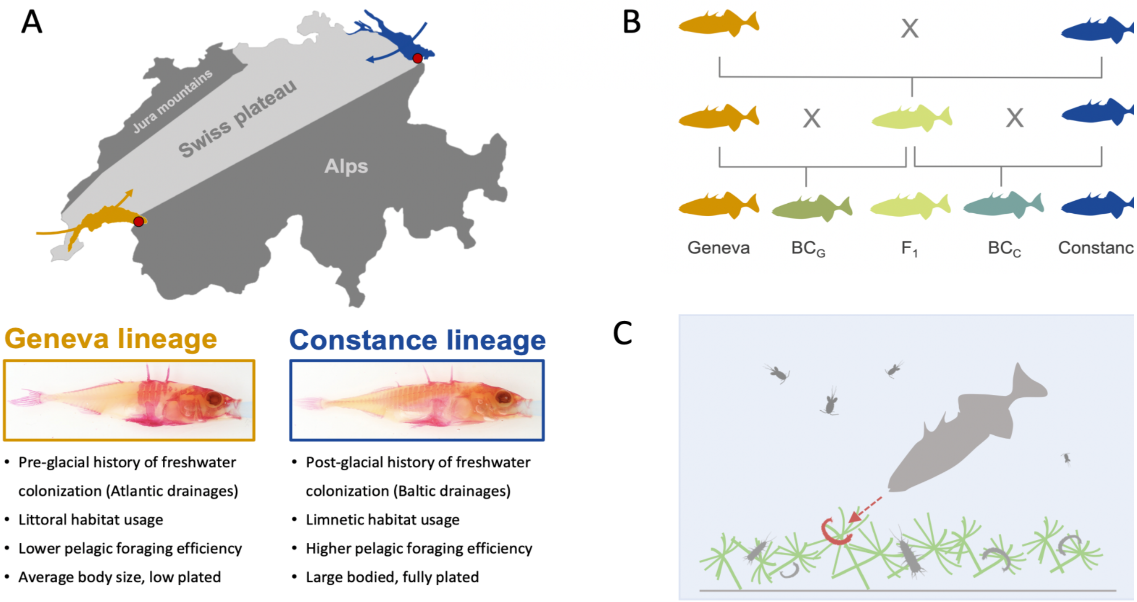

Figure 1. The Swiss plateau was colonized by two lineages of stickleback originating from different European watersheds in recent historical times via Lake Geneva (orange) and Lake Constance (blue) (a). These lineages differ in their history of freshwater colonization, and in various ecological, morphological and physiological traits. Since their introduction to Lake Constance and Lake Geneva, both lineages have undergone range expansion into the Swiss Plateau, where they came into secondary contact, and formed a hybrid zone that includes both lakes. The sampling locations for the stock populations used in this experiment are indicated with red dots. To obtain an experimental population with an increased phenotypic variance, we bred Lake Constance and Lake Geneva fish in a line-cross scheme (c). Individual variation in diet and foraging behaviour was then assessed in a common garden foraging experiment with a complex prey community and related to morphological variation at an individual and cross-type level (c).

grow in the open water of the lake, where they are hyper abundant, but migrate to spawn both in littoral zones and inflowing streams (Alexander et al. 2016). Stickleback from Lake Constance express phenotypes that are more typical of marine populations (e.g. extensive body armour, large body size), whereas those from Lake Geneva are restricted to nearshore habitats and express phenotypes more typical of freshwater populations (e.g. reduction of body armour) (Berner et al. 2010; Lucek et al. 2010, 2013, 2014b). Wild populations also differ in various traits related to resource-use, such as morphology (body depth, gill racer length and eye diameter), physiology (copy number of genes related to fatty acid metabolism) and foraging performance (zooplankton feeding efficiency) (Best et al. 2017, Hudson et al. 2021a). Furthermore, Lake Geneva fish had stronger effects on benthic prey communities in short term mesocosm experiments, suggesting different resource use (Best et al. 2017). Given these ecological and phenotypic differences between the lineages, it has been hypothesised that they also exhibit divergent resource-use in their natural environment, with the pelagic foraging population of Lake Constance specialising on zooplankton (Bretzel et al. 2021; Lucek et al. 2012), and the more littoral populations of Lake Geneva specialising on benthic invertebrates (Best et al. 2017; Hudson et al. 2021b).

Foraging experiment

Line-cross to generate a phenotypically variable consumer population

For our experiments, we created a line-cross between a pelagic population from Lake Constance and a littoral resident population from Lake Geneva stickleback. For this purpose, we used stock populations of the parental lineages and F1 hybrids that we maintained in husbandry (Figure 1B) as described in Hudson et al. (2021a). The stock populations originate from fish sampled in 2013 and 2015 (Ecotype / Sampling locations: lake ecotype / Marina Rheinhof, Lake Constance (47°29'51.5148N, 09°33'33.2064E); littoral lake ecotype / Le Grande Canal, Lake Geneva (46°23´48.372N, 06°53´14.2224E)) (Best et al. 2017). These sampling locations were chosen to represent evolutionary lineages with a minimal history of recent introgression according to previous genetic analyses (Marques et al. 2019; Roy et al. 2015; Best et al. 2017). In May of 2017 we crossed fish in five different parental combinations: Geneva × Geneva (G; 2 families), Geneva × F_1 Hybrid (BC_G; 5 families), Geneva × Constance (F_1; 6 families), Constance × F_1 Hybrid (BC_C; 4 families) and Constance × Constance (C; 6 families). The five resulting offspring populations (from here onwards referred to as cross-types) were reared in the laboratory for approximately 20 months on a diet of frozen chironomid larvae before they were used in the experiment.

Experimental procedure

For the foraging trials, fish (150 total, 30 individuals per cross-type) were individually placed in 12L tanks (20 x 30 x $20cm^3$) that were supplemented with zooplankton, benthic invertebrates, and plant material, and were allowed to forage for 16 minutes (Figure 1C). The foraging trials were conducted in a randomised block design, such that each temporal block contained five fish, with one representative of each line-cross group (i.e. G, BC_G, F_1, BC_C, C), being tested simultaneously. By doing so, we could statistically account for variation that may arise from changes in the environment (e.g. temperature) and prey communities sampled in the wild that occurred throughout the course of our experiments. The zooplankton and benthic invertebrates for the trials were collected from Lake Lucerne (47°00'09.0"N, 8°20'03.2"E) each day before the experiments by pelagic plankton net sampling, and by collecting plant material from a littoral *Chara* bed, respectively. To homogenise the benthic prey availability within experimental blocks, we rinsed off plant material through a sieve (mesh size 0.5mm) retaining macroinvertebrates but washing out fine particles that would otherwise cause turbidity. The separated plant material and the macroinvertebrates were then divided into eight equal parts. Similarly, the zooplankton samples were split into eight equal parts using a plankton splitter. Five samples of zooplankton, plants, and benthos were randomly added to the experimental tanks and one sample was retained as a reference to estimate prey availability and composition (Figure S1). On average, 51g of plant material (wet weight; SE = 2g), 423 (SE = 23) benthic prey items, and 563 (SE = 38.1) pelagic prey items were added to each tank. Thus, our tanks included a diverse prey community that taxonomically overlaps with the invertebrate communities of both Lake Constance and Lake Geneva (Mulattieri 2022; Mulattieri 2022). The most common prey items were isopods (332 individuals per tank on average), calanoid (240) and cyclopoid (210) copepods, chironomid larvae (71), and Daphnia (54). For a more detailed list of prey items and their rank abundance see Figure S1. Shortly after supplementing the tanks with the prey, fish were added behind a divider that separated them from the prey for 10 minutes before the beginning of the feeding trial. Prior to the trials, fish were not fed for 24h. To initiate the trials, we raised the dividers simultaneously in all five experimental tanks, releasing the fish into the foraging chamber. The foraging trials were video recorded for 16 minutes with two cameras per tank (GoPro Hero 7) positioned from the top and the front. To conclude the foraging trials, the fish were removed from the tank, euthanized with an overdose of MS-222, and frozen at -80°C. We conducted all trials between January 15 and February 12, 2019.

Part 1: Detecting prey specific trait-utility

Analysis of foraging behaviour

To analyse fish foraging behaviour, we inspected the video recordings using the event-logging-software BORIS (Friard and Gamba 2016). Four different strike types were manually scored by one observer: *benthic strikes* - those directed at the substrate/ground; *pelagic strikes* - those occurring in the water column; *wall strikes* - those directed at the walls of the tank; and *surface strikes* - those directed at the water surface. While observing these strikes we could neither determine which prey items were being targeted, nor which strikes resulted in successful ingestions. As a result, we relied on gut content analysis to determine individual diet variation (see below). We additionally measured *non-foraging behaviour* as a summation of: *inactivity* - the time a fish spent motionless in the plant material (e.g. hiding), and *self-reflection* - the time moving up and down the glass walls of the foraging tanks, presumably interacting with their own reflection. For all strike types we used generalized linear models (GLM, family = quasipoisson) to test for the effects of non-foraging behaviour and cross-type on the number of strikes.

Measuring prey-specific foraging performance

We measured prey-specific foraging performance as the number of prey items consumed during the 16min foraging trial. We removed the guts of the fish, and inspected their contents via dissection microscope, creating a diet abundance matrix distinguishing 22 prey types (Table 1). In order to simplify subsequent analyses and increase our power to detect trait-utility, we decided to pool prey types into categories. For this purpose, we Hellinger-transformed the diet-matrix, to reduce the influence of prey types with low and zero values, and analysed it with a PCA (Borcard et al. 2011) using the R package vegan (Oksanen et al., 2019). Three prey-types - Cyclops, Chironomidae, and Isopoda - were the most important both in terms of absolute abundance (Table 1) as well as in driving dietary divergence along PC1 and PC2 (see loading depicted in (Figure S2). The relative occurrence of these major prey types in the guts was associated with other, less abundant prey, as is apparent from the angles between the loadings (Figure S2). Using the loadings on PC1 and PC2 as criteria we created three prey categories that tended to co-occur in the guts:

- *'Plankton group'*: All plankton species and the small bodied dipteran larvae of the family Ceratopogonidae; loadings on PC1 < 0

- *'Chironomid group':* represented by chironomidae (91.2%) and associated species; loadings on PC1 > 0 and on PC2 > 0)
- *'Isopod group'*: *Asellus aquaticus* (79.7%) and associated species (loadings on PC1 > 0 and on PC2 < 0)

We additionally measured prey-specific foraging performance as the absolute number of prey items ingested that belonged to the "Plankton group", the "Chironomid group", and the "Isopod group", respectively. More detailed information on the prey composition and their relative contribution to the prey-categories are described in Table 1. We excluded five prey types from the analysis (Pleuroxus sp., Chydorus sp. and Harpacticoida, adult insects, and digested chironomidae remains) that occurred in the guts, but resulted from the feeding history in our husbandry tanks despite a 24h fasting period prior to the trials. To obtain an estimate of the total ingested biomass the gut contents of each fish were dried at 50° C for 12h and weighed.

Analysis of individual morphological variation

After dissection (for gut removal and sexing), we stained the fish with alizarin red and bleached them with hydrogen peroxide to highlight bony features (McGee and Wainwright 2013). The fish were then photographed laterally twice, once with a closed mouth, and once with their mouths open and their jaws fully protruded. On these photographs we placed 11 landmarks to obtain linear trait measurements using the high throughput phenotyping pipeline *phenopype* (Lürig 2021). These landmarks comprise five traits and trait systems that have been previously associated with foraging performance variation in stickleback (McGee et al. 2013; Schmid et al. 2018): i) gape width, ii) epaxial height, iii) jaw protrusion, iv) lever ratio of lower jaw, and v) the kinematic transmission of the opercular four-bar linkage. Because all landmarks measure bony structures of the head they were likely not affected by the staining and the dissection of the fish. For more details see Figure S3 and Table S1.

Table 1: Summary of prey items contained in the guts of the 105 experimental fish used in the analysis. Most experimental fish (93/105) had digested chironomid remains in their guts (N=1071), stemming from the feeding history in husbandry. Similarly, some guts contained species that inhabited the husbandry tanks (adult dipterans (N=5) and the microzoobenthos chydoridae (N=572) and harpacticoida (N=532)) that were not found in the experimental prey communities. These non-experimental prey were thus excluded from any further analyses.

Prey category	Prey type	Total count	% of prey category
Plankton group	Cyclops sp.	1031	72.0%
	Daphnia sp.	192	13.4%
	Eudiaptomus sp.	165	11.5%
	Ceratopogonidae	21	1.5%
	Bythotrephes sp.	12	0.8%
	Bosmina sp.	7	0.5%
	Nauplius	4	0.3%
Chrionomid group	Chironomidae	992	91.2%
	Oligochaeta	92	8.5%
	Ephemeroptera (Larva)	3	0.3%
	Hirudinea	1	0.1%
Isopod group	Isopoda	377	79.7%
	Trichoptera	38	8.0%
	Gammaridae	32	6.8%
	Platyhelminthes	15	3.2%
	Gastropoda	7	1.5%
	Diptera (Pupa)	4	0.8%

Path analysis to identify trait utility

To gain a more comprehensive understanding of how phenotypic variation contributes to prey-specific variation in foraging performance among the individuals, we fitted a Bayesian path analysis to explain the number of ingested individuals that belonged the ‘plankton group’, the ‘chironomid group’, and ‘isopod group’ based on sex and body size, a suite of functional traits (jaw protrusion, epaxial height, gape width, lower jaw lever ratio, opercular-four bar linkage); the number of benthic and pelagic strikes; the total number of available benthic and pelagic prey items; and temporal block as a random effect. In this model, the total weight of the ingested biomass during the experiment was included, and modelled as a function of the abundance of three main prey types. For the hierarchical structure of the model see Figure S4. This full experimental path analysis allowed us to include all predictor and response variables and model the dependencies between traits and performance while simultaneously accounting for other sources of variation. For example, we were not reliant on size correcting trait values, but could both include body size and the (biologically more relevant) raw trait values into the model (Schmid et al. 2018). We did not include cross-type as an explanatory variable in this analysis, because in this step we aimed at understanding trait utility across the full phenotypically diverse experimental population. This gave us more power to detect trait utility relative to analyses either within or between cross-types. We form our conclusions on the basis of interpreting those parameter estimates whose 95% credible intervals of effect size do not overlap with zero (Schmid et al. 2018). To implement the path analysis, we used the R package brms (Bürkner 2018).

Part 2: Relating functional trait variation to dietary divergence between lineages

Dietary divergence between cross-types and parental lineages

To test for dietary divergence between the cross types in general, and the parental lineages specifically, we conducted a distance-based redundancy analysis (db-RDA) using the capscale() function in the R package vegan (Oksanen et al., 2019). This constrained ordination yielded the axis of dietary variation, that are best explained by sex and cross-type. Having reared and tested all fish in the same resource environment, these axes thus represent the evolved dietary divergence between the lineages. We tested for the significant differences in dietary composition on the Hellinger-transformed diet-matrix by permuting cross-type and sex within blocks 999 times. We conducted this analysis including all five cross-types, and including only the two parental lineages (Constance and Geneva). With the reduced dataset we also tested for lineage-effects on the absolute number of ingested items per prey categories using a GLMM model with block as a random effect. Because the distribution of the data was over-dispersed we set family to negative binomial. We estimated p-values in an ANOVA type II analysis with the function ANOVA() from the R package car, which uses Wald Chi-squared tests to estimate p-values (Fox and Weisberg, 2019).

Morphologically divergence

To explore morphological variation among cross-types and sexes, we performed an ANOVA to compare standard length between cross types, and type III ANCOVAs on the traits, including standard length as a covariate using the R package car (Fox and Weisberg, 2019). Because trait-body size relationships often follow power laws, we log transformed all traits in order to linearize this relationship (McCoy et al. 2006). We repeated this same analysis including only Constance and Geneva fish, in order to specifically understand the level of phenotypic divergence between the parental lineages when reared in common garden. Four fish were removed from all morphological analysis because they were damaged during the staining process.

Relating functional phenotypes to dietary variation

While the full-experimental path analysis aimed at identifying traits that are associated with prey-specific foraging performance at the individual level across all individuals, we also wanted to understand if functional trait variation is driving dietary (i.e. compositional) differences between cross-types and sex. To do this, we tested for correlations between the mean trait values of cross-types and sex and their mean position on the first two axes of the constrained ordination of the gut contents - 'RDA1 (full)' and 'RDA2 (full)'. To specifically test for the phenotypic determinants of dietary divergence between Constance and Geneva fish, we conducted a second path analysis on a reduced dataset with only Constance and Geneva fishes. In this path analysis 'RDA1 (Constance-Geneva)' and 'RDA2 (Constance-Geneva)' were the response variables, and lineage, sex, body size and the set of candidate traits were explanatory variables (for path structure see Figure 5).

The experiments were conducted in accordance with animal experimentation permits, that were ethically reviewed, approved, and issued by the Kanton Lucerne (Permit #29801: Effects of environmental variation in resource availability on ecological divergence in stickleback).

Results

Part 1: Detecting prey specific trait-utility

Foraging behaviour

On average, fish made 28.5 feeding strikes during the 16 min trial. The number of total strikes was correlated with the total amount spent engaged in non-foraging behaviours (F(1,144)=45.05, p>0.001), but there was no effect of cross type (F(4,144)=2.209, p=0.07) (Figure S5, Table S2). Pelagic strikes were the most common (mean 13.9/trial), followed by benthic strikes (11.2/trial), wall strikes (2.25/trial) and surface strikes (1.14/trial). Cross-types significantly differed in the number of wall strikes (Cross: F(4,144)=3.549, p=0.009), with

Constance and F_1-Hybrid fish engaging in fewer wall strikes than Geneva fish (TukeyHSD; p=0.031 and p=0.017, respectively). Cross-types did not differ in the number of pelagic strikes (Cross: F(4,144)=1.853, p=0.122), benthic strikes (Cross: F(4,144)=1.132, p=0.344), and surface strikes (Cross: F(4,144)=1.166, p=0.3284). In subsequent analyses, in order to ensure that fish with low feeding activity were not driving patterns of dietary variation, fish in the lower quartile of feeding activity (i.e. the 25% of fish with the lowest feeding activity; < 8 feeding strikes; 41 fish) were excluded. Including inactive fish would have distorted our results, since they are not suitable to estimate dietary variation. Furthermore, because many fish had zero occurrences of wall and surface strikes, these strike types were not considered in subsequent analyses.

Path analysis: identifying functional traits

To identify traits that explain foraging performance (i.e. functional traits in the experimental context) we built a full-experimental path model, that allowed us to identify correlations between morphological and behavioural variation and the foraging performance on different prey categories (i.e. the number of ingested ‘isopod group’, ‘Chironomid group’, and ‘plankton group’) (Figure 2). We identified four morphological traits and behaviours that appeared relevant and functional in this experimental context. The number of ingested prey items of the ‘Chironomid group’, was positively correlated with the number of *benthic strikes*, and positively correlated with the opercular-four bar linkage. The number of ‘isopod group’ individuals ingested was positively associated with the number of *benthic strikes* and fish *gape width*, and negatively associated with the number of *pelagic strikes* and fish *epaxial height.* Additionally, males ingested more of the ‘isopod group’ than females. The number of ingested ‘plankton group’ prey was positively associated with both the number of *benthic and pelagic strikes* (i.e. overall activity), and negatively associated with the *lower jaw lever ratio*. Males ingested more ‘plankton group’ prey items than females, and there was a direct negative effect of *body size* on the number of ingested ‘plankton group’ (i.e. not mediated via another trait). The total ingested biomass was positively correlated with the number of ingested prey of the ‘isopod group’ but not with the other prey types. To see the results of the full path model results including all significant links see Table S3 and Figure S4.

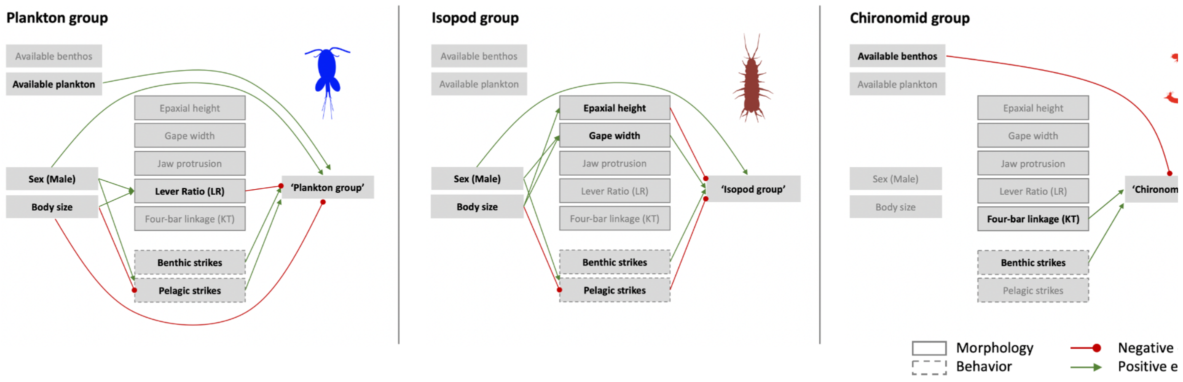

Figure 2. Traits that explain the number of ingested prey items per prey category. For visualization purposes, we only show the arrows directly or indirectly connecting explanatory variables with the number of ingested prey items. The number of ingested prey items is associated with morphological and behavioural variation for all prey types, and trait utility is prey-specific. For the parameter estimates and the complete set of significant correlations see Table S3 and Figure S4.

Part 2: Relating functional trait variation to dietary divergence between lineages

Dietary divergence in common garden

To identify the axes of dietary composition that were best explained by cross-types and sex, we conducted a constrained ordination to the diet matrix containing all fish, as well as a reduced diet matrix only containing Constance and Geneva fish only. Cross (F(4,102)=2.6951, p=0.005) but not sex (F(1,102)=2.0450, p=0.070) had a significant effect on the diet composition both in the model containing the full line-cross (Figure 3A), and in the model containing only the Constance and Geneva lineages (Lineage: F(1,44)=3.9533, p=0.01; Sex: F(1,44)=1.2649 p=0.250) (Figure S6). For both models, the first axes of variation ('RDA 1 (full)', and 'RDA1 (Constance - Geneva)') represent a compositional gradient from the 'chironomid group',' to 'plankton group' and the 'isopod group'. The second axis ('RDA2 (full)', and 'RDA2 (Constance - Geneva)') represents a shift from the 'isopod group' to 'plankton group'.

Consistent with the multivariate analysis of diet composition, Geneva fish ingested more prey items of the 'isopod group' (Lineage: $\chi^2(1)=13.55$, p<0.001) than Constance fish, but fewer of the 'chironomid group' (Lineage: $\chi^2(1)=6.99$, p=0.008). Males of both lineages ingested more 'chironomid group' prey than females (Sex: $\chi^2(1)=7.61$,p=0.006). The lineages did not differ

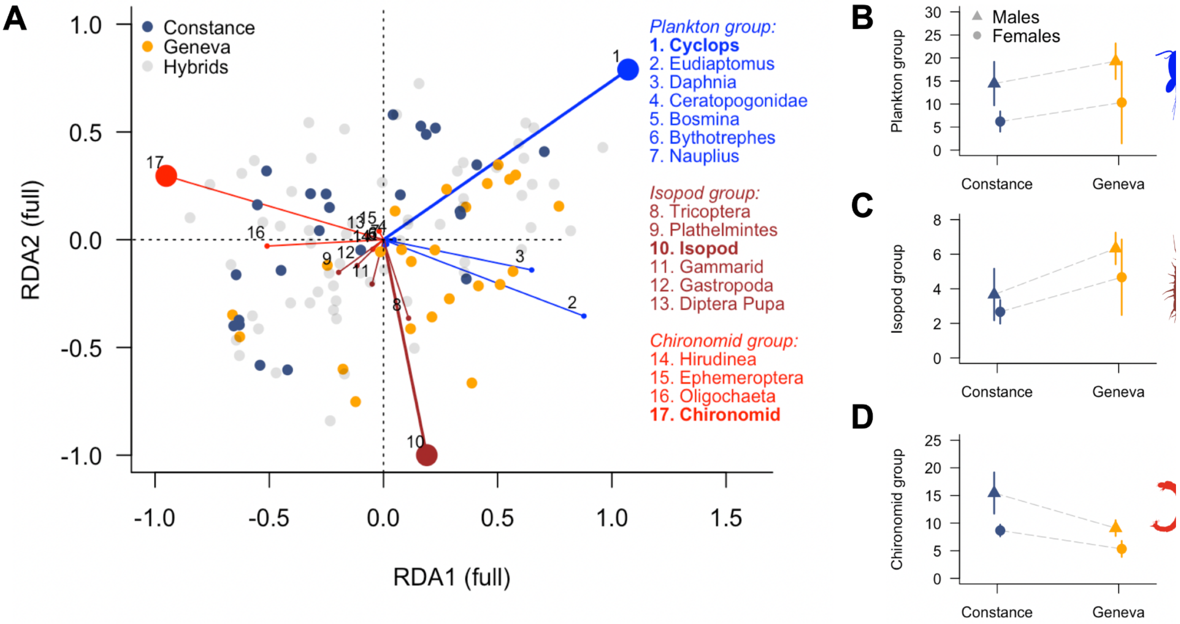

Figure 3. Dietary variation between the crosses, highlighting the dietary variation between the Constance and Geneva lineages. RDA1 (full) and RDA2 (full) are the axes of dietary variation that are best explained by cross-type. To see the distribution of the hybrids along RDA1 (full) and RDA2 (full), see Figure 4. With respect to composition (c), we found that Geneva fish ingested more 'isopod group' and 'plankton group' prey items, whereas Constance fish ingested more of the 'chironomid group'. With respect to numbers of prey consumed (b-d), Geneva fish had significantly more 'isopod group' prey and fewer 'chironomid group' prey than Constance fish (c-d), while there were no differences in the consumption of plankton (Table S5).

significantly in the number of ingested plankton (Lineage: $\chi^2(1)=6.99$, p=0.008), and sexes only marginally differed between the sexes (Sex: $\chi^2(1)=0.82$, p=0.364) (Figure 3 B-D, Table S4).

Morphological variation among cross-types and parental lineages

Using ANCOVAs we explored the morphological variation between sexes and the five cross-types (Table S5). The three linear traits (gape width, jaw protrusion, and epaxial height) scaled positively with standard length, whereas the two ratio-based functional trait systems (lower jaw lever ratio, and the opercular four-bar linkage) were size independent. Sex affected all traits except epaxial height. Gape width (F(4,135)=4.227, p<0.001) and jaw protrusion differed between cross-types (F(4,135)=3.297, p=0.013), and jaw protrusion was additionally affected by a significant cross-by-sex interaction (F(4,135)=5.013, p<0.001).

Repeating the same analysis including only Constance and Geneva fish (table S6), we found that in the parental lineages only gape width differed between the sexes, with males having larger gapes than females (Sex: F(1,52)=9.2486, p=0.004). The lineages did not significantly differ in gape width and epaxial height, and only marginally in the lower jaw lever ratio (F(1,52)=3.64, p=0.06). Jaw protrusion was affected by a lineage-by-sex interaction (F(1,52)=10.02, p=0.004), such that in Geneva fish, males had larger jaw protrusion than females (Geneva only; Sex : F(1,25)=1.77, p=0.19), while the sexes did not differ in Constance fish (Constance only; Sex: F(1,26) = 10.02, p = 0.004).

Explaining among cross-type and linage dietary variation with functional traits

To test whether the observed differences in the dietary composition among the cross-types can be explained by differences in the functional traits, we tested for correlations between mean functional trait values and the mean position on the axes of dietary variation of cross-types and sexes (Table S7). The mean position on the dietary axis 'RDA1 (full)' was positively correlated with the among cross-types and sex variation in the number of pelagic strikes (t(8)=3.370, r=0.77, p=0.01). Thus, the shift from a diet containing more of the 'chironomid group' to one with more 'plankton group' was associated with variation in mean pelagic foraging activity between the cross-types and sexes (Figure 4A). 'RDA2 (full)' was negatively correlated with body size (t(8)=-3.22, r=-0.75, p = 0.01), gape width (t(8)=-3.89, r=-0.81, p = 0.00), and epaxial height (t(8)=-2.43, r =0.65, p=0.04) (Figure 4B-C). Both gape width and epaxial height scale with body size, and the patterns might thus be partially driven by body size variation among the cross-types (Figure 4C). However, as the full experimental model path analysis suggests that body size has a direct negative effect on the performance on the 'plankton group', the positive effect of body size on the 'isopod group' is mediated via gape width (Figure 2). Because epaxial height scales with body size, the negative effect of epaxial height on feeding the 'isopod group'' found at the individual level, is likely overshadowed by the differences in body size among populations.

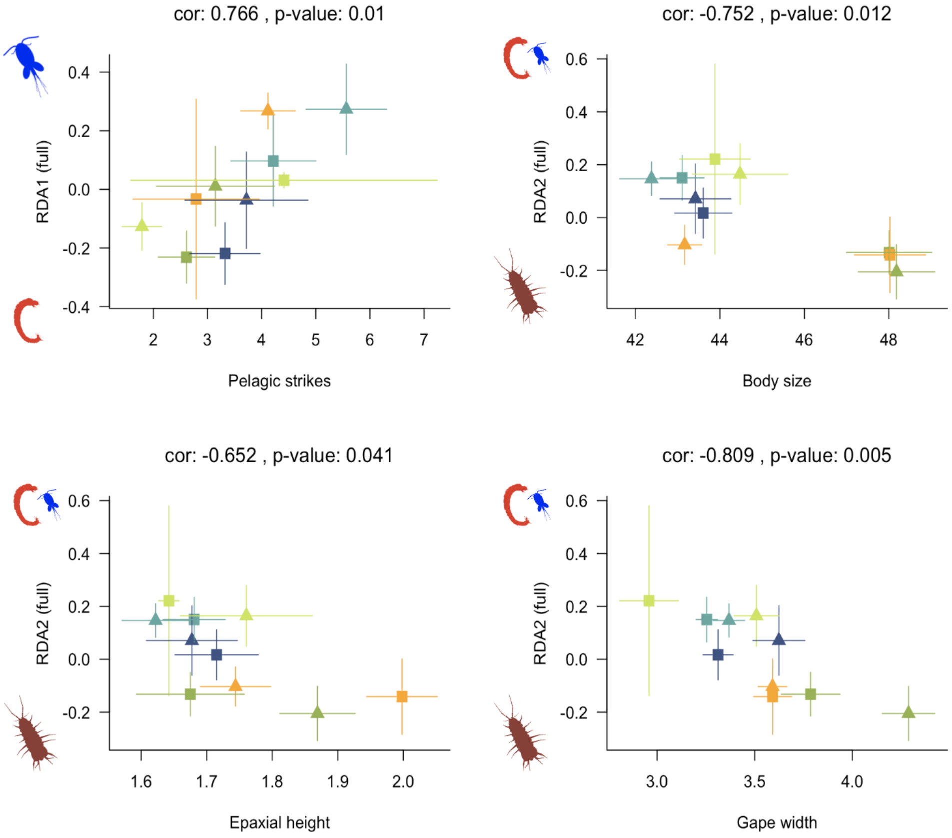

Figure 4. Correlations among cross/sex variation in dietary composition and functional traits. RDA1 reflects the proportion of plankton in the diet and is correlated with variation in the number of pelagic strikes. RDA2 reflects the proportion of the 'isopod group' in the guts and is correlated with the among-cross variation in body size, epaxial height and gape width.

In a second path analysis we specifically tested for determinants of divergence in the diet composition between Constance and Geneva fish (Figure 5). This analysis revealed a direct correlation between Lineage and the first axis of dietary divergence ('RDA1 (Constance - Geneva)') but not the second axis, consistent with the results of the permutation test. However, there was no indirect link between lineage and the diet axes, suggesting that the dietary differences between Constance and Geneva fish are not mediated by the traits included in the analysis (Table S8).

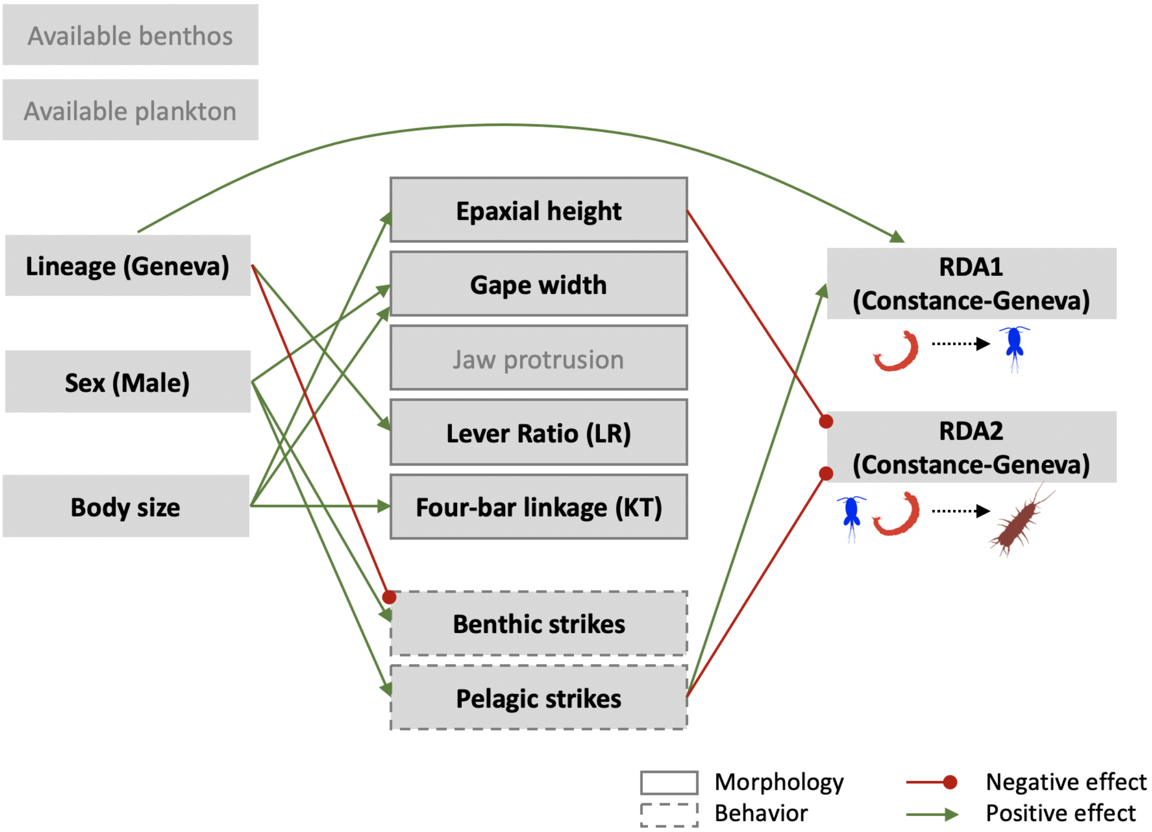

Figure 5. Path analysis to uncover the phenotypic determinants of dietary variation between Lake Constance and Lake Geneva fish in common garden. Lineage has a direct effect on the first axes of dietary divergence between the Lake Constance and Lake Geneva lineage, however, there is no indirect links connecting Lineage with dietary variation, indicating that the dietary divergence is not associated with any of the traits found to be functional at the individual level.

Discussion

In our common garden experiment, we observed divergent resource-use between two phenotypically and genetically distinct lineages of lake dwelling stickleback when foraging in a complex resource setting (Figure 3). To investigate the underlying phenotypic causes of this dietary divergence, we tested for associations between traits and foraging performance (trait utility) on three relevant prey categories (Figure 2), using individuals from a line-cross to increase the phenotypic variance. We found that morphological and behavioural traits explained both performance variation among individuals, and dietary variation among cross-types (Figure 2). However, these same traits did not explain the observed dietary divergence between the parental lineages (Figure 4, 5). In the following sections, we discuss these findings in the context of the ecomorphological paradigm.

Trait utility: Identifying functional traits

Previous work has identified traits and trait systems that predict the biomechanical forces and kinematics that fish predators apply on their prey, and that may thus play an important role in explaining prey-capture success of sticklebacks (McGee et al. 2013; Thompson et al. 2017; Schmid et al. 2018). However, how variation in these traits (and their predicted functions) translates into variation in foraging performance depends on the habitat-context and composition of available prey. We therefore tested a set of candidate traits for their utility with respect to catching prey items in a mixed resource context. We emphasise that we measured foraging performance as the number of prey items ingested during the experiment, and not biomechanical performance parameters related to foraging (e.g. suction forces, strike velocities, etc.).

In general, our results confirm that trait variation can explain some variation in foraging performance among individuals, albeit in a prey-specific manner (Figure 2). However, some of the trait-performance relationships we found were contrary to what we expected based on their predicted biomechanical function. Firstly, we found that the number of ingested plankton was positively correlated with both benthic and pelagic strikes, which is unsurprising given that plankton, unlike benthic invertebrates, were distributed throughout the tank, and therefore fish could ingest plankton using both benthic and pelagic strikes. However, the number of ingested plankton was negatively associated with both body size, and the lower jaw lever ratio. This result is surprising, since an increasing lever ratio conveys a higher degree of kinematic displacement advantage of the lower jaw, and is therefore typically associated with populations feeding on evasive limnetic prey, such as copepods (Alfaro et al. 2004). Secondly, the number of ingested 'chironomid group' prey items was positively associated with benthic feeding activity and the calculated kinematic transmission of the opercular-four bar linkage, which affects the force and speed of jaw opening and protrusion (Westneat 2004). Like with the lower jaw lever ratio, increased kinematic transmission of the opercular four-bar linkage has been previously associated with limnetically foraging stickleback populations (McGee et al. 2013; Thompson et al. 2017). Finally, the number of ingested 'isopod group' prey items was negatively correlated with pelagic activity and epaxial height, and positively correlated with benthic activity and gape width. Epaxial height and gape are counteracting traits in generating the suction force applied on prey items: epaxial musculature generates the suction force, but larger gape increases the volume of water this force is applied to, reducing the overall suction performance (Wainwright et al. 2007). Therefore, foraging on benthos (i.e. prey whose capture requires high suction force) is typically associated with large epaxial height relative to gape (McGee et al. 2013) . However, the ingestion of large prey items is also limited by gape width (i.e. gape limitation), which is likely causing the positive correlation between gape width and the ingestion of large bodied prey items like isopods.

Some of these surprising relationships between morphological and performance variation might be due to variation in behaviour, which is known to complicate relationships between *maximal biomechanical* performance (predicted by morphological traits) and *foraging* performance (Garland and Losos 1994). In our experiment, for example, individuals that largely engaged in non-feeding behaviours had a low feeding activity (number of total strikes) (Figure S5). In this trivial case, behavioural variation (engaging in non-feeding behaviours) disrupted the relationship between morphology and foraging performance, because individuals with low feeding activity exhibit poor performance irrespective of their morphology. More importantly, variation in prey choice in species-rich prey communities might alter trait-performance relationships due to variation in preference and species-specific encounter rates. For example, previous work with stickleback has both predicted (McGee et al. 2013) and observed (Schmid et al. 2019) that jaw protrusion improves pelagic foraging efficiency on plankton. However, in our current study with in similar experimental setup as Schmid et al. (2019) (e.g. same tank volume, and plankton community from Lake Lucerne) albeit with a benthic community added, jaw protrusion was neither an important predictor of diet variation for prey categories (Figure 2) nor of dietary divergence between the two lineages (Figure 5).

In natural settings, variation in habitat choice among consumers can largely determine the prey availability and composition, and, consequently, which traits are functionally important for prey capture. Such variation in how individuals interact with their environment might result in differential selection pressures, and the behavioural variation that is underlying these interactions can be highly context dependent. Although such effects of behaviour are often studied in the context of choosing abiotic environmental conditions (e.g. Bogert effect in relation to thermoregulation and physiological adaptation) (Muñoz 2021), behaviour can similarly situate individuals into foraging habitats with contrasting prey communities that influence the evolution of morphological-foraging traits. Thus, behavioural variation might be a fundamental driver of how other traits relate to performance and, ultimately, fitness (Garland and Losos 1994). For example, in the emergence of trophic novelty in Caribbean pupfishes, genes associated with foraging behaviour underwent selective sweeps prior to those associated with foraging morphology (Richards et al. 2021).

Do functional traits explain dietary differences between cross-types and lineages?

For traits to explain dietary differences between groups of individuals (e.g. lineages) they i) must be functional (i.e. they explain performance differences among individuals) and ii) differ between the groups. We found that the number of pelagic strikes and three morphological traits that explained performance variation among individuals, also explained dietary variation among the cross-types (split by sex) (Figure 4). Among cross-type/sex variation in the number of pelagic strikes was positively correlated with variation in the first diet axis (‘RDA1 full’), which captures a shift from ‘plankton group’ to ‘chironomid group’. Body size, gape, and epaxial height were negatively correlated with the among cross/sex variation on the second diet axis, (‘RDA2 full’), which represents a decreasing proportion of large benthos (predominantly isopods). Body size, gape, and epaxial height covary, and this makes it challenging to interpret their independent effects. However, the path analysis has revealed that - at the individual level - epaxial height was negatively associated with feeding on the ‘isopod group’’ (Figure 2). Because epaxial height scales with body size, and because cross-types and sexes differ in size, this could explain the positive relationship at the cross/sex level. The same may be true for gape, however, the path analysis has revealed that gape mediates the positive relationship between size and ‘isopod group’ at the individual level, (i.e. large individuals have high performance on the ‘isopod group’ *because* they have a large gape width; note the path from body size to ‘isopod group’ benthos via gape in Figure 2). Thus, we conclude that the dietary variation among cross-types/sexes at least partially arises from variation in pelagic feeding activity (predicting the ingestion of plankton), and gape width (limiting the ingestion of large benthic prey items).

While some traits explained among cross-type variation in diet, those same traits did not explain the dietary divergence between the Constance and Geneva lineages (Figure 3, 5). Previous work on wild-caught fish has documented a wide range of morphological differences between the lineages (e.g. body depth, gill raker length, eye diameter, lateral plate number), and differences in foraging efficiency on plankton (Lucek et al. 2013; Best et al. 2017;). In the current study, using common-garden reared fish, we found limited morphological divergence between the two lineages for several functional foraging traits (Table S6). On the one hand, perhaps there was insufficient morphological variation to explain the observed diet divergence. On the other hand, much of the divergence in functional foraging traits observed in the wild may result from phenotypic plasticity, and may only be expressed if the individuals develop in their natural environment. Such context-specific expression of the traits related to foraging performance is common in many species (Olsson et al. 2007; Levis et al. 2017), including stickleback (Oke et al. 2016), and can obscure our detection of foraging trait utility, particularly for consumers feeding on diverse prey communities in natural populations.

So, what explains the dietary differences between Lake Constance and Lake Geneva fish in our experiment? Although we observed clear dietary divergence between Lake Constance and Lake Geneva fish (Figure 3, 5, S6), it is possible this could be explained by behavioural variation in prey choice, rather than a strict morphology-mediated ability to catch prey (Figure 5). In our experiment, Lake Constance fish predominantly ingested 'chironomid group' prey items while Lake Geneva fish ingested more 'isopod group' (Figure 5, Table S4), while there were no significant differences in the consumption of 'plankton' (Figure 3B). In the wild, Lake Constance fish feed predominantly on pelagic prey outside of the breeding season (Bretzel et al. 2021, Hudson et al. 2021b). However, Lucek et al. (2012) found that those Lake Constance fish that move into small streams to spawn during the breeding season largely forage on benthos (and specifically Chironomidae), suggesting that prey choice is habitat specific even in pelagic populations. Some of the experimental dietary differences between the lineages may reflect the ability to deal with the different anti-predator strategies of benthic prey species. Many benthic chironomidae are tube-dwelling, which provides protection against predation (Hershey 1987; Robinson 2000). However, this mechanism was largely lost in the experiment when the invertebrates were separated from the plant material, potentially turning them into 'easy-to-catch' prey, even for fish that may otherwise be less proficient at capturing benthos (i.e. Lake Constance fish). However, isopods, which have both cryptic coloration and chitinous exoskeletons, were mainly preyed upon by Geneva fish, but we were unable to find a trait that could explain preference for, or feeding proficiency on, isopods. Another possibility is that the dietary differences between the lineages might reflect different degrees of neophobia (Thomas et al. 2010). All the fish were reared in the laboratory on a fixed diet of chironomids and so did not encounter any of the other prey items during their lifetime. More generally, it also always possible that our set of candidate traits does not include the most relevant morphological or behavioural traits that are responsible for the dietary divergence between the lineages.

While there may be more putative morphological traits that we did not include in our analysis and that may determine prey capture success (e.g. gill raker morphology), we suggest that in mixed-prey contexts prey-choice may be an important driver of dietary divergence. Our experimental set-up did not allow us to distinguish which prey was attacked in each bout, and we could thus not quantify the degree to which fish differed in attack rates on different prey. Therefore, future experiments should aim at quantifying prey-choice and prey capture success/failure, in order to put them into relation with functional morphology.

Common garden foraging experiments: benefits and limitations

Common garden experiments are a practical approach to test for genetic underpinnings of phenotypic variation, however, some additional considerations are necessary when testing traits such as diet, whose expression depends on the underlying prey community. Firstly, it is important to both rear individuals and perform foraging trials in a common environment, because diet variation can arise from multiple physiological, morphological, and behavioural traits, which may themselves be subject to phenotypic (and developmental) plasticity. Thus, if individuals are tested for dietary divergence, but not reared in a common garden, it remains unclear whether the observed variation is heritable or arises from different developmental histories. Secondly, it is important that the experimental conditions allow for dietary differences to be expressed. It is therefore crucial to provide an ecologically relevant prey community context (e.g. with prey species that vary in vulnerability and profitability), within which individuals can exhibit ecologically-relevant dietary preferences. These considerations also mean that the outcomes of a common garden experiment will often depend on the rearing environment and the experimental set up (i.e. prey community provided). For example, the trait-performance relationships found in this study may have not arisen in other prey community contexts (e.g. only zooplankton (Schmid et al. 2018)). However, while the specific findings of our experiment may not fully represent natural conditions, they still allow us to draw general conclusions about the genetic and functional basis of dietary divergence between lineages, which we discuss below.

Conclusion

Differential resource-use is a common feature of phenotypically divergent consumer populations; however, we often lack an understanding of what drives populations to interact differently with their prey communities. The ecomorphological paradigm suggests that variation in performing ecologically relevant tasks like foraging is the product of functional phenotypic differences among individuals. While our individual level analysis supports this notion (i.e. Figure 2), we also found that evolved dietary differences in common garden between parental lineages could not be explained by functional traits identified at the individual level. We suspect this is due to unmeasured behavioural differences between the members of each lineage affecting prey choice, which can only be expressed under complex resource conditions (i.e. diverse prey assemblages). The role of behavioural variation in connecting functional traits with fitness variation is still very much ambiguous; behavioural variation may lead to differential selection by exposing individuals to different selective agents, and simultaneously shield traits from selection by disrupting links between form and function (Muñoz 2021). In nature, behavioural variation such as prey and habitat choice may largely determine prey availability and composition, and, consequently, dictate which traits become functionally important. Thus, to understand the functional underpinnings of performance variation traits must be understood in the context of the behavioural variation of individuals expressed in the appropriate environmental contexts. For foraging traits in particular, this will require more work on the expression and functional significance of behavioural traits in as natural prey-community contexts as possible.

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Supplementary Material for:

The phenotypic determinants of diet variation between divergent lineages of threespine stickleback

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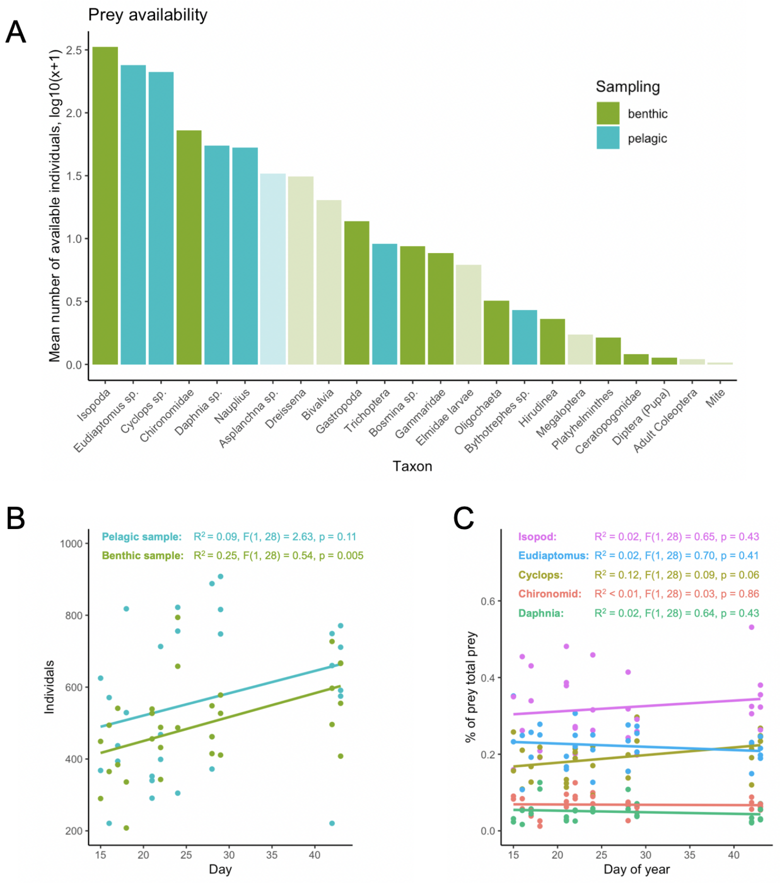


Figure S1: Prey availability in the experiment. Prey items were directly sampled from the wild, thus their rank abundance represents natural prey composition in Lake Lucerne. Bars indicate the mean number of individuals found per block and colors indicate the if the prey items came from the benthic samples (green: plant material) or the pelagic samples (blue: pelagic net tow). Dark bars represent taxa that were also found in the guts, shaded bars taxa that were not consumed by fish (A). Overall the number of benthic individuals per tank significantly increased over the course of the experiment, while the number of plankton did not (B). This did not affect the composition of the prey community, as the relative contribution of the five most common prey (which together comprise on average 85.3 % of prey individuals) remained stable over the course of the experiment (C).

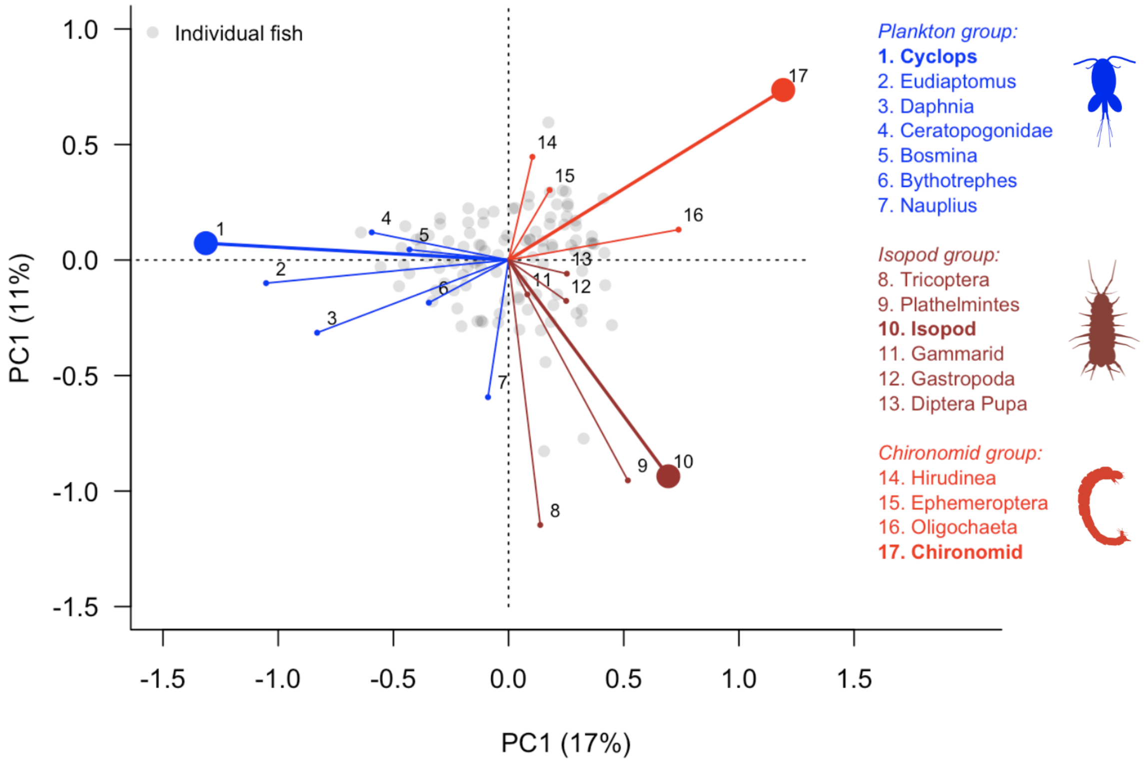


Figure S2. Unconstrained ordination of prey items ingested during the foraging trials based on gut contents. The first PC reflects a pelagic-benthic contrast of ingested prey, while the second PC appears to reflect a contrast between isopods and chironomids. Based on the co-occurrence between prey types we grouped prey into three prey categories (colors). The three highlighted prey types (Cyclops, Isopoda and Chironomids) together accounted for 80% of the prey items found in the guts.

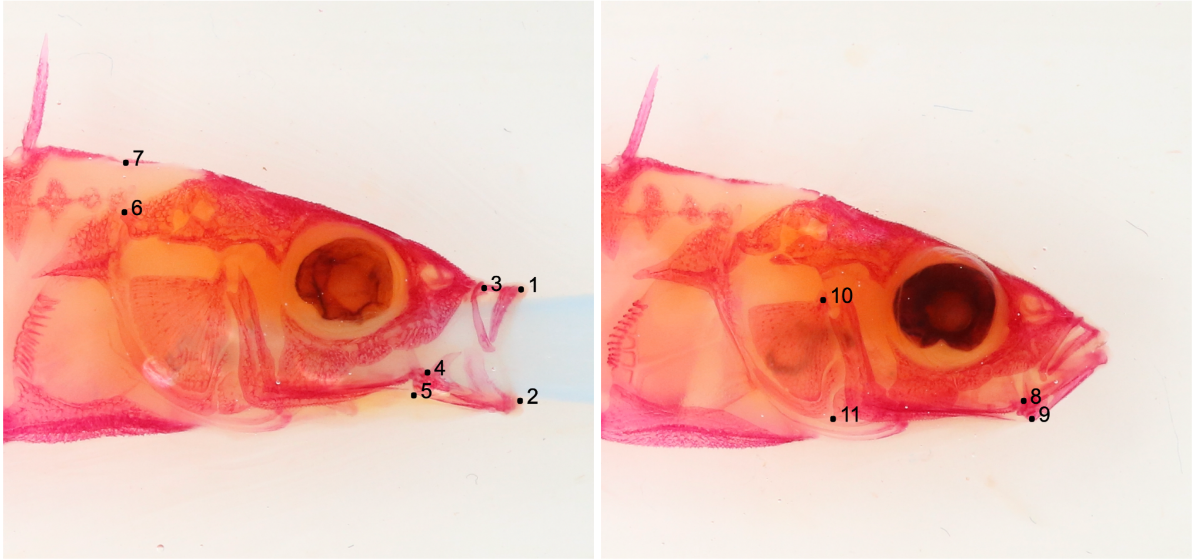


Figure S3: Landmarks used for morphological analysis (McGee et al. 2013).
1. anterior most extent of premaxilla; 2. anterior most extent of dentary; 3. anterior most extent of maxilla; 4. quadrate-articular jaw joint; 5. insertion of the interopercular-articular ligament; 6. point of articulation between the supracleithrum and post-temporal; 7. dorsalmost extent of epaxial muscle, measured dorsal to landmark 6; 8. quadrate-articular jaw joint; 9. insertion of the interopercular-articular ligament; 10. opercular joint; 11. posteroventral extent of interopercule.

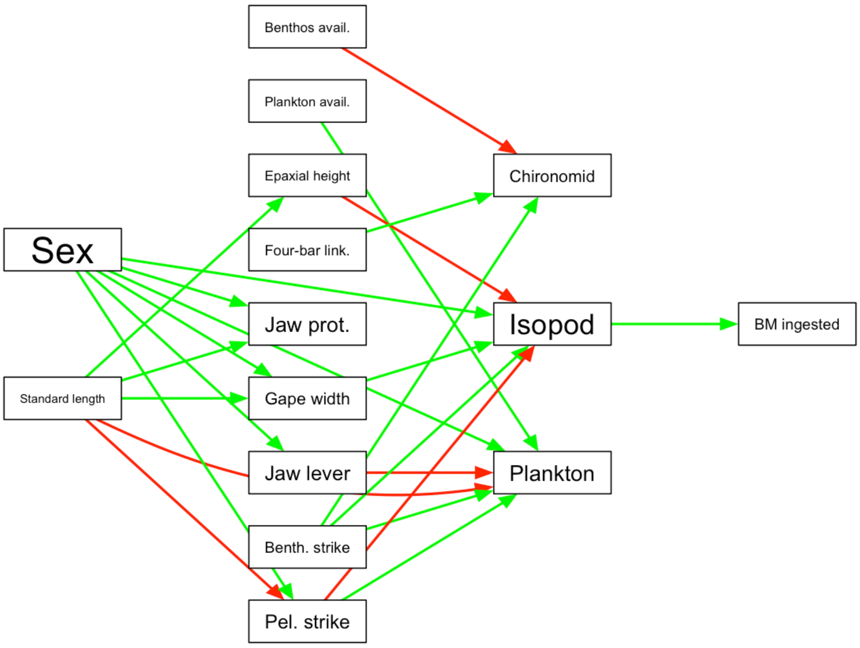


Figure S4: Full hierarchical structure of the bayesian path analysis. Green and red arrows indicate positive and negative parameter estimates with confidence intervals that did not overlap zero, and were thus deemed significant. The path model was composed of 10 individual regression models:

benthic_strike ~ Sex+SL

pelagic_strike ~ Sex+SL

Jaw_protrusion ~ Sex+SL

Epaxial_height~ Sex+SL

KT ~ Sex+SL

Disp_adv ~ Sex+SL

Chironomid ~ Sex + SL + Jaw_protrusion + Gape + Epaxial_height + KT +Disp_adv + benthic_strike + pelagic_strike+ BenthosAvail + PlanktonAvail + (1|Block)

Isopod ~ Sex+SL + Jaw_protrusion + Gape+ Epaxial_height + KT + Disp_adv + benthic_strike + pelagic_strike+ benthosAvail + PlanktonAvail+ (1|Block)

Plankton ~ Sex+SL + Jaw_protrusion + Gape + Epaxial_height + KT + Disp_adv + benthic_strike + pelagic_strike + benthosAvail + PlanktonAvail + (1|Block)

BM_ingested ~ Chironomid + Isopod + Plankton + (1|Block)

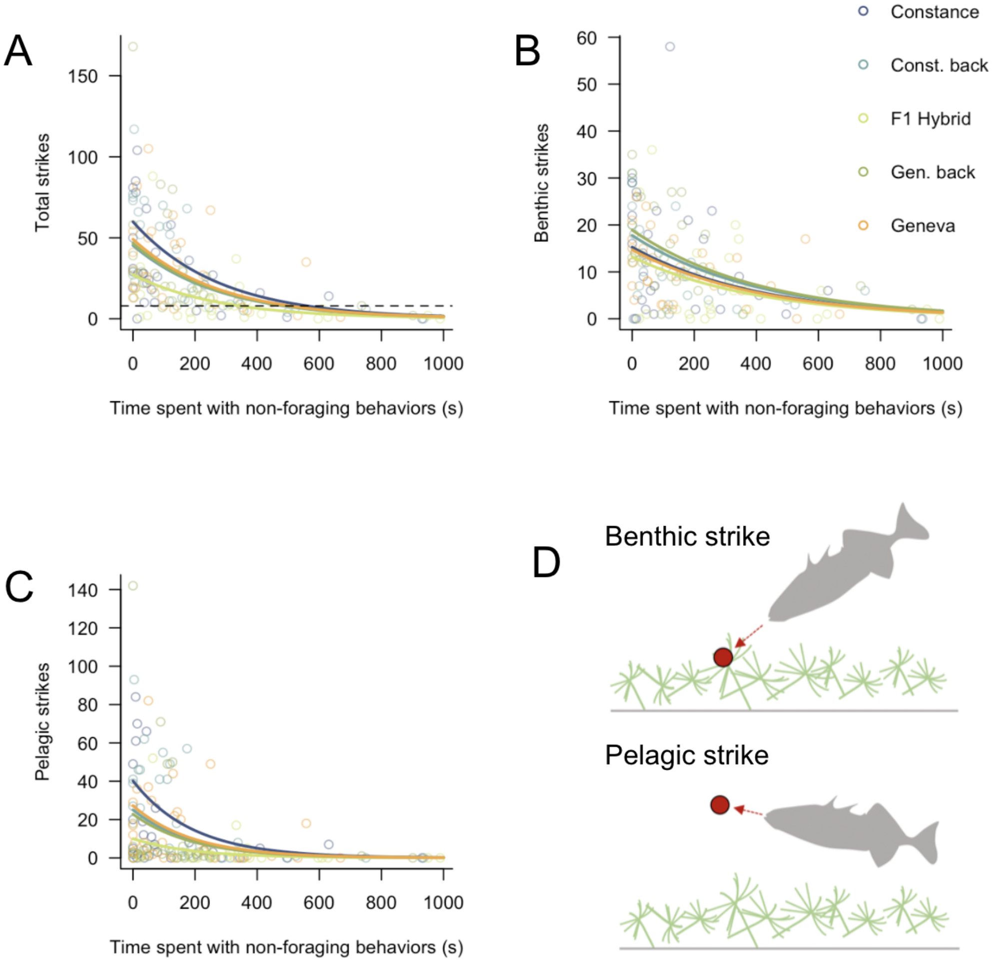


Figure S5. The number of feeding strikes was dependent on the time spent with non-foraging behaviors ‘hiding’ and ‘self-reflection’. To avoid bias in the dietary outcomes by individuals with low feeding activity, we excluded all individuals with less than 8 total feeding strikes (dashed line) (A). The crosses did not differ in the number of the most common strike types (benthic and pelagic strikes) (B-C). We considered benthic strikes those that were directed at prey items at the tank floor or the plant material, and pelagic strikes those that were directed at prey items in the open water (D).

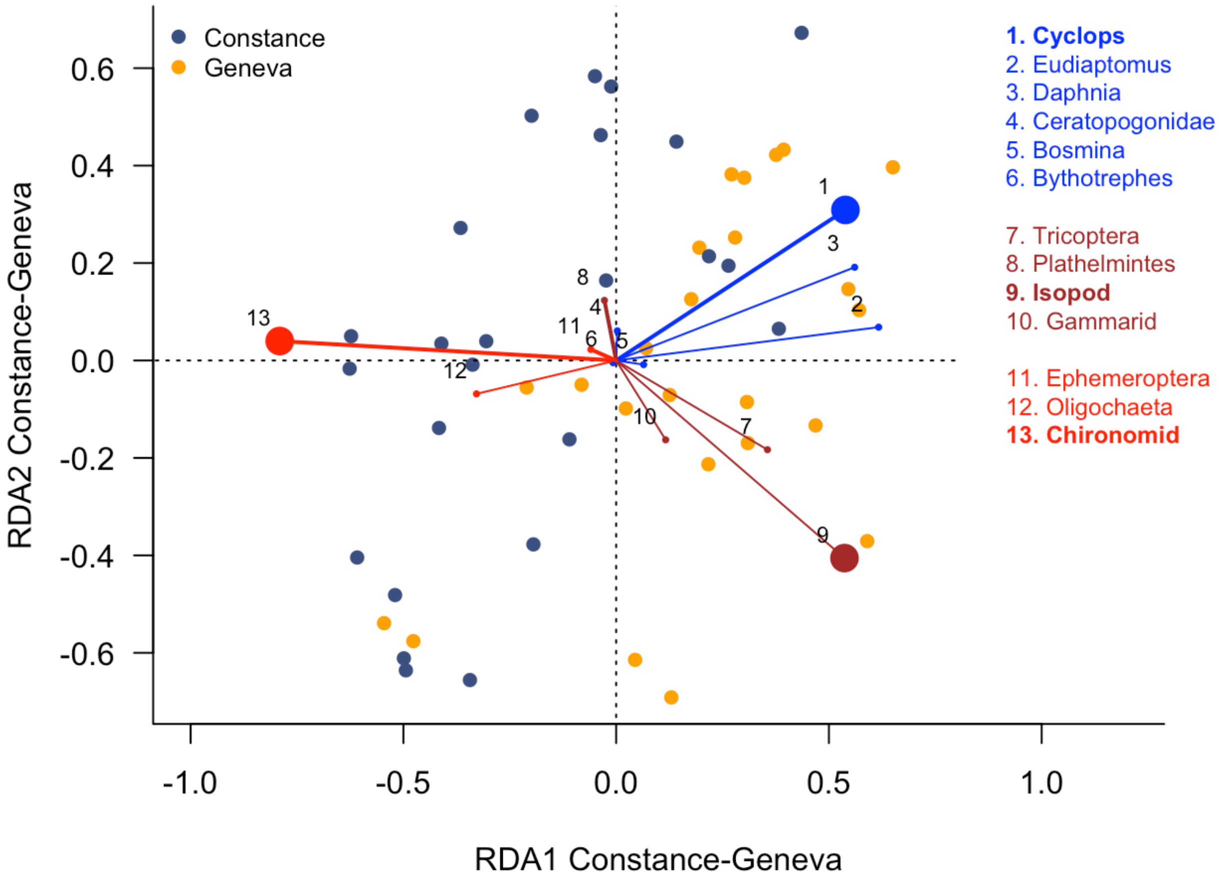


Figure S6. Constrained ordination of prey items including the Constance and Geneva lineage. The axes represent the variation that best explain the compositional differences between the two parental lineages.

Table S1: Candidate traits and trait systems (including component traits) used in the analysis, and their putative function during prey capture.

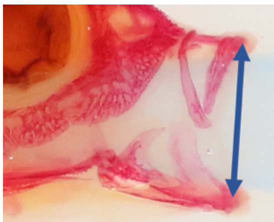
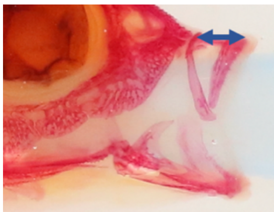
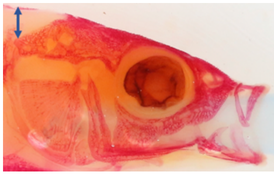
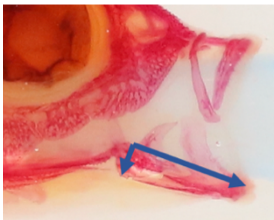
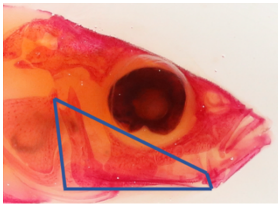
Traits	Landmarks		Function	References
Gape width	1,2		Determines the size of prey item gape limited predators can ingest. At the same time, it is negatively associated with suction force generated during suction feeding by increasing the buccal volume.	Wainwright et al. 2007
Jaw protrusion	1,3		Impacts the hydrodynamic disturbance during suction feeding, which strain-sensitive prey utilize to sense and evade predation.	McGee et al. 2013; Holzman and Wainwright 2009
Epaxial height	6,7		The epaxial height measures the thickness of the epaxial musculature, which generates negative pressure applied to prey items during suction feeding. It is a component trait of the suction index (SI), together with gape among other traits. To avoid non-independence of gape and SI, we forwent calculating SI and used epaxial height as linear measurement instead, as it largely explained variation in calculated SI.	Wainwright et al. 2007
Lower jaw lever ratio			Two-lever system that measures a trade-off between force and speed that is generated when rotating the lower jaw.	McGee et al. 2013; Thompson et al. 2017; Westneat 2004
In lever	4,5			
Out lever	4,2			
Opercular-four-bar linkage			Predicts how rotation of the input link translates into rotation of the output link (kinematic transmission), which affects the force and speed of jaw opening and protrusion.	McGee et al. 2013; Thompson et al. 2017; Westneat 2004; Holzman et al. 2008
Fixed link	8,10			
Coupler link	9,11			
Input link	10,11			
Output link	8,9			

Table S2: Results of the GLMM on the effects of non-foraging behavior and cross-type on the number of strikes.

		Df	**Sum Sq**	**Mean Sq**	**F value**	**p value**	
Total strikes	Non-foraging	1	27511	27511	45.054	4.10E-10	***
	Cross	4	5395	1349	2.209	0.0709	.
	Residuals	144	87931	611			
Benthic strikes	Non-foraging	1	2587	2586.5	33.217	4.82E-08	***
	Cross	4	353	88.2	1.132	0.344	
	Residuals	144	11213	77.9			
Pelagic strikes	Non-foraging	1	9414	9414	19.943	1.60E-05	***
	Cross	4	3498	875	1.853	0.122	
	Residuals	144	67972	472			
Surface strikes	Non-foraging	1	33.9	33.92	4.559	0.0344	*
	Cross	4	34.7	8.67	1.166	0.3284	
	Residuals	144	1071.4	7.44			
Wall strikes	Non-foraging	1	147.8	147.82	17.296	5.47E-05	***
	Cross	4	121.3	30.34	3.549	0.00856	**
	Residuals	144	1230.7	8.55			

Table S3: Results from the Bayesian full experimental model path analysis:

Response	**Explanatory**	**Estimate**	**Est.Error**	**95% CI**
Ingested Biomass	Isopod group	0.07	0.01	0.05 to 0.09
Ingested Biomass	Plankton	-0.01	0	-0.01 to 0
Ingested Biomass	Chironomid group	0	0.01	-0.01 to 0.01
Chironomid group	Available benthos	-0.41	0.18	-0.78 to -0.04
Chironomid group	Available plankton	-0.02	0.19	-0.39 to 0.37
Chironomid group	Benthic strike	0.02	0	**0.02 to 0.03**
Chironomid group	Body size	0.21	0.13	-0.04 to 0.47
Chironomid group	Lower jaw lever ratio	0.01	0.07	-0.14 to 0.14
Chironomid group	Epaxial height	-0.09	0.1	-0.28 to 0.1
Chironomid group	Four-bar linkage	0.16	0.07	**0.02 to 0.31**
Chironomid group	Gape width	-0.03	0.14	-0.3 to 0.25
Chironomid group	Jaw protrusion	0.02	0.1	-0.18 to 0.21
Chironomid group	Pelagic strike	0	0	**-0.01 to 0**
Chironomid group	Sex (Male)	0.12	0.08	-0.03 to 0.28
Isopod group	Available benthos	0.02	0.22	-0.41 to 0.46
Isopod group	Available plankton	-0.18	0.24	-0.63 to 0.3
Isopod group	Benthic strike	0.03	0.01	**0.02 to 0.04**
Isopod group	Body size	0.36	0.19	-0.02 to 0.74
Isopod group	Lower jaw lever ratio	-0.12	0.1	-0.33 to 0.08
Isopod group	Epaxial height	-0.42	0.15	**-0.71 to -0.14**
Isopod group	Four-bar linkage	0.06	0.11	-0.16 to 0.28
Isopod group	Gape width	0.4	0.2	**0 to 0.79**
Isopod group	Jaw protrusion	-0.23	0.14	-0.51 to 0.04
Isopod group	Pelagic strike	-0.01	0	**-0.02 to 0**
Isopod group	Sex (Male)	0.55	0.13	**0.29 to 0.8**
Plankton	Available benthos	-0.11	0.18	-0.46 to 0.25
Plankton	Available plankton	0.52	0.19	**0.14 to 0.91**
Plankton	Benthic strike	0.03	0	**0.02 to 0.04**
Plankton	Body size	-0.68	0.14	**-0.95 to -0.42**
Plankton	Lower jaw lever ratio	-0.53	0.08	**-0.68 to -0.37**
Plankton	Epaxial height	-0.04	0.1	-0.23 to 0.15
Plankton	Four-bar linkage	0.02	0.07	-0.13 to 0.16
Plankton	Gape width	0.06	0.14	-0.22 to 0.33
Plankton	Jaw protrusion	0.08	0.09	-0.1 to 0.27
Plankton	Pelagic strike	0.03	0	**0.02 to 0.03**
Plankton	Sex (Male)	0.29	0.08	**0.14 to 0.45**
Benthic strike	Body size	0.04	0.05	-0.06 to 0.14
Benthic strike	Sex (Male)	0	0.05	-0.1 to 0.1
Pelagic strike	Body size	-0.5	0.05	**-0.6 to -0.4**
Pelagic strike	Sex (Male)	0.15	0.05	**0.06 to 0.24**
Four-bar linkage	Body size	0.1	0.1	-0.09 to 0.3
Four-bar linkage	Sex (Male)	-0.14	0.1	-0.33 to 0.06
Displacement advantage	Body size	0.01	0.1	-0.18 to 0.21

Displacement advantage	Sex (Male)	0.2	0.1	**0 to 0.39**
Epaxial height open	Body size	0.39	0.09	**0.21 to 0.58**
Epaxial height open	Sex (Male)	0.12	0.09	-0.06 to 0.3
Gape width	Body size	0.79	0.06	**0.68 to 0.9**
Gape width	Sex (Male)	0.35	0.06	**0.24 to 0.47**
Jaw protrusion	Body size	0.51	0.09	**0.34 to 0.67**
Jaw protrusion	Sex (Male)	0.26	0.09	**0.09 to 0.43**

Table S4: Results of the GLMM on the number of consumed prey between the sexes and the Constance and Geneva lineage.

Prey type	**Variable**	**Chisq**	**Df**	**P-value(>Chisq)**	
Isopod group	Lineage	13.55	1	0.0002	***
	Sex	1.08	1	0.2989	
	Lineage:Sex	0.51	1	0.4867	
Chironomid group	Lineage	6.99	1	0.008	**
	Sex	7.61	1	0.006	**
	Lineage:Sex	0.01	1	0.924	
Plankton group	Lineage	0.82	1	0.364	
	Sex	3.70	1	0.054	.
	Lineage:Sex	0.07	1	0.797	

Table S5: Test for comparing the body size and functional traits among the full line-cross. The table shows sums of squares (Sum Sq), degrees of freedom (DF), F-values and p-values from Type III ANOVAs with standard length, cross-type, and sex as factors.

		Sum Sq	**DF**	**F-value**	**p-value**
Standard length	Cross	0.060	4	20.221	<0.001 ***
	Sex	0.000	1	5.991	0.016 *
	Cross:Sex	0.000	4	1.310	0.269
	Residuals	0.1	136		
Gape width	log10(SL)	0.162	1	173.618	<0.001 ***
	Cross	0.016	4	4.227	0.003 **
	Sex	0.042	1	44.614	<0.001 ***
	Cross:Sex	0.005	4	1.348	0.256
	Residuals	0.126	135		
Jaw protrusion	log10(SL)	0.236	1	48.889	<0.001 ***
	Cross	0.064	4	3.297	0.013 *
	Sex	0.098	1	20.280	<0.001 ***
	Cross:Sex	0.097	4	5.013	<0.001 ***
	Residuals	0.653	135		
Epaxial height	log10(SL)	0.044	1	13.159	<0.001 ***
	Cross	0.010	4	0.742	0.565
	Sex	0.000	1	0.057	0.812
	Cross:Sex	0.018	4	1.357	0.252
	Residuals	0.455	134		
lower jaw lever ratio	log10(SL)	0.000	1	0.015	0.904
	Cross	0.009	4	1.557	0.189
	Sex	0.006	1	4.033	0.047 *
	Cross:Sex	0.004	4	0.639	0.636
	Residuals	0.201	135		
Opercular-four-bar linkage	log10(SL)	0.003	1	1.057	0.306
	Cross	0.003	4	0.351	0.843
	Sex	0.010	1	4.050	0.046 *
	Cross:Sex	0.006	4	0.606	0.659
	Residuals	0.328	135		

Table S6: Test for comparing the body size and functional traits between lab reared Constance and Geneva fish. The table shows sums of squares (Sum Sq), degrees of freedom (DF), F-values and p-values from Type III ANOVAs with standard length, cross-type, and sex as factors.

		Sum Sq	**DF**	**F-value**	**p-value**	
Standard length	Lineage	0.003	1	6.402	0.014	*
	Sex	0.004	1	7.802	0.007	**
	Lineage:Sex	0.003	1	6.438	0.014	*
	Residuals	0.026	53			
Gape width	log10(SL)	0.018	1	16.450	>0.001	***
	Lineage	0.000	1	0.224	0.638	
	Sex	0.010	1	9.249	0.004	**
	Lineage:Sex	0.000	1	0.072	0.790	
	Residuals	0.056	52			
Jaw protrusion	log10(SL)	0.020	1	4.386	0.041	*
	Lineage	0.011	1	2.434	0.125	
	Sex	0.011	1	2.463	0.123	
	Lineage:Sex	0.058	1	12.762	0.001	***
	Residuals	0.236	52			
Epaxial height	log10(SL)	0.006	1	1.801	0.186	
	Lineage	0.004	1	1.103	0.298	
	Sex	0.002	1	0.640	0.427	
	Lineage:Sex	0.000	1	0.009	0.925	
	Residuals	0.184	52			
lower jaw lever ratio	log10(SL)	0.001	1	0.823	0.369	
	Lineage	0.005	1	3.638	0.062	.
	Sex	0.000	1	0.006	0.940	
	Lineage:Sex	0.000	1	0.171	0.681	
	Residuals	0.071	52			
Opercular-four-bar linkage	log10(SL)	0.015	1	6.970	0.011	*
	Lineage	0.004	1	1.853	0.179	
	Sex	0.000	1	0.178	0.675	
	Lineage:Sex	0.001	1	0.446	0.507	
	Residuals	0.114	52			

Table S7: Correlations between the means of functional traits per cross/sex and the mean position along the axes of dietary divergence.

Diet axis	Trait	Pearson correlation coefficient	P-value	
RDA1 (full)	Benthic strikes	-0.12	0.74	
	Pelagic strikes	0.77	0.01	*
	Body size	-0.48	0.16	
	Four-bar linkage	-0.31	0.38	
	epaxial height	-0.15	0.68	
	displacement advantage	-0.06	0.88	
	Gape width	-0.14	0.70	
RDA2 (full)	Benthic strikes	0.41	0.24	
	Pelagic strikes	0.38	0.28	
	Body size	-0.75	0.01	*
	Four-bar linkage	-0.02	0.97	
	epaxial height	-0.65	0.04	*
	displacement advantage	-0.19	0.60	
	Gape width	-0.81	0.00	***

Table S8: Results from the second path analysis, relating the axes of dietary divergence between the Constance and Geneva lineage with phenotypes.

Response	**Explanatory**	**Estimate**	**Est.Error**	**l-95% CI**
RDA1 (C-G)	Lineage (Geneva)	0.46	0.13	**0.2 to 0.72**
RDA1 (C-G)	Sex (male)	0.04	0.16	-0.26 to 0.36
RDA1 (C-G)	Body size	-0.05	0.16	-0.38 to 0.27
RDA1 (C-G)	Jaw protrusion	0.08	0.11	-0.14 to 0.28
RDA1 (C-G)	Gape	0.21	0.14	-0.07 to 0.48
RDA1 (C-G)	Epaxial height	-0.05	0.12	-0.29 to 0.2
RDA1 (C-G)	Four-bar linkage	-0.07	0.12	-0.3 to 0.17
RDA1 (C-G)	Lower jaw lever ratio	-0.08	0.12	-0.33 to 0.16
RDA1 (C-G)	Benthic strike	0	0.01	-0.01 to 0.01
RDA1 (C-G)	Pelagic strike	0.01	0	**0.01 to 0.02**
RDA1 (C-G)	Available benthos	0.02	0.13	-0.24 to 0.27
RDA1 (C-G)	Available plankton	0.02	0.13	-0.25 to 0.28
RDA2 (C-G)	Lineage (Geneva)	0.25	0.15	-0.04 to 0.55
RDA2 (C-G)	Sex (male)	-0.19	0.17	-0.52 to 0.14
RDA2 (C-G)	Body size	0.3	0.18	-0.05 to 0.65
RDA2 (C-G)	Jaw protrusion	0.06	0.12	-0.17 to 0.3
RDA2 (C-G)	Gape	0.08	0.15	-0.22 to 0.38
RDA2 (C-G)	Epaxial height	-0.33	0.13	**-0.59 to -0.07**
RDA2 (C-G)	Four-bar linkage	0.12	0.13	-0.15 to 0.38
RDA2 (C-G)	Lower jaw lever ratio	0.16	0.13	-0.11 to 0.43
RDA2 (C-G)	Benthic strike	0	0.01	-0.01 to 0.02
RDA2 (C-G)	Pelagic strike	-0.01	0	**-0.02 to -0.01**
RDA2 (C-G)	Available benthos	0.02	0.13	-0.23 to 0.27
RDA2 (C-G)	Available plankton	-0.14	0.13	-0.4 to 0.12
Benthic strike	Sex (male)	0.33	0.1	0.15 to 0.52
Benthic strike	Body size	0.1	0.08	-0.07 to 0.26
Benthic strike	Lineage (Geneva)	-0.42	0.09	**-0.59 to -0.24**
Pelagic strike	Sex (male)	0.43	0.09	**0.26 to 0.6**
Pelagic strike	Body size	-0.02	0.07	-0.17 to 0.12
Pelagic strike	Lineage (Geneva)	-0.12	0.08	-0.28 to 0.03
Jaw protrusion	Sex (male)	0.05	0.2	-0.33 to 0.43
Jaw protrusion	Body size	0.07	0.17	-0.25 to 0.4
Jaw protrusion	Lineage (Geneva)	-0.08	0.19	-0.45 to 0.29
Epaxial height	Sex (male)	-0.11	0.18	-0.46 to 0.25
Epaxial height	Body size	0.34	0.15	**0.04 to 0.63**
Epaxial height	Lineage (Geneva)	0.2	0.17	-0.14 to 0.53
Four-bar linkage	Sex (male)	0.08	0.18	-0.27 to 0.43
Four-bar linkage	Body size	0.41	0.15	**0.11 to 0.7**
Four-bar linkage	Lineage (Geneva)	-0.24	0.17	-0.57 to 0.1
Lower jaw lever ratio	Sex (male)	0.05	0.18	-0.32 to 0.4
Lower jaw lever ratio	Body size	-0.18	0.15	-0.48 to 0.13

Lower jaw lever ratio	Lineage (Geneva)	-0.34	0.17	-0.68 to 0
Gape width	Sex (male)	0.47	0.16	**0.15 to 0.79**
Gape width	Body size	0.49	0.13	**0.22 to 0.75**
Gape width	Lineage (Geneva)	-0.02	0.15	-0.32 to 0.28

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Chapter 3

Predator species, their traits, and abiotic environments jointly shape prey community structure

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All authors carried out the field work. MM and RO processed the zooplankton samples in the laboratory. MM, RG and BM conceptualized the project. MM obtained the morphological data and led the data analyses and writing. All authors gave critical feedback to the manuscript.

Abstract

Phenotypic diversification between consumer populations often involves divergence in foraging morphology, suggesting changes in the ecological interactions between consumers and their prey. Theoretical and experimental work suggests that such intraspecific variation can affect community structure, however, only few studies have investigated these effects in nature. Here, we use a comparative approach to explore the relative importance of different ecological factors in driving macrozooplankton composition among 78 Greenlandic lakes. The lakes vary along gradients in lake size, water chemistry and in the configuration of their fish community (fishless, only arctic char (*Salvelinus alpinus*), only threespine stickleback (*Gasterosteus aculeatus*), or both species). First, we explored how abiotic gradients and fish configuration jointly drive the zooplankton composition. Second, we focused on the community-wide effects of intraspecific-variation in trophic morphology among stickleback populations. Overall, our analyses highlight the role of abiotic gradients and stickleback in structuring zooplankton communities, and the role of char in altering the effects of stickleback. Furthermore, we find that in lakes with both fish species present, variation in stickleback jaw protrusion affects community structure, but not in lakes with only stickleback present. This study adds to the growing body of literature that highlights a potential interplay between evolutionary and ecological dynamics, where communities impose selection on foraging morphology and foraging morphology shapes communities.

Introduction

Intraspecific variation among consumer populations arises frequently when they are embedded in different community contexts (Sanford and Worth 2010; Levis et al. 2017; Holding et al. 2018; terHorst et al. 2018; Harmon et al. 2019; Oester et al. 2022). In such cases, the nature of intraspecific variation among populations can involve a myriad of traits (e.g. morphology and behavioral) underlying species interactions. There is both ample theory and experimental evidence suggesting that intraspecific variation can alter the ecological interactions among species (Hiltunen and Becks 2014), and may affect the structure of communities (i.e. species abundances and richness) (Holdridge et al. 2017; McPeek 2017; Des Roches et al. 2018; Govaert et al. 2021). When community context causes rapid evolution of traits that underlie species interactions, there are possibilities for feedbacks to develop between phenotypic evolution and changes in community structure (Post and Palkovacs 2009; Hendry 2016; terHorst et al. 2018). Numerous experiments have documented phenotypic effects of consumers on communities (Des Roches et al. 2018), which in some cases might feedback to affect traits distributions or trait expression of subsequent generations (Matthews et al. 2016; Best et al. 2017; Brunner et al. 2017). However, only a few studies have investigated the role of intraspecific variation in driving community structure in natural systems (Post et al. 2008; Post and Palkovacs 2009).

The relative importance of consumer traits for shaping prey communities likely depends on the complex interplay between various abiotic and biotic factors that can shape natural communities (Polis and Strong 1996; Paquette et al. 2022). Physical and biogeochemical environments, for example, can alter resources so as to affect community structure (Leibold et al. 1997; Wærvagen et al. 2002; Betini et al. 2016; Paquette et al. 2022), while at the same time, either top-predators or parasites can have cascading ecological effects on lower trophic levels (Polis et al. 2000; Schmitz et al. 2004; Anaya-Rojas et al. 2019). However, it is largely unclear how the outcome of such ecological dynamics might interact with potential feedbacks between trait evolution and community structure.

The trophic interaction between threespine stickleback (*Gasterosteus aculeatus*) and their prey community is a useful starting point to investigate how consumer trait variation can affect ecosystems (Harmon et al. 2009; Matthews et al. 2016). Stickleback show high degrees of intraspecific variation in foraging traits that is often associated with local feeding habitats and resource use (Schluter 1993; Matthews et al. 2010; Lucek et al. 2013; McGee et al. 2013; Arnegard et al. 2014). Previous work has related phenotypic variation in some of these traits to prey-specific foraging performance in different environmental contexts, proving their utility (Schluter 1993; Robinson 2000; Higham et al. 2017; Schmid et al. 2018). A separate body of experimental work has investigated the effects of lineage divergence on ecosystem processes in semi natural systems (mesocosms) (Harmon et al. 2009; Rudman et al. 2015; Matthews et al. 2016; Best et al. 2017), and has related divergence in functional morphology of stickleback to changes in prey communities (Schmid et al. 2018). Combined, these previous studies have argued that phenotypic divergence of stickleback populations, and the resulting changes in species-interactions, can play a structuring role in prey communities. Nevertheless, relationships between traits and performance might be highly context-specific in relation to both species interactions and abiotic environmental conditions. In natural ecosystems, it therefore remains an open question to what extent intraspecific variation of a predator can explain variation in prey community structure among systems.

Here, we use a comparative approach to study the environmental drivers of zooplankton community composition among 78 Greenlandic lakes, with a particular focus on the role of stickleback trait variation. Our study system is well suited to address this question for several reasons. Firstly, the lakes in our sampling region are inhabited by comparatively simple zooplankton communities (Røen 1994), which allows us to reduce meaningful compositional changes in the zooplankton community to a few relevant dimensions. Secondly, the lakes are inhabited by only two fish species, and combinations of their presence/absence are highly replicated across a regional landscape with constrained variation in abiotic conditions (*i.e.* similar climate and underlying geology). Specifically, following postglacial emergence, many lakes and ponds were independently colonized by threespine stickleback (a planktivore/invertivore) and arctic char (*Salvelinus alpinus*; a piscivore/invertivore salmonid), resulting in a patchwork of lakes with either no fish, only char, only stickleback or both species. Finally, the lakes in our sample vary in size and water chemistry, providing ecologically relevant gradients in abiotic conditions, which may drive both variation in zooplankton community structure and phenotypic divergence among stickleback populations. This natural set up allows us to investigate how variation in lake size, water chemistry and fish community configuration influence zooplankton communities across our full set of lakes. Furthermore, we can focus on lakes with stickleback to test for associations between ecologically relevant trait variation of stickleback and variation in zooplankton community structure.

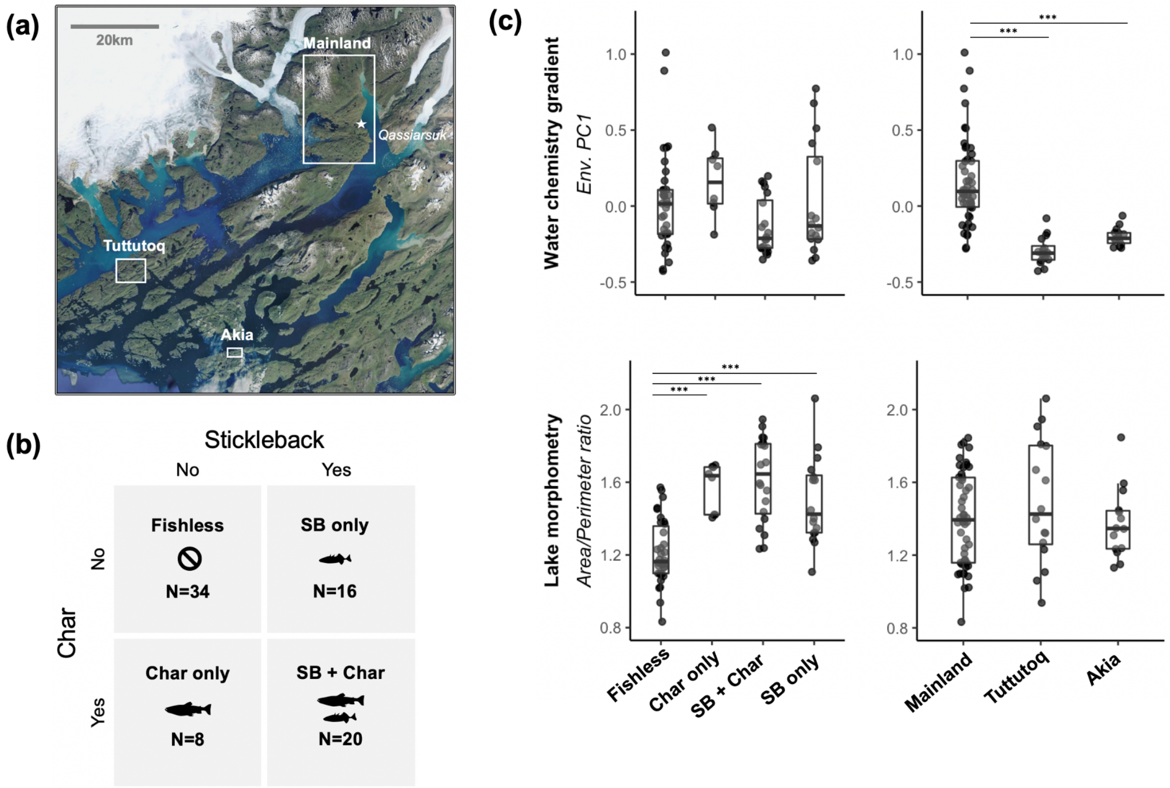

Figure 1: Environmental context of this study. (a) Zooplankton communities were sampled in 78 lakes in three geographic regions of southern Greenland. (b) The sampled lakes differed in the configuration of their fish communities (presence and absence of arctic char and threespine stickleback) in a factorial design. (c) Water chemistry varied regionally, but was not associated with fish community composition, contrary to lake morphometry: Fishless lakes in our sample were smaller and shallower than lakes harboring fish communities of any kind.

Material and Methods

Study Area and Sample Collection

In August 2018 and July-August 2019 we sampled zooplankton communities from 78 lakes in the Kujalleq municipality of southern Greenland. The sampling effort focused on three regions: the islands Akia (60°40' 42" N, 46°5'39" W) and Tuttutoq (60°49'14" N, 46°27'51" W), and on the peninsular mainland surrounding the settlement Qassiarsuk (61°09′00″N 45°31′00″W) (Figure 1a). Lakes in this area are inhabited by two fish species - threespine stickleback and arctic char. In each lake we determined the configuration of the fish community (i.e. the presence and absence of stickleback and char, respectively). 34 lakes were fishless (hereinafter referred to as *fishless lakes*), 8 lakes contained only char (*char-only lakes*), 16 lakes contained only sticklebacks (*stickleback-only lakes*), and 20 lakes contained stickleback and char (*stickleback-and-char lakes*).

Environmental Variation

We assessed maximal lake depth by lowering a weighted rope at the estimated deepest point of the lake and obtained surface area and lake perimeters from analyses of Google Earth satellite images. Lakes were often highly irregular in shape, which affects the relationships between area, maximal depth and perimeter. We used the ratio between lake area and perimeter (log10(area/perimeter)) as a simplified metric of lake morphometry that was nevertheless highly correlated with lake area ($r=0.93$, $t_{(76)}=21.35$, $p<0.001$). The ratio is also more strongly correlated with lake depth ($r=0.75$ $t_{(76)}=9.77$, $p<0.001$), than lake depth and lake area are to each other ($r=0.64$, $t_{(76)}=7.26$, $p<0.001$). This is because more irregularly shaped lakes (i.e. lakes with lower area/perimeter ratio for a given size) tend to be shallower than more circular lakes. As such, the area/perimeter ratio (hereinafter *lake morphometry gradient)* integrates the different aspects of lake morphometry into one measurement.

We used a multiparameter sonde (EXO2 modular sensor platform [YSI- WTW]), to obtain vertical profiles of four chemical parameters (conductivity, fluorescent dissolved organic matter (fDOM), oxygen concentration, and chlorophyll-*a*) at the deepest point of the lake (Table S1). To obtain one sample per lake for each parameter, we integrated the top layer of the vertical profiles, by taking the mean of the measurements obtained between 0 and 3 m depth. We then performed a PCA on the water chemistry parameters using the R package vegan (Oksanen et al., 2019). Conductivity, fDOM and chlorophyll-*a* had equally strong loadings on PC1, which explained 41 % of the total variance in the data (Table S1). Subsequently, we used the environmental PC1 (env. PC1) as a measurement for the chemical lake condition (hereinafter *water chemistry gradient)*. We used ANOVA to test if lake morphometry and water chemistry gradients differed between sampling region (Akia, Tuttutoq, mainland) and fish community configurations, respectively.

Zooplankton sampling and processing

For zooplankton sampling, we performed vertical tows with a plankton net (mesh size 90 μm) at the deepest point of each lake. The zooplankton samples were fixed with an iodine solution (Lugol's solution), for subsequent analysis of taxonomy (typically to Genus, focusing on crustaceans (Røen 1994)). To do so, we first inspected the samples with a dissecting microscope to get an overview of the abundance and composition of the sample. We then split the sample using a plankton splitter until we reached a density of 300-400 individuals for counting, identification and body size measurements. In samples that contained few individuals, the entire sample was counted without splitting. If the initial inspection revealed that rare plankton species were present in the sample, we counted and measured those at a lower split. From the counts per sample we calculated the abundance per liter in the lake by using the diameter of the plankton net, the depth of the vertical tow, and the number of sample splits. This resulted in an abundance matrix containing 13 taxa (Table S2). To simplify subsequent analysis, we combined members of the Chydoridae (Chydorus, Acropercus, Alona, Eurycercus) into one category, as many of them occurred in only a few lakes and at low abundance.

Fish sampling and processing

We used a combination of visual inspection and trapping to determine the presence and absence of arctic char and stickleback in the lakes (minnow traps for stickleback, gill nets for char). To collect adult stickleback, we placed 5-10 unbaited steel minnow traps in the littoral zones of the lakes between 1-5 m from the shore. Traps were set for a specific amount of time (typically less than 2 h), with a total trapping effort per lake ranging from 5.5 - 64 h. This allowed us to calculate the catch per unit effort as an estimate of stickleback population density (CPUE = total catch/total trapping hours). In two cases traps were left in the lakes overnight due to exceptionally low stickleback densities, which amounted to a total trapping effort of 97.5 h and 95 h, respectively. We euthanized 50 fish per lake with 1 g/L of ethyl-3-aminobenzoate methanesulfonate, and took tissue samples (caudal fin and caudal peduncle muscle). The fish were then fixed in an aqueous solution of formaldehyde (4 %) for shipping to the laboratory where they were transferred to water and dissected for gut removal. The dissected fish were cleared using a trypsin solution and stained using alizarin red to highlight bony structures, and ultimately stored in glycerin (Dingerkus and Uhler 1977; McGee et al. 2013).

Morphological Measures

We photographed the cleared and stained fish from three perspectives: laterally with their mouths closed, latterly with their mouths open and their jaws fully protruded, and dorsally. Using the high throughput phenotyping pipeline *phenopype* (Lürig 2021) we placed a total of 13 landmarks on each specimen (Figure S1). With the 13 landmarks, we measured jaw protrusion (**JP**), and the component traits of three functional trait systems: the lever system of the lower jaw, the epaxial-buccal cavity, and the opercular four-bar linkage (Table S3). Mechanical models predict the biomechanical function of these trait systems, which allowed us to calculate biomechanical functions rather than relying on raw morphological measures. This is important because these traits systems can be functionally redundant, i.e. different morphologies can result in the same biomechanical function (Wainwright et al. 2005; Thompson et al. 2017). For the lever system of the lower jaw, we calculated the lever ratio (**LR**), which predicts the displacement advantage of the lower jaw during prey capture (Westneat 2004; McGee et al. 2013). For the epaxial-buccal cavity, we calculated the suction index (**SI**), which reflects the fish's ability to generate suction force on prey items during suction feeding (Carroll et al. 2004; Wainwright et al. 2007). For the opercular four-bar linkage, we calculated the kinematic transmission (**KT**), which affects the force and speed of jaw opening and protrusion (Holzman et al. 2008; Thompson et al. 2017). Despite being a ratio based measurements (Thompson et al. 2017), KT and LR were size dependent, i.e. fish change in shape as they grow larger. To avoid variation in these traits that could arise from differences in mean body size between populations, we standardized to KT, DA, and the linear measurement JP to a common body length (42 mm ≙ mean body size of all fish) following (Paccard et al. 2020). For this purpose, we used the formula $S = M_0 (L_S/L_0)^b$, where S is the standardized trait, M_0 is the linear trait measurement, L_S is the mean body size of all fish, L_0 is the standard length of the fish, and *b* is the allometric scaling exponent. We estimated *b* for each lake based on the slopes from mixed effects models (using the r package *lme4*) regressing the linear traits against standard length (both log10 transformed) with lake as a random factor. After size standardization we took the mean trait value per population and used linear regression and the function *anova* in the R package *car* (Fox and Weisberg, 2019) to test for the effects of char presence on the all four traits and stickleback density ($\log_{10}$(CPUE)).

Statistical analysis of community structure

To analyze the effects of abiotic gradients, fish community configuration, stickleback density, and phenotypic variation of stickleback on zooplankton communities, we conducted a series of distance-based redundancy analysis (db-RDA) on the Hellinger transformed abundance-matrix, using the R package *vegan* (Table 1). This ordination method constrains axes of community dissimilarity to be a linear combination of environmental variables. Firstly, using the full dataset, we tested for the effects of lake morphometry, water chemistry, and fish community configuration on community structure (db-RDA-1.0). Finding that community composition was affected by interactions between abiotic gradients and fish configurations, we

Table 1: Table of the set of db-RDAs conducted to explore different drivers of community composition.

Model	Dataset	Constraints
db-RDA-1.0	Full dataset	~ fish.config. × (chem. + morph.)
db-RDA-1.1	Fishless	~ chem. + morph.
db-RDA-1.2	Char only	~ chem. + morph.
db-RDA-1.3	Char-and-stickleback	~chem. + morph.
db-RDA-1.4	Stickleback-only	~ chem. + morph.
db-RDA-2.1	Stickleback lakes	~ fish.config. × (chem. + morph.) + KT + SI + LR + JP + Density
db-RDA-2.2	Stickleback lakes	~ KT + SI + LR + JP + Density
db-RDA-3.1	Stickleback-only	~ chem. + morph. + KT + SI + LR + JP + Density
db-RDA-3.2	Char-and-stickleback	~ chem. + morph. + KT + SI + LR + JP + Density

additionally explored the effects of the abiotic gradients for each lake-type separately (db-RDA-1.1 - 1.4). Secondly, using the *stickleback-only* and *stickleback-and-char lakes* (total 33 lakes) we tested for the effects of phenotypic variation and population density of stickleback on the zooplankton community. For this purpose, we performed a db-RDA using the four functional traits, CPUE, fish configuration and the abiotic gradients as constraining variables (db-RDA-2.1). Because some traits and density covaried with environmental variables (see below), we additionally conducted a db-RDA on the combined stickleback lakes using only the traits and density as constraining variables (db-RDA-2.2). Finally, we performed db-RADs on *stickleback-only* (db-RDA-3.1) and *stickleback-and-char lakes* (db-RDA-3.2) separately (14 and 19 lakes, respectively), with the four functional traits, CPUE and the two abiotic gradients as constraining variables. We used permutation tests (anova.cca()) to test for the overall effects of the constraining variables on the community structure (by="axis"), and for the significance of individual variables in explaining community variation (by="margin).

Results

Environmental variation

Lakes ranged between 0.1– 64.5 ha in surface area and 0.75 m – 49.5 m in maximal depth. The lake morphometry gradient (Area/Perimeter) did not differ between sampling regions (ANOVA: $F_{(3,75)}$=0.944, p=0.394), but differed between lakes with different fish configurations (ANOVA: $F_{(3,74)}$=20.37, p<0.001). Posthoc test (TukeyHSD) revealed that fishless lakes were smaller/shallower than lakes that contained fish, but the later did not differ amongst fish community configurations (Figure 1b). Water chemistry differed between sampling regions ANOVA: $F_{(2,75)}$=29.17, p<0.001), with the two islands having lower values (i.e. lower conductivity, fDOM and chlorophyll) than the mainland (Figure 1b). Water chemistry did not differ between fish configurations (ANOVA: $F_{(3,74)}$=2.308, p=0.08). The lake morphometry and water chemistry gradients were not correlated with each other ($r_{(76)}$=-0.05, t=-0.47, p=0.64).

Phenotypic variation and stickleback density

Of the four functional systems, only KT significantly differed between *stickleback-only* and *stickleback-and-char lakes*, with mean trait values being larger in *stickleback-and-char lakes* (KT: $F_{(1,31)}$=4.191, p=0.049; JP: $F_{(1,31)}$=3.385, p= 0.075); LR: $F_{(1,31)}$=0.146, p=0.705; SI: $F_{(1,31)}$=0.003, p= 0.958) (Figure 2). Our estimate of stickleback population density (CPUE) did not differ between *stickleback-only* and *stickleback-and-char lakes* ($F_{(1,31)}$=0.874, p= 0.357).

Fish configuration and abiotic gradients as drivers of community structure

The db-RDA on the full dataset (db-RDA1.0) explained 41.0 % (adjusted R^2) of the community structure, with the interactions between fish-configuration and water chemistry (fish.config.:chem., $F_{(3,66)}$=4.63, p<0.001), and between fish-configuration and lake morphometry (fish.config.:morph., $F_{(3,66)}$=2.58, p<0.012) having significant effects. These constraints resulted in two significant axes of community dissimilarity ($RDA1_{full}$: $F_{(1,67)}$=35.97, p<0.001; $RDA2_{full}$: $F_{(1,67)}$=24.35, p<0.001). $RDA1_{full}$ reflects a shift in the dominance of the calanoid copepod *Leptodiaptomus minutus*, relative to other taxa, while $RDA2_{full}$ reflects which taxa replace *L. minutus* as it becomes less dominant along $RDA1_{full}$ (Figure 3).

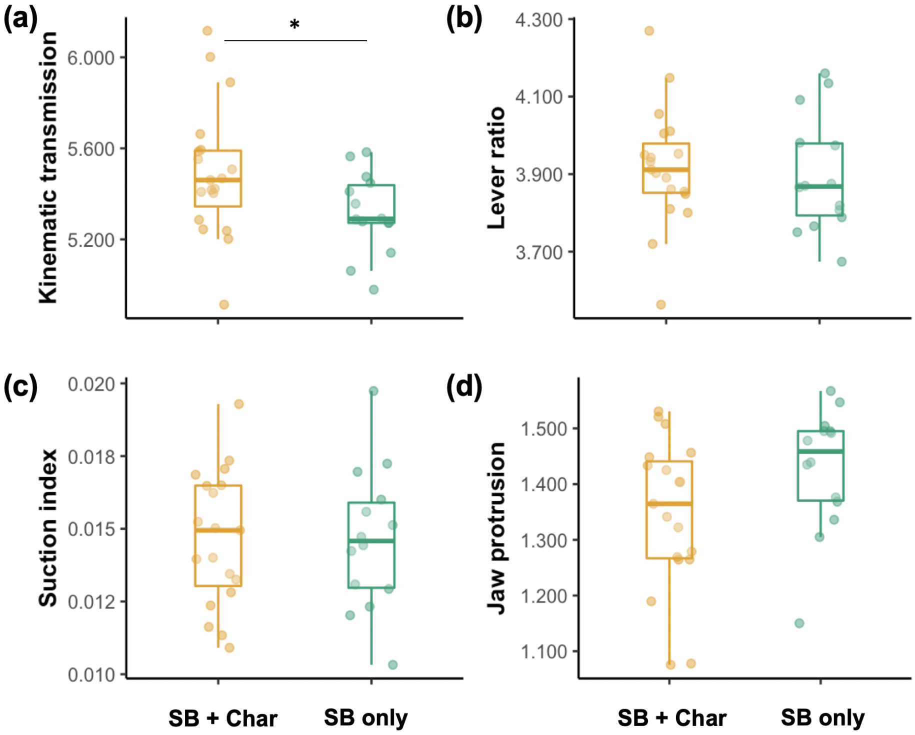

Figure 2: Phenotypic variation among stickleback populations, plotted as population means for the (a) kinematic transmission of the opercular four bar system (KT), (b) displacement advantage of the lower jaw lever ratio (LR), (c) Suction index (SI) and, (d) jaw protrusion (JP). Phenotypic divergence between lakes with and without char occurred only in KT.

To explore how the effects of the abiotic gradients depend on fish-configuration, we ran db-RDAs including water chemistry and lake morphometry for each lake-type separately (db-RDA1.1 - 1.4). For all lake-types RDA1 was associated with a shift in the dominance of *L. minutus*. This axis was significant in all lake-types, except for *stickleback-only-lake.* ($RDA1_{fishless}$: $F_{(1,31)}=17.41$, $p<0.001$; $RDA1_{char\text{-}only}$: $F_{(1,5)}= 4.89$, $p=0.03$; $RDA1_{SB\&char}$: $F_{(1,17)}=14.20$, $p=0.002$; $RDA1_{SB\text{-}only}$: $F_{(1,13)}=3.62$, $p=0.160$). In *fishless-lakes* community structure was driven by water chemistry (chem., $F_{(1,31)}=17.41$, $p= 0.001$; morph., $F_{(1,31)}=0.79$, $p=0.54$), in *char-only-lakes* by lake morphometry (chem., $F_{(1,5)}=9.59$, $p= 0.001$; morph., $F_{(1,5)}=0.79$, $p=0.54$), and in *stickleback-and-char-lakes* by both water chemistry and lake morphometry (chem., $F_{(1,17)}=8.59$, $p=0.002$; morph., $F_{(1,17)}=6.02$, $p=0.011$). In *stickleback-only-lakes* water chemistry and lake morphometry had no effect on community structure (chem., $F_{(1,13)}=0.64$, $p=0.53$; morph., $F_{(1,13)}=3.36$, $p= 0.08$) (Figure 4).

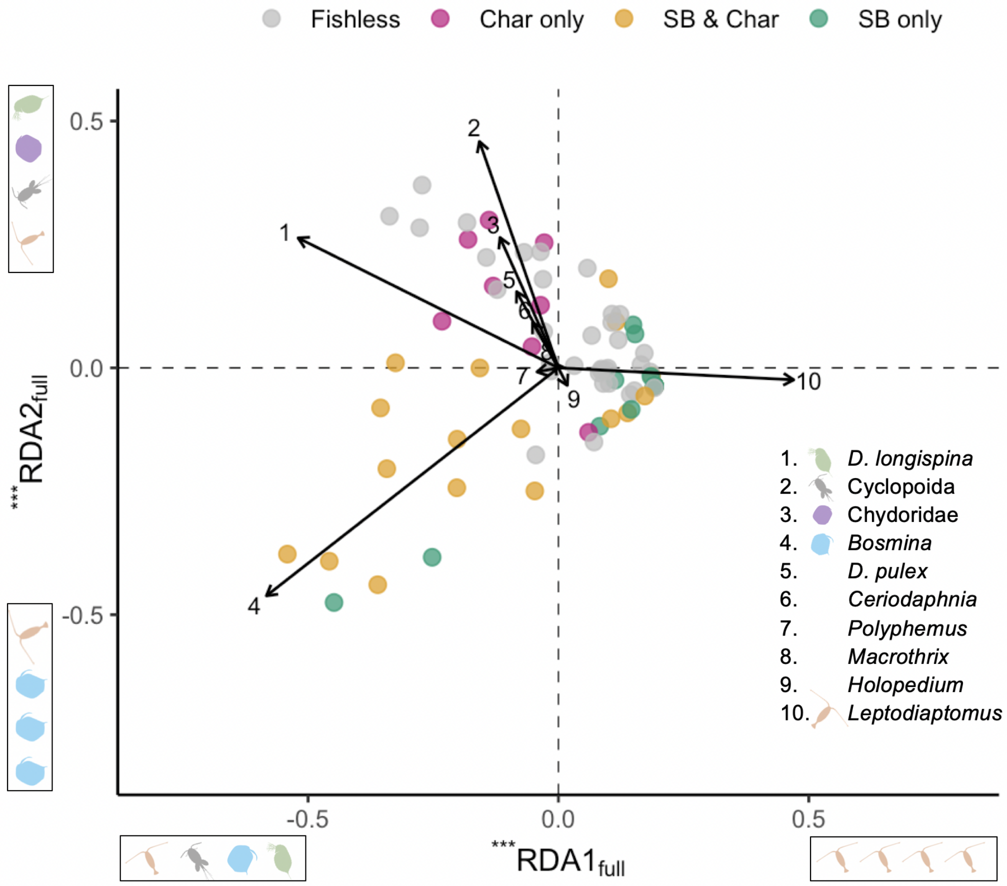

Figure 3: Db-RDA of all lake communities. The first two db-RDA axes significantly explain community variation, and were associated with interactions between fish community composition and lake morphometry and water chemistry, respectively . The first axis reflects a shift in the dominance of the calanoid copepod *Leptodiaptomus minutus* relative to all other common taxa (i.e. Daphnia, Bosmina, cyclopoids and chydoridae). The second axis reflects which taxa replace calanoids as they become less dominant. In the case of stickleback-and-char lakes *L.minutus* became predominantly replaced by *Bosmina,* and by *Daphnia* and cyclopoids in fishless and char-only lakes. Most stickleback-only lakes were dominated by *L. minutus.*

Stickleback density and phenotypes as drivers of community structure

Db-RDA on the combined *stickleback-and-char* and *stickleback-only-lakes* (db-RDA-2.1) including stickleback density and traits, alongside the fish configuration and abiotic gradients significantly explained zooplankton community structure (adj. R^2=0.41; RDA1: $F_{(1,22)}$=28.75, p=0.001), and was driven by the interaction between fish-configuration and water chemistry (fish.config.:chem., $F_{(1,22)}$=4.70, p=0.019), with weak evidence for an effect of jaw protrusion (JP, $F_{(1,22)}$=3.25, p=0.059). When we repeated this db-RDA including only the traits and

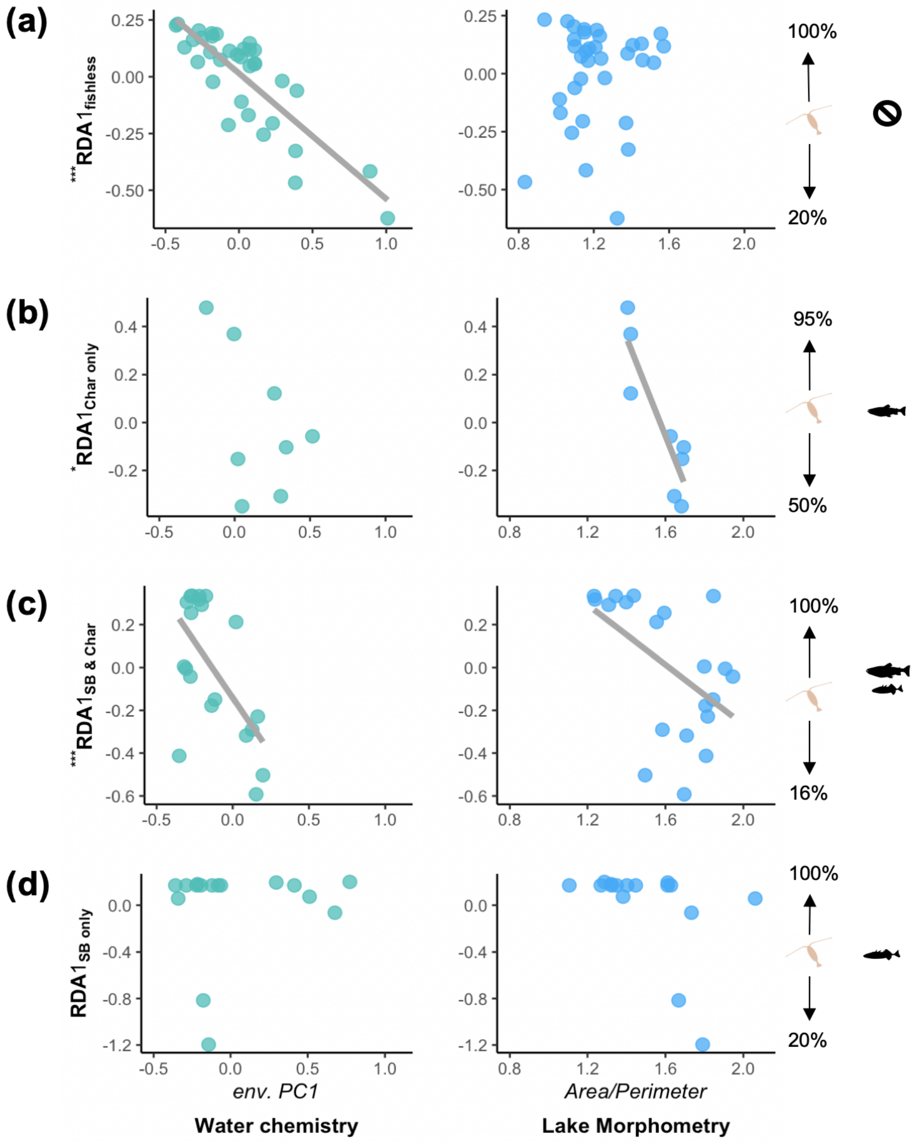

Figure 4: The effects of gradients in water chemistry and lake morphometry on community structure. In fishless lakes (a) water chemistry is strongly associated with a shift in the dominance of *L.minutus* relative to all other taxa, whereas in char-only lakes (b) this shift in dominance is associated with lake morphometry. In stickleback-and-char lakes (c) the shift from calanoid to Bosmina dominated systems is associated with changes in both water chemistry and lake morphometry. With few exceptions, stickleback-only lakes (d) are dominated by calanoids, irrespective of abiotic conditions.

stickleback density (db-RDA-2.2), community structure could not be significantly explained (adj. R^2=0.12; RDA1: $F_{(1,27)}$=7.98, p=0.11), however, there was a significant effect of the kinematic transmission of the opercular 4-bar linkage (KT , $F_{(1,22)}$=5.16, p=0.014).

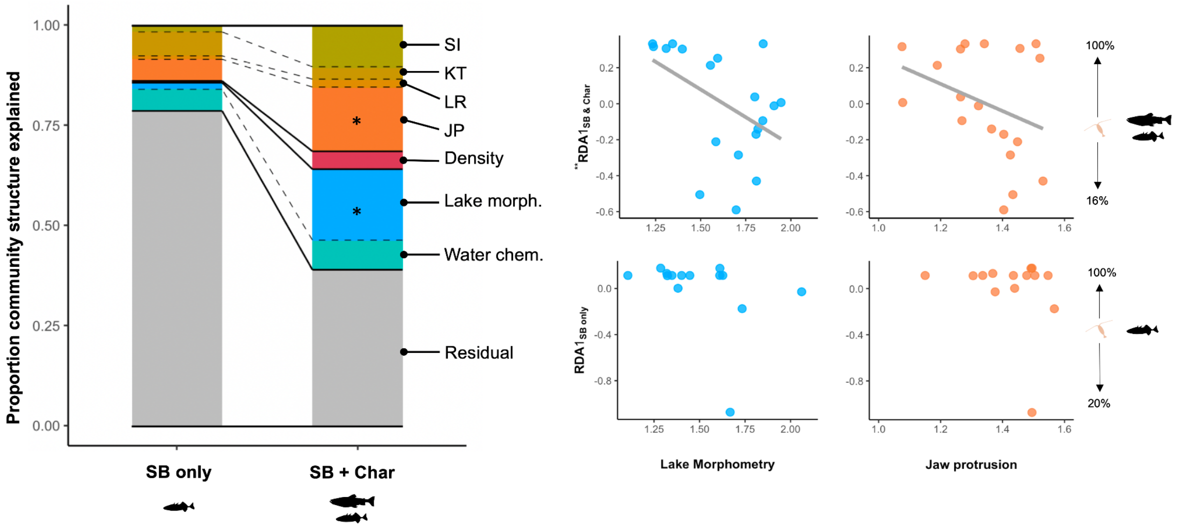

Figure 5: The effects of intraspecific-trait variation in driving community structure, relative to other ecological factors. Most stickleback-only lakes have very similar zooplankton communities (near 100% calanoid copepods), while stickleback-and-char lakes have more variable communities that are driven by lake morphometry and jaw protrusion.

To explore the effects of stickleback traits and density independent of fish-configuration, we also conducted db-RDAs on *stickleback-and-char* (db-RDA-3.1) and *stickleback-only-lakes* (db-RDA-3.2) separately, including stickleback density, traits and the abiotic variables as constraining factors. This model significantly explained community structure in *stickleback-and-char* lakes (adj. R^2=0.47; RDA1: $F_{(1,11)}$=19.49, p= 0.004), and was driven by lake morphometry (morph., $F_{(1,11)}$=4.98, p=0.014) and variation in jaw protrusion (JP, $F_{(1,11)}$=4.51, p=0.028), such that *L. minutus* was dominant in small lakes and when stickleback had low jaw protrusion, and was increasingly replaced by *Bosmina* as lakes became deeper and jaw protrusion became larger. The same model could not explain community structure in *stickleback-only-lakes* (adj. R^2=-0.59; RDA1: $F_{(1,7)}$=1.93, p=0.89), as none of the variables had a significant effect (Figure 5).

Discussion

Intraspecific variation in foraging traits arises frequently when predator populations evolve independently and under different environmental conditions. Ample experimental evidence suggests that such phenotypic variation can have community-wide effects. However, the importance of intraspecific variation in driving community structure in natural systems often remains unclear, especially in relation to other putative abiotic and biotic factors (El-Sabaawi

2017). Here, we use a set of 78 Greenlandic lakes to elucidate how lake morphometry, water chemistry, species interactions, and intraspecific variation drive variation in zooplankton community structure (Figure 1). We find that the response of the zooplankton community to water chemistry and lake morphometry is highly dependent on the configuration of the fish community (i.e. the presence and absence of planktivores and piscivores) (Figure 3,4). Furthermore, we report evidence for divergence in foraging morphology of stickleback in response to the presence of a piscivore top-predator (arctic char) (Figure 2). We find that intraspecific variation in the jaw protrusion of stickleback is correlated with prey community structure, but that this phenotypic effect is conditional on the presence of an arctic char (Figure 5). Below, we elaborate on how the observed relationships between ecological drivers and community structure may help us understand the relative role of phenotypic variation in shaping communities and the conditions under which such effects may arise, and discuss our results in the context of previous work.

Abiotic and biotic divers of community structure

The zooplankton composition among the 78 lakes varied along two major axes (Figure 3). The first axis reflects a shift in the dominance of the calanoid copepod *L.minutus* relative to all other common taxa (i.e. *Daphnia*, *Bosmina*, cyclopoid copepods and chydoridae). The second axis reflects which taxa replace calanoids as they become less dominant. In fishless lakes, the shift in calanoid dominance was strongly associated with water chemistry (Figure 4a). Lakes with low levels of conductivity, fDOM and chlorophyll were dominated by calanoids, whereas other taxa (*Daphnia*, cyclopoids etc.) became more frequent as the values of these environmental parameters increased. Such species turnover of zooplankton along this environmental gradient may be related to changes in the availability of essential resources and/or productivity. The variation in water chemistry among our sampling sites likely arises from differences in geology (granite bedrock vs. sandstone/carbonatite) and land-use (pristine vegetation vs. pasture) (cite this), which are important sources of conductivity variation (Wetzel 2001). Conductivity is a measure of dissolved ions, some of which can be limiting for specific aquatic organisms. For example, zooplankton species differ widely in their calcium requirements depending on their stoichiometric demand and body size (Wetzel 2001; Wærvagen et al. 2002; Betini et al. 2016). In particular, large-bodied and calcium-demanding species, such as *Daphnia*, are more likely to occur in calcium-rich environments, whereas species with low calcium-demands (e.g. calanoids, *Bosmina*) can thrive in calcium-poor environments (Pinel-Alloul et al. 1995; Wærvagen et al. 2002). Shifts from calanoid to cladoceran dominated systems have also been associated with increasing primary production, resulting from a competitive advantage of cladocerans over calanoids in more productive systems due to different feeding modes (McNaught 1975).

The zooplankton community composition of *char-only lakes* largely overlapped with that of *fishless lakes*, suggesting that char have limited direct effects on these zooplankton community (Figure 3). This result is somewhat contrary to previous findings demonstrating significant suppression of cladocerans by char in Greenlandic lakes *(Jeppesen et al. 2001)*. However, arctic char populations often undergo adaptive divergence, which can result in trophic polymorphisms ranging from planktivores to piscivores (McCarthy et al. 2004; Doenz et al. 2019). As a result, the ecological impact of char may depend on the ecotypes present in a lake. Only a few of our study lakes have multiple species of char, and so we do not have sufficient replication to test for the effects of char speciation on either stickleback phenotype or overall trophic structure. Calanoid dominance in *char-only lakes* was correlated with increasing lake size, but not with water chemistry (Figure 4b). However, we may lack sufficient power to detect effects of water chemistry on community structure because our sample is restricted to only eight *char-only lakes*.

The zooplankton communities in lakes with stickleback markedly differed from those in fishless and char-only lakes (Figure 3). With few exceptions, *stickleback-only lakes* are dominated by calanoid copepods, irrespective of lake conditions (Figure 4d). Calanoids are well known to be highly evasive prey items due to their efficient sensory-motor system and escape response (Yen et al. 2015) and potentially cryptic coloration (Oester et al. 2022). As such, they may be the only macrozooplankton taxon in the system that can sustain viable populations under intense predation pressure in *stickleback-only lakes*. This is broadly consistent with previous evidence from other natural systems (Rudman and Schluter 2016; Jeppesen et al. 2017) and experimental contexts (Harmon et al. 2009; Rudman et al. 2015; Matthews et al. 2016; Best et al. 2017) demonstrating that stickleback can strongly alter the pelagic prey community. For example, Harmon et al. (2009) found that only the limnetic stickleback species was able to eliminate *Diaptomus* copepods in mesocosm ecosystems, whereas these copepods persisted in the presence of a benthic species and population with an intermediate morphology.

In *stickleback-and-char lakes* the controlling effects of stickleback were less pronounced and other taxa than calanoids could rise to high frequency in some lakes. However, contrary to *fishless lakes*, calanoids were predominantly replaced by *Bosmina,* rather than *Daphnia* and Cyclopoids (Figure 3). This pattern of species turnover was associated with increasing conductivity/fDOM/chlorophyll and lake size. Contrary to *Daphnia, Bosmina* has relatively low Ca^{2+} requirements (Jeziorski et al. 2014). The shift in relative abundance along a water chemistry gradient may therefore arise from increasing primary production associated with higher chlorophyll levels, rather than (directly) from changes in conductivity. *Bosmina* often co-occur with fish, likely because they can evade predation more efficiently than other cladocerans due to their small body size (Brooks and Dodson 1965; Jeppesen et al. 2017). Thus, the presence of char may reduce planktivory by stickleback and allow *Bosmina* to establish populations, in the absence of competing cladocera such as *Daphnia.* Greater lake depth may further limit the exposure of *Bosmina* to stickleback predation (Korosi et al. 2013), especially if char presence reduces the pelagic foraging activity of stickleback.

Overall, our analyses highlight the role of stickleback in structuring zooplankton communities (Harmon et al. 2009; Des Roches et al. 2013; Matthews et al. 2016; Rudman and Schluter 2016), and the role of char in altering the top-down effects of stickleback (Jeppesen et al. 2017). Given the notable similarity between *fishless* and *char-only lakes* it is unlikely that char and zooplankton have strong direct interactions in our particular study system (but see (Jeppesen et al. 2001)). We therefore suggest that the community differences between stickleback systems with and without char arise from an indirect ecological interaction between char and zooplankton that is mediated via stickleback. Such indirect ecological effects can arise, for example, when top-predators reduce the density of the consumers and thereby lessen predation pressure on lower trophic levels (Polis et al. 2000). This can lead to shifts in community composition, when prey species respond differentially to changes in consumer densities ("species-level trophic cascade") (Polis et al. 2000). Alternatively, top-predators may affect consumer traits and thereby change the interaction between consumers and prey. Such trait-mediated indirect effects may involve behavioral changes, such as decreasing in consumer activity or habitat switching (Křivan and Schmitz 2004), and plastic or heritable changes in morphology, physiology and life-history (Utsumi et al. 2010).

Phenotypic divergence between predation regimes and its effects on community structure

Of our four candidate traits, only the kinematic transmission of the opercular four bar linkage (KT) diverged between lakes with different predation regimes, with populations in stickleback-and-char lakes having higher KT values (Figure 2). Theory suggests that high KT conveys a performance advantage when capturing plankton (Westneat 2004), and has been previously associated with limnetic foraging in threespine stickleback (McGee et al. 2013; Schmid et al. 2018). Increased limnetic performance is contrary to the expectation that stickleback reduce pelagic feeding when co-occurring with char due to an increased risk of predation in the open-water habitat (Vamosi and Schluter 2002). A possible adaptive explanation for the observed pattern is that there is a lower availability of less-evasive pelagic in lakes with char, whereas in stickleback-only lakes the pelagic is largely deprived of available resources, as there are only the more evasive calanoids remaining. This may facilitate the adaptation to pelagic resources in stickleback-and-char lakes, despite the increased mortality risk when foraging in the open water. Alternatively, the divergence in KT may arise if lakes with and without char differ in resource limitation (e.g. due to competition, predation, or differences in the resource base) (Moosmann et al. Chapter 3). In fishes, resource limitation often leads to smaller average body size (“stunting”), but can also affect body proportions, especially of the cranium (Ylikarjula et al. 1999; Chizinski et al. 2010), and may as such drive divergence in functional trait systems (Moosmann et al. Chapter 3).

Our comparative data is ambiguous with respect to whether KT is casually affecting prey community structure. This is because among-population variation in KT was associated with community structure across all stickleback lakes, but not when char-presence was included as an explanatory variable. This is because char-presence and KT covary and thus overlap in the proportion of community structure they explain. Hence it is unclear if KT and community structure are mechanistically related, or if they share a common underlying cause of variation (e.g. char predation). Experiments would be helpful to distinguish between the effects of morphology and other mechanisms (e.g. changes in behavior). For example, planktivorous fish with divergent morphology (with KT as a suitable candidate trait) could be transplanted into lakes (or mesocosms) with and without piscivorous predators in a fully factorial design. This would allow us to independently assess the effects of planktivore morphology and other predator-related effects in driving community structure.

Trait-mediated community-wide effects depend on predation regime

In *stickleback-and-char lakes* the shift from calanoid- to *Bosmina*-dominated communities was associated with increasing mean jaw protrusion (Figure 5), supporting the prediction of previous experimental findings that jaw protrusion may act as a community-effect trait (Schmid et al. 2018). In stickleback, divergence in jaw protrusion has been repeatedly associated with local adaptation to pelagic habitats (McGee et al. 2013; Schmid et al. 2018). Biomechanical analysis of different teleosts has provided evidence that greater jaw protrusion improves prey-capture performance when foraging on zooplankton in general, and evasive copepods in particular (Holzman and Wainwright 2009; Yen et al. 2015; Schmid et al. 2018). In an experiment, Schmid et al. (2018) tested the utility of stickleback traits when foraging in a mixed pelagic community. In this small-scale (15 L tanks), short-term (8 min) experiment, greater jaw protrusion increased the performance of capturing the most evasive prey items (copepods) but was unrelated to prey capture success of less evasive cladocera (*Bosmina* and *Daphnia*). The same study analyzed the resulting community change, and compared it to the outcomes of a longer-term (~12 weeks) mesocosm experiment using the same populations (Matthews et al. 2016). While the overall effects of stickleback predation was strong on all zooplankton taxa, populations with larger jaw protrusion removed a proportionally higher biomass of copepods than of cladocera, at both the tank (15L) and the mesocosm (1000L) scale.

The outcomes of these experimental studies are strikingly similar to the patterns we observe among *stickleback-and-char lakes* (but not in *stickleback-only lakes*), where greater jaw protrusion of stickleback populations is associated with a shift towards more cladocera-dominated systems. But why does increased jaw protrusion benefit *Bosmina* over calanoids, and why is this response dependent on the presence of char? Calanoid copepods are exceptionally nutritionally valuable (Hudson et al. 2021; Twining et al. 2021) but are also highly evasive prey (Yen et al. 2015). As such, calanoids might be preferentially targeted by stickleback populations that are capable of catching them (e.g. due to high jaw protrusion (Holzman and Wainwright 2009)). We propose that variation in jaw protrusion alters the interaction-strength between stickleback and calanoids, and thereby directly or indirectly affects the performance of *Bosmina* populations. For example, increased predation on calanoids may reduce predation pressure on *Bosmina* below a threshold that allows *Bosmina* populations to establish. Predation pressure on *Bosmina* may only fall below this threshold in *stickleback-and-char lakes*, where we expect that overall predation pressure from stickleback is relatively low. In *stickleback-only lakes*, the negative effects of increased predation pressure by stickleback (as a consequence of release from piscivory) may outweigh the more subtle, indirect positive effects of increased jaw protrusion on *Bosmina*.

Conclusion

The present comparative study links phenotypic variation in functional morphology among stickleback populations with variation in the structure of prey communities. This link may be interpreted in three ways: i) functional morphology and community structure are not mechanistically related but share underlying causes of variation, ii) variation in functional morphology arises as a (plastic or evolutionary) response to differential community structure, and iii) variation in functional morphology affects community structure, via changing species interactions. It is notoriously challenging to infer causal relationships from comparative data, especially since, in our case, the latter two interpretations are not mutually exclusive. However, in the context of previous findings, our results yield important insights. Previous experimental work has successfully established a mechanistic relationship between jaw protrusion and both foraging performance and zooplankton community change (Schmid et al. 2018). These results have highlighted the potential for an interplay between evolutionary and ecological dynamics, where communities impose selection on foraging morphology and changes in foraging morphology shape communities. This study adds to a growing body of evidence that such an interplay may play a role in shaping the community dynamics of ecosystems in nature. Our results also provide new insights about the possible context dependence of such dynamics (Catford et al. 2022), an aspect that has found little attention in previous studies (Rudman et al. 2015; terHorst et al. 2018) and is largely unexplored in natural systems. In different ecological contexts, the effects of intraspecific variation may be overridden by the effects of top-predator presence, abiotic conditions or phenotypic variation of other taxa. For example, Oester et al (2022), found that *L. minutus* expresses variation in color that is likely shaped by a trade-off between radiation exposure and stickleback predation. Presently it is unclear how such phenotypic variation may affect predator-prey interactions and community dynamics in the system. We propose that more experimental and comparative work is necessary to understand the interplay between evolutionary trait changes and community dynamics in nature, such as tests of differential selection between resource environments (Bolnick and Lau, 2008), and transplant experiments to disentangle trait-mediated effects from alternative mechanisms.

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Supplementary material for:

Predator species, their traits, and abiotic environments jointly shape prey community structure

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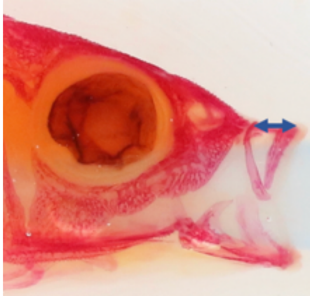
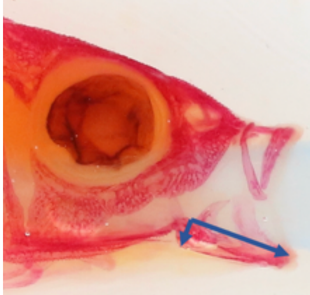
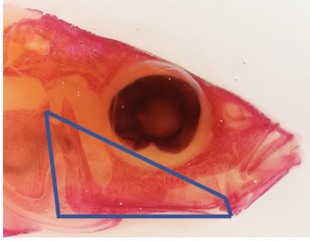
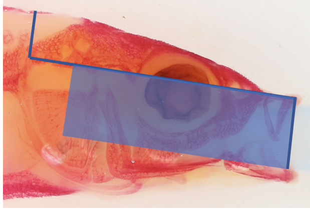
Table S1: Specifics on the chemical parameters obtained from multiparameter sondes. And their loading on the first two principal component axes of the environmental PCA. Proportion of explained variation is reported in brakes.

Parameter	**Unit**	**Sensor type**	**env. PC1 (41%)**	**env. PC2 (26%)**
Conductivity	µS × cm−1	4-electrode cell	1.51	0.91
ODO	mg/L	Optical, luminescence	-0.46	1.94
fDOM	fluorescence	Optical, fluorescence	1.56	-0.28
Chlorophyll A	fluorescence	Optical, fluorescence	1.50	-0.04

Table S2: Summary of the zooplankton communities, including the number of lakes a taxon was present (total 78 lakes), the mean number of individuals per liter (where present), and mean body sizes. Mean body sizes and standard errors are based on lake means. Members of the family Chydoridae (*) were summarized into one category.

Taxon	**No. of lakes**	**Abundance (ind./l)**	**Body size (mm)**
Leptodiaptomus minutus	78	36.024	0.62 ±0.01
Bosmina sp.	54	2.477	0.51 ±0.01
Cyclops/Megacyclops sp.	49	2.316	0.86 ±0.02
Daphnia longispina	44	3.187	0.82 ±0.02
* *Chyorus sp.*	35	9.626	0.30 ±0.01
Daphnia pulex	27	1.445	1.60 ±0.05
Polyphemus sp	23	0.091	0.83±0.02
* *Acropercus sp.*	20	0.071	0.64 ±0.03
* *Alona sp.*	17	0.263	0.39 ±0.07
Ceriodaphnia sp.	17	0.461	0.67 ±0.02
* *Eurycercus sp.*	17	0.174	1.01 ±0.10
Macrothrix sp.	11	0.032	1.22 ±0.14
Holopedium sp.	10	1.645	1.22 ±0.10

Table 3: Candidate traits used in the analysis of community structure**.**

Trait	**Landmarks**		**Function**	**Reference**
Jaw protrusion (JP)	1,3		Impacts the hydrodynamic disturbance during suction feeding, which strain-sensitive prey utilize to sense and evade predation.	McGee et al. 2013; Holzman and Wainwright 2009
Lever ratio of the lower jaw lever (LR)			Two-lever system that measures a trade-off between force and speed that is generated when rotating the lower jaw during prey capture	McGee et al. 2013; Thompson et al. 2017; Westneat 2004
In lever	4,5			
Out lever	4,2			
Kinematic transmission of the opercular four-bar linkage (KT)			Predicts how rotation of the input link translates into rotation of the output link (kinematic transmission), which affects the force and speed of jaw opening and protrusion.	McGee et al. 2013; Thompson et al. 2017; Westneat 2004; Holzman et al. 2008
Fixed link	9,11			
Coupler link	10,12			
Input link	11,12			
Output link	9,10			
Suction index (SI)			Predicts pressure gradient (suction) a fish can generate as a function of the force of the epaxial musculature, the moment arm of the epaxials, the moment arm of the buccal cavity and the area of the buccal cavity.	(Carroll et al. 2004; Wainwright et al. 2007)
Gape width	1,2			
Epaxial height	7,8			
Epaxial width	13,14			
Buccal length	1,6			
Neurocranium outlever	1,7			

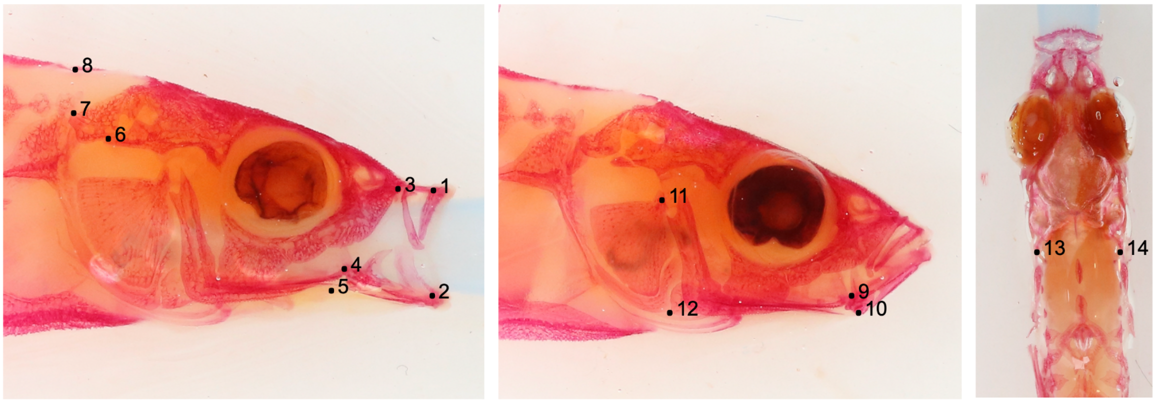


Figure S1: Landmarks used for morphological analysis (McGee et al. 2013)
1. anteriormost extent of premaxilla; 2. anteriormost extent of dentary; 3. anteriormost extent of maxilla; 4. quadrate-articular jaw joint; 5. insertion of the interopercular-articular ligament; 6. posteriormost extent of buccal cavity; 7. point of articulation between the supracleithrum and post-temporal; 8. dorsalmost extent of epaxial muscle, measured dorsal to landmark 6; 9. quadrate-articular jaw joint; 10. insertion of the interopercular-articular ligament; 11. opercular joint; 12. posteroventral extent of interopercule; 13. point of articulation between the supracleithrum and post-temporal left; 14. point of articulation between the supracleithrum and post-temporal left.

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Chapter 4

Differential response of body size and head size to predator mediated resource limitation causes phenotypic variation among stickleback populations

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JB organized the logistics of the fieldwork. MM, RG and BM conducted the fish and zooplankton sampling. BM and MM conceived and formulated the ideas presented. MM processed the fish and led the analysis and the writing. All authors provided critical feedback on the manuscript.

Abstract

The phenotypic variation of consumer populations is jointly shaped by their predators and prey communities, however in natural food webs, we often lack an understanding of the ecological mechanisms that affect consumer phenotypes. In a comparative study, we explore how phenotypic variation of 34 Greenlandic stickleback populations is affected by the presence and absence of an intraguild predator (arctic char). We find that while char-presence positively affects overall body size, it does not affect head size. This differential response to predation, results in a change in the allometry (i.e. the relative size) of the head, and is consistent with changes in growth patterns in response predation and resource limitation observed in other fishes. Using path analysis, we show that the observed shift in relative head size of stickleback is, in part, explained by predator induced changes in the species composition and availability of zooplankton biomass. Our study suggests that predators can indirectly affect resource limitation via changes in the prey communities of consumers, and thereby influence the allometry of consumer traits, likely via allocation tradeoffs. These results have important implications for interpreting the allometric patterns among populations and for using comparative studies to dissect the ecological causes of the phenotypic variation of natural populations.

Introduction

Species-interactions are important drivers of phenotypic variation among lineages in nature, but understanding the mechanisms by which predators, competitors, and resources jointly shape phenotype-distributions remains a challenge (terHorst et al. 2018). Predation, for example, is often used to explain among-population variation in the body size of prey species. Predators can affect prey body size distributions via multiple mechanisms including changes in demography, life-history and resource availability (Rodd & Reznick 1997; Ylikarjula et al. 1999; Nakazawa et al. 2007; Billman et al. 2011; Czorlich et al. 2022). Often predator effects on size are attributed to shifts in life-history traits (e.g. growth, size at maturity, fecundity), many of which are jointly determined by allocation tradeoffs expressed in a given resource environment (Reznick et al. 2019). Compared to effects of predation on life-history tradeoffs, and the resulting effects for body size (Palkovacs 2003; MacColl et al. 2013), we have a much weaker understanding about the effects of predator-induced allocation-tradeoffs on the allometry (i.e. their scaling relationship with body size) of other traits that underlie organismal performance (Chizinski et al. 2010). However, substantial variation in such fitness-relevant traits can arise due to allometry (Klingenberg & Zimmermann 1992). For example, mismatches between body size and either resource acquisition or escape performance could be detrimental in certain ecological contexts (Langerhans 2009). In order to better understand the causes of the phenotypic variation in nature, we need to determine how the allocation tradeoffs that govern body size variation might also influence the expression of other fitness-relevant traits (Shingleton et al. 2009).

Predators can affect the body size distributions of their prey populations directly, for example via selection on body size and life-history traits, or indirectly, for example by effecting resource availability and population densities (Palkovacs 2003; Heino *et al.* 2015; Singkam & MacColl 2019; Czorlich *et al.* 2022). For many predators, especially in aquatic ecosystems, the size of available prey items is limited by the extent of their gape (gape-limitation), which can result to selection for larger body size in prey species (Janzen *et al.* 2000; Langerhans 2007). Life-history theory predicts that the increased risk of mortality before reproduction leads to the evolution of faster growth and earlier maturation, resulting in smaller body size - as has been experimentally documented in guppies (Reznick *et al.* 1990). At the same time, predator-induced mortality can reduce prey densities and weaken the competition for resources among prey individuals, which may foster faster growth and larger individuals when predators are abundant and growth is density dependent (Ylikarjula *et al.* 1999). In nature, these mechanisms are likely at play simultaneously, and their relative importance for shaping body size variation may be highly context specific (Billman *et al.* 2011; Singkam & MacColl 2019).

By reducing prey density and increasing per-capita resource availability, predators may indirectly affect the phenotypic development of prey (Chizinski *et al.* 2010). Through an organism's life-cycle its overall somatic growth and phenotype trajectory depends on the allocation of resources to constituent body parts (Shingleton *et al.* 2009). Allocation tradeoffs between body parts may emerge when resources are limited and/or when body parts make significantly different contributions to different fitness-relevant functions (e.g. maintaining survival, reproduction, storage etc.). For example, when reproductive investment occurs at the expense of other traits (e.g. overall somatic growth), this can substantially alter the trait-allometry of organisms (Post & Parkinson 2001). In fishes, resource limitation often leads to small sized (i.e. “stunted”) individuals, with large heads relative to their body (Olsson *et al.* 2007; Chizinski *et al.* 2010). This effect on the head-allometry likely arises because the investment into the growth of more caudal body parts is reduced in resource poor environments, while the bony structures of the cranium grow continuously irrespective of the environmental conditions (Olsson *et al.* 2007). Such differential sensitivity of trait expression to resource conditions is a common feature of organisms (Felmy *et al.* 2022), and can strongly impact how we interpret the causes of phenotypic variation among populations in nature.

Here, we do a comparative analysis of the body size and the allometry of the head and other morphological traits for stickleback *(Gasterosteus aculeatus)* populations with and without intraguild predation by arctic char (*Salvelinus alpinus*). We analyze 34 stickleback populations in southern Greenland that are either solitary (N=14) or in sympatry with arctic char (N=20), and focus on the underlying causes of head size variation relative to total body size. Relative head size has been previously associated with food limitation in multiple fish species (Olsson *et al.* 2007; Chivers *et al.* 2008; Chizinski *et al.* 2010), and with feeding habitat in stickleback (McGuigan *et al.* 2010). We find that stickleback have larger body sizes in the presence of arctic char, consistent with predation increasing greater per capita food availability, and that stickleback have relatively larger heads in the absence of char, possibly symptomatic of food limitation culminating in reduced allocation to body growth relative to investment in heads. Such phenotypic outcomes might be partly explainable by arctic char having indirect effects on the prey community of stickleback, specifically reducing the predation pressure of stickleback on the zooplankton community, and, thereby, allowing the persistence of less evasive zooplankton species. Furthermore, we find that relative head size is the primary driver of the allometry of other functional cranial traits. Altogether, our results have important implications for interpreting the allometric patterns of fitness-relevant traits, and for using comparative studies to dissect the ecological causes of the mosaic-like phenotypic variation of natural populations (Felmy *et al.* 2022).

Material and Methods

Study Area and Sample Collection

In August 2018 and July-August 2019 we sampled zooplankton communities and threespine stickleback (*Gasterosteus aculeatus*) populations from 34 lakes in the Kujalleq municipality of southern Greenland. The sampling effort focused on three regions: the islands Akia (60°40' 42" N, 46°5'39" W) and Tuttutoq (60°49'14" N, 46°27'51" W), and on the peninsular mainland surrounding the settlement Qassiarsuk (61°09′00″N 45°31′00″W) (Figure S1, Table S1). Lakes in this area are inhabited by two fish species - threespine stickleback and arctic char (*Salvelinus alpinus*). In each lake we determined the configuration of the fish community (i.e. the presence and absence of stickleback and char, respectively) using a combination of visual inspection and trapping (minnow traps and gill nets). In total we sampled 14 lakes with only stickleback, and 20 lakes that contained both stickleback and char (hereafter *stickleback-and-char lakes* and *stickleback-only-lakes*, respectively).

Fish sampling and processing

Adult fish were captured using 5–10 unbaited steel minnow traps that were placed in the littoral zones of the lake between 1–5m from the shore. Traps were set for a specific amount of time (typically less than 2 h), with a total trapping effort per lake ranging from 5.5–64h, allowing us to calculate the catch per unit effort as an estimate of stickleback population density (CPUE = total catch/total trapping hours). In two cases (T04 and T08, Table S1) traps were left in the lake overnight due to low fish densities, which amounted to a total trapping effort of 97.5h and 95h, respectively. Captured fish were euthanized on-site with 1g/l of ethyl-3-aminobenzoate methanesulfonate, and tissue samples (caudal fin and muscle) were obtained. The fish were then fixed in an aqueous solution of formaldehyde (4%) for shipping to the laboratory, where they were transferred to water and dissected for gut removal. The dissected fish were cleared using a trypsin solution and stained using alizarin red to highlight boney structures, and ultimately stored in glycerin (Dingerkus & Uhler 1977; McGee *et al.* 2013). We photographed each fish three times: laterally with their mouths closed, latterly with their mouths open and their jaws fully protruded, and dorsally. On these photographs we placed 19 landmarks (Figure S2) to obtain linear trait measurements using the high throughput phenotyping pipeline phenopype (Lürig 2021). Based on these landmarks, we determined standard length, head length, and 11 additional cranial traits that are associated with foraging performance (for details see Figure S2 and Table S2). Following (Paccard et al. 2020) we standardize the linear traits to a common body length (45mm ≙ mean body size of all fish), by using the formula $S = M_0 (L_S/L_0)^b$, where S is the standardized trait, M_0 is the linear trait measurement, L_S is the mean body size of all fish (45mm), L_0 is the standard length of the fish, and b is the allometric scaling exponent *b*. We obtained within population estimates for b, by extracting the slopes from mixed effects models using the r package lme4 (Bates et al. 2015) regressing the linear traits against standard length (both log10 transformed) with lake as a random factor (Paccard *et al.* 2020).

Zooplankton sampling and processing

For the zooplankton sampling we performed vertical tows with a plankton net (mesh size: 90 μm) at the estimated deepest point of each lake. The zooplankton samples were fixed with an aqueous iodine solution (Lugol's solution), for subsequent analysis of taxonomy (typically to *Genus*), abundance, and body size. To do this we first inspected the samples with a dissecting microscope to get an overview of the abundance and composition of the sample. We then split the sample using a plankton splitter until we reached density of 300-400 individuals for counting, identification and body size measurements. In samples that contained few individuals, the entire sample was counted without splitting. If the initial inspection revealed that rare plankton species were present in the sample, we counted and measured those at a lower split. We calculated the biomass per prey species contained in the sample by using published length-weight regressions (Table S3) (Dumont *et al.* 1975; Bottrell *et al.* 1976). From the counts and biomass estimations per sample we calculated the abundance/biomass per liter in the lake by using the diameter of the plankton net, the depth of the vertical tow, and the number of sample splits.

The effects of char on resource conditions

To understand the effects of char on zooplankton community composition, we conducted an unconstrained ordination (RDA) on the Hellinger transformed plankton-abundance matrix using the *capscale* function in the r package vegan (Oksanen et al., 2019). This ordination revealed that *stickleback-only lakes* are largely dominated by the calanoid copepod *Leptodiaptomus minutus*, whereas *stickleback-and-char* are more variable in their composition and typically contain higher proportion of other taxa such as cladocerans and cyclopoid copepods (Figure 1A). This shift is likely due to the higher evasiveness of calanoid copepods relative to cladoceran species and large-bodied cyclopoid copepods (see discussion, chapter 3). We thus grouped the plankton biomass into *evasive* (Calanoids) and *non-evasive* prey (sum of remaining prey types). We then tested for differences in the total plankton biomass, as well as biomass of evasive and non-evasive plankton between *stickleback-and-char* and *stickleback-only lakes* using Mann-Whitney U tests. Similarly, we tested for difference in stickleback densities (CPUE) between *stickleback-only lakes* and *stickleback-and-char lakes*.

Body size variation and head allometry.

We tested for the difference in body size, head size, and relative head size (i.e. standardized to body size) between *stickleback-and-char-lakes* and *stickleback-only* populations using linear mixed effects models with char absence/presence as fixed variable, and lake as a random effect to account for the non-independence among individual fish in the same lake using the r package lme4. To obtain p values we used the r package lmerTest (Kuznetsova et al. 2017). We also tested for a correlation between the population means of body size and mean relative head size (standardized) using a Spearman's rank correlation test. Mean body size as a measure of the size distribution is likely affected by the age structure of the population (e.g. the abundance of young-of-the-year). However, young-of-the-year fish were typically not sampled due to the mesh size of the minnow trap, and we thus assume that the population means adequately represents variation in adult body size among populations (Shin *et al.* 2005).

Bayesian path analysis: the effects of predation, stickleback density and prey biomass on head size

The focal hypothesis of this study is that variation in the allometry of head size is at least partially driven by the effects of char predation on resource availability. To test this hypothesis, we conducted a Bayesian path analysis, in which we modeled standardized head length as a function of char absence/presence, stickleback density and the biomass of evasive and non-evasive plankton biomass. Specifically, we used the *brm* and *bf* functions in the r package *brms* (Bürkner 2018) to combine four linear regression models: i) stickleback density as a function of char absence/presence, ii) evasive prey biomass as a function of stickleback density and char absence/presence, iii) non-evasive prey biomass as a function of stickleback density and char absence/presence, and iv) head size as a function of char absence/presence, stickleback density, evasive prey biomass and non-evasive prey biomass (for the resulting path structure see Figure 3). To set up the analysis, all continuous variables were $\log_{10}$ transformed and standardized to have a mean of 0 and a standard deviation of 0.5, with a normal prior of mean 0 and standard deviation 1 for both slope and intercept terms (Carpenter et al. 2017). Using the function *se* in *brms* we specified standard errors of the population head size means (Bürkner 2017). Three lakes with overall low pelagic biomass (A01, A09, A07) contained no non-evasive prey (i.e. only calanoid copepods were present). To facilitate data transformation and subsequent analysis, we added a value of 0.0125 ug/l non-evasive biomass for these lakes, which is half of the minimal value observed in the dataset and corresponds to one daphnia in ~300L. To test the hypothesis that head size is driven by variation in the zooplankton community, we also performed a mixed effects model that included community RDA1 (Figure 1A), total prey biomass (log10) and CPUE (log10) as fixed effects and lake as random variable. We excluded char from this model because RDA1 and char presence are highly correlated, and the former is a continuous variable and so better suited for this hypothesis test.

Analysis of other fitness-relevant morphological traits

To understand the consequences of variation in head-length for other cranial traits, we performed a morphological PCA on a set of 11 linear traits that are associated with ecological performance (Table S2). All traits used in the morphological PCA were standardized to a common body size as described above. We then performed an additional path analysis (*path analysis 2*) to explore the drivers of population variation in the first two morphological PC axes. The data were transformed and standardized the same way as in the first path analysis. Specifically, we combined three linear models: i) relative head size as a function of char absence/presence, stickleback density, total available plankton biomass, and lake surface area, and ii)/iii) populations mean position on the morphological PC axes 1 and 2, respectively, as a function of all of the above and head size (see Figure 4C for the resulting path structure).

Results

The effects of char and stickleback on the zooplankton community

The RDA on the zooplankton community revealed a shift in the dominance of the calanoid copepod *Leptodiaptomus minutus* that was associated with the presence and absence of char.

While *stickleback-only lakes* where dominated by *L.minutus*, *stickleback-and-char*-lakes contained more mixed communities including cyclopoid copepoda and cladocera (Figure 1A). We found that total zooplankton biomass (W = 118, p = 0.46) did not differ between lakes with and without char (W = 166, p = 0.38). While the evasive-prey biomass (i.e. *L. minutus*) was not affected by the presence/absence of char in the lakes (W = 130, p-value = 0.74), non-evasive-prey biomass (i.e. sum of all other species) was significantly lower in *stickleback-only lakes* (W = 55, p = 0.003) (Figure 1B). The presence of char did not affect the density (CPUE) of the stickleback populations (W = 166, p = 0.38).

Body and head size variation among lakes

Individuals in *stickleback-only lakes* had shorter bodies than those in *stickleback-and-char-lakes* (char effect = -0.035 [-0.058, -0.01], $t_{(32)}$ = -2.89, p = 0.007), while absolute head size did not differ between lake types (char effect = -0.012 [-0.04, -0.01], $t_{(32)}$ = -1, p = 0.33). This difference in the response of body-length and head-length, resulted in *stickleback-only* populations having larger standardized heads, i.e. larger heads relative to the body (char effect

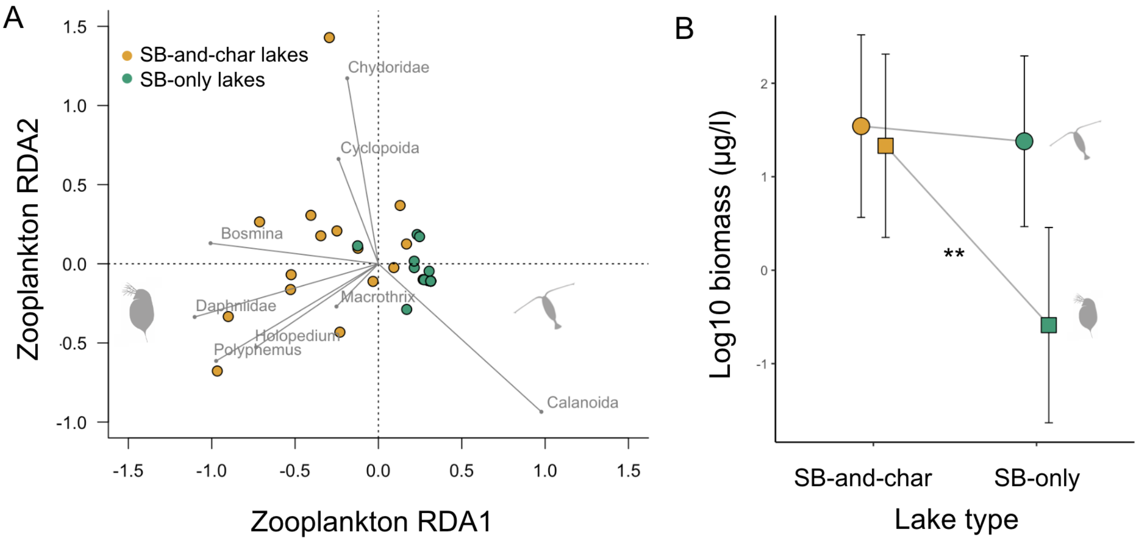

Figure 1: Effects of char presence on zooplankton community composition and biomass. The zooplankton communities of *stickleback-only lakes* were largely dominated by highly evasive calanoid copepods, while *stickleback-and-char* lakes have more diverse pelagic communities that contain higher abundances of other species, most notably less-evasive cladoceran (A). This shift in community composition lead to a collapse of non-evasive prey biomass (squares) when char were absent, while the biomass of evasive prey (circles) remained unaffected by the presence or absence of char (B).

= 0.02 [0.01, -0.03], $t_{(32)} = -4.66$, $p < 0.001$). Among populations, mean body size and mean standardized head size were negatively correlated (Spearman's rank correlation S = 8968, $\rho_{(33)} = -0.37$, p = 0.032), such that larger bodied populations had smaller relative heads (Figure 2).

Bayesian path analysis: the effects of predation, stickleback density and prey biomass on head size

The first path analysis confirmed the results of the mixed models that the presence/absence of char does not affect the density of stickleback or the biomass of evasive zooplankton, and that char-presence has a positive effect on non-evasive zooplankton biomass (population-level estimate: -0.46 [-0.5, -0.42]) (Figure 3). Relative head size was negatively correlated with the presence of char (-0.4 [-0.45, -0.36]), the density of stickleback (-0.2 [-0.23, -0.16]), the evasive-prey biomass (-0.1 [-0.15, -0.05]) and the non-evasive-prey biomass (-0.14 [-0.2, -0.09]). Notably, and as we discuss further below, there were two paths by which char absence/presence could explain variation in head size; a direct path, and an indirect path via the effect char has on non-evasive prey biomass. For the full results table see table S4: path analysis I. To directly test the effect of prey community differences on head size we conducted

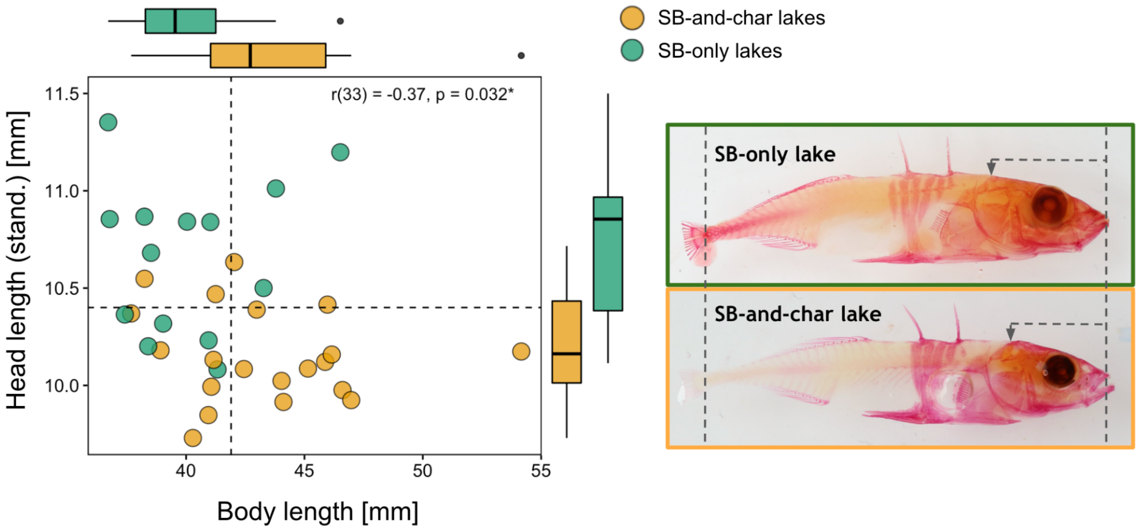

Figure 2: Morphological variation in body length and head length among populations. Mean body and standardized head length were negatively correlated, such that larger bodied populations had smaller relative heads. Stickleback in the presence and absence of char significantly differed in body lengths and relative head sizes, such that populations that co-occurred with char (orange) typically were large-bodied and small-headed, whereas solitary populations (green) were small-bodied and large-headed.

a mixed effects model including zooplankton RDA1, CPUE and total prey biomass, which confirmed that the zooplankton community composition had a significant effect on head allometry (RDA1 effect = 0.015 [0.001- 0.029], $t_{(32)} = 2.05$, p-value = 0.049) (Table S4).

Analysis of other fitness-relevant morphological traits

In our morphological PCA analysis of 11 cranial linear traits, all eigenvalues had positive loading on the first PC axis, which accounts for 48% of the variance (Figure 4A). Variation in morphological PC1 among populations was positively correlated with relative head size (lm: slope= 0.23, t = 6.32, p = 4.26e-07), and, to a lesser degree, negatively correlated with body size (slope estimate = -0.012, t = -3.02 , p = 0.005). Morphological PC2, which accounts for 12% of the variation, is mainly associated with the height and width of the epaxial musculature. Morphological PC2 is not correlated with body size (lm: slope estimate = -0.0004, t = -0.14, p = 0.89) and only marginally with relative head size (lm: slope estimate = -0.045, t = -1.82, p = 0.078). The second path analysis (path analysis II, Figure 4B) confirmed that relative head size

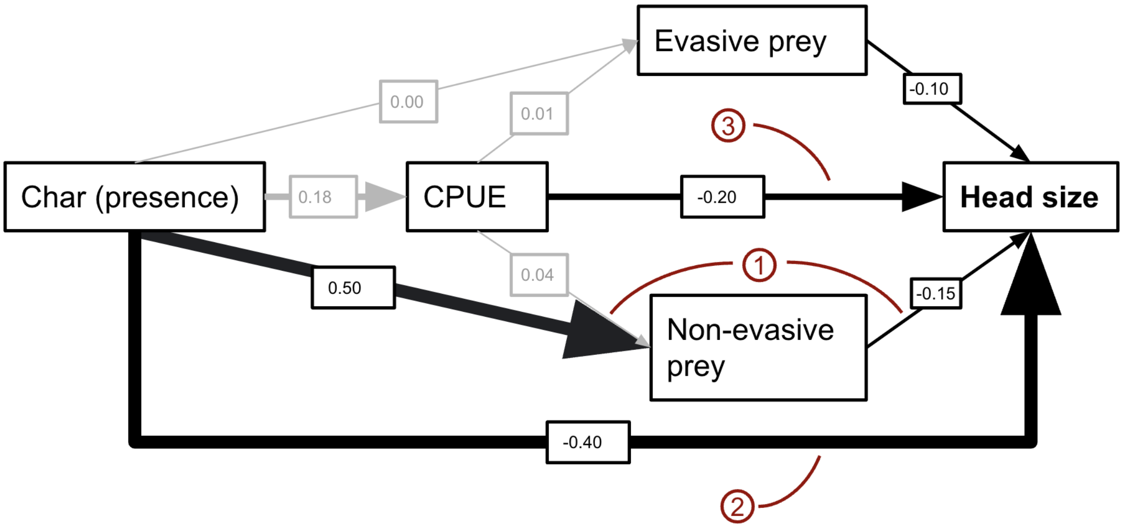

Figure 3: Three major findings of path analysis I. 1) Char presence had a strong positive effect on the non-evasive prey biomass (but not the evasive prey biomass), which in turn had a significant negative effect on relative head size. Thus, there exists an indirect path via prey biomass composition by which char affected head size. 2) Besides the indirect path, char had a strong direct effect on relative head size. 3) Contrary to our expectation, we found that stickleback population density (CPUE) was negatively associated with relative head size, i.e. in more dense populations expressed smaller heads. For our interpretation of these three major findings see the discussion.

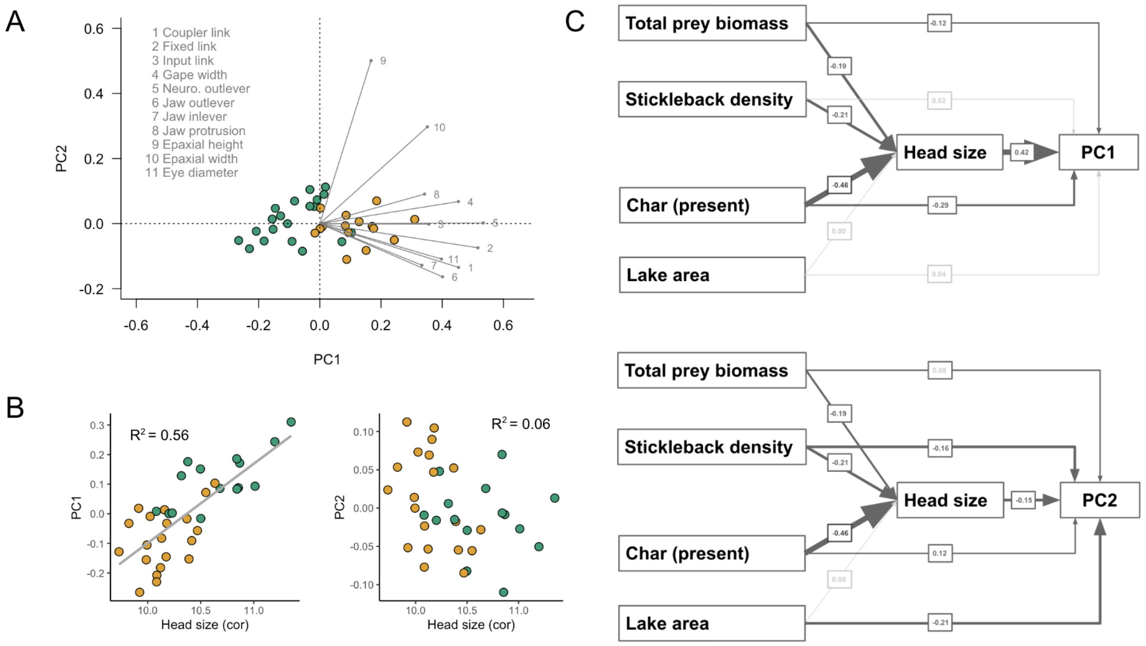

Figure 4: Consequence of predation and head size variation for other cranial traits. A morphological PCA of body-size-standardized linear traits of the cranium reveals an effect of char-presence on the multivariate trait along PC1 (A). PC1 is largely driven by relative head size, i.e. individuals with larger heads also have greater linear traits (B). However, some traits (PC2: epaxial width and height) are less driven head size (B). The effect of char presence on PC1 is largely explained by the effect of char on relative head size (C), whereas variation in PC2 is most strongly correlated with lake surface area.

is affected by char-presence (population-level estimate: -0.46; 95% [-0.5, -0.42]), stickleback density (-0.21 [-0.24, -0.17]) and the total available plankton biomass (-0.19 [-0.23, -0.16]), and that relative head size is strongest driven by variation in morphological PC1 (-0.43, [0.3, 0.56]). Additional variation in morphological PC1 was directly correlated with char presence (-0.29 [-0.36, -0.22]) and prey biomass (-0.12 [-0.16, -0.07]). The strongest driver of variation in morphological PC2 was lake surface area (-0.21 [-0.25, -0.17]), followed by weaker effects of the other environmental variables. For a full list of results see table S4: path analysis II.

Discussion

In this study, we explored how predation regimes (char presence/absence) and variation in prey community structure (composition and biomass) jointly affect patterns of phenotypic variation among Greenlandic stickleback populations. We focused on the size of the head relative to the body, as previous studies have found that this trait can be affected by resource allocation trade-offs arising from food limitation in fishes (Olsson *et al.* 2007; Chizinski *et al.* 2010). In agreement with these studies, we found that in the presence of char, populations grew to larger average body sizes and had smaller relative heads (Figure 2). Such effects could be mediated via different ecological mechanisms, including direct selection inflicted by predation, changes in consumer population densities or changes in the availability of resources (Chizinski *et al.* 2010; Reznick *et al.* 2019; Singkam & MacColl 2019). Using a path analysis, we provide support for the hypothesis that the effects of predation on head size is, at least in part, mediated by differences in prey availability between lake types (Figure 3). This difference in resource availability arises from shift in the biomass composition of the prey community between lakes with and without char (Figure 1). The finding that environmental conditions (predation and resource availability) can differentially affect growth patterns of different body parts, may have implications for how we interpret phenotypic variation among populations (Figure 4).

The presence of char changes zooplankton community

In our set of 34 zooplankton communities, we observed that *stickleback-only lakes* were largely dominated by calanoid copepods (*Leptodiaptomus minutus*), whereas *stickleback-and-char-lakes* had more diverse zooplankton communities that included higher biomass of cladocera and cyclopoid copepods (Figure 1). This result indicates an indirect ecological effect of char on the zooplankton community, where the presence of a top-predator alters the interaction between stickleback and their prey (Křivan & Schmitz 2004; Utsumi *et al.* 2010). Similar to previous studies (Jeppesen *et al.* 2017) we found that the release of stickleback from a top-predator negatively affects cladocerans and cyclopoid copepods, but has no effect on the biomass of calanoid copepods (Figure 1B). Overall, this results in a dramatic change in the zooplankton biomass composition, but not in the overall zooplankton biomass (Figure 1B). It is well known that calanoid copepods are better at evading fish predation than the other zooplankton species (Yen *et al.* 2015; Ogorelec *et al.* 2022), and that stickleback are more voracious planktivores than char for a given body size.

The decrease of less-evasive zooplankton species in the presence of char is consistent with a species-level (trophic) cascade (Polis *et al.* 2000), though in our case neither stickleback biomass nor zooplankton biomass differs among lakes with or without char. As we did not quantify variation in char biomass, and did not see a strong reduction in either stickleback or overall zooplankton biomass, we have limited support for potential variation in the strength of trophic cascades among lakes with and without char. Nevertheless, char may affect zooplankton communities via effects on stickleback habitat use and foraging behavior (Křivan & Schmitz 2004). For example, if the presence of char behaviorally restricts stickleback to the littoral zones, this might allow non-evasive prey to grow in a pelagic refuge. While this may indicate a trait-mediated mechanism, we cannot fully rule out density-mediated effects of char on zooplankton using our comparative dataset. This is because, other ecological drivers, such as lake productivity, may be important contributors to variation in stickleback density across the landscape that vary independently from char predation. Disentangling the importance of density- and trait mediated effects of char predation on the zooplankton community would require either a larger comparative survey or manipulative whole-ecosystem experiments (e.g. biomass manipulation of char and/or stickleback).

Phenotypic effects of char predation on body size and head allometry

On average, individual sticklebacks in *stickleback-and-char-lakes* had longer bodies than those in *stickleback-only lakes*. Predation likely affects body size distributions of fish populations through a series of mechanisms that involve changes in demography, resource conditions, and growth rates (Reimchen 1991; Rodd & Reznick 1997; Nakazawa *et al.* 2007; Singkam & MacColl 2019). For example, size selective predation by piscivores may shape body sizes distributions depending on the predators preferred prey size, and lead to the evolution of life-history traits, such as faster somatic growth rates and lower age at maturity (Rodd & Reznick 1997; MacColl *et al.* 2013). On the other hand, larger body size may arise as an adaptation to gape-limited predators - a hypothesis that has been put forward to explain large body size in sticklebacks (Reimchen 1991). Furthermore, predation often reduces population densities and thereby lowers intra-specific competition, which increases per capita food availability (Ylikarjula *et al.* 1999). Singkam and MacColl (2019) report that where predation and resource availability covary resources are more important in driving size at maturity of stickleback. This leads to larger individuals when resource conditions are good, even when predation is high. In our system, predator presence does not appear to covary with overall pelagic biomass, but it does change biomass composition, such that in predator presence pelagic resources may be more available to individual stickleback.

Contrary to the length of the body, absolute head-length did not differ between *stickleback-and-char* and *stickleback-only* populations. This disparity in the responses of the head and the body changes the allometry of the head (i.e. the relative size of the head to the body), such that *stickleback-char* populations have smaller relative heads than *stickleback-only* populations. Similar variation in relative head size has been observed in other fishes, where it was associated with resource limitation (Olsson *et al.* 2007; Chizinski *et al.* 2010). Olsson et al. (2007) argue that under limiting conditions, resources are mainly invested into maintaining survival, and little surplus energy is available for somatic growth ((Ylikarjula *et al.* 1999; Kooijman & Lika 2014). However, different body parts may respond differently to the resulting allocation trade-offs, which changes the allometry and the covariance among traits (Post & Parkinson 2001; Felmy *et al.* 2022). In the case of resource-limited fish, boney structures (such as the head) may grow continuously irrespective of resource conditions, while the rest of the body remains undifferentiated, resulting in the typical "small-bodied large-headed" phenotype of stunted fish populations (Olsson *et al.* 2007; Chizinski *et al.* 2010).

We argue that variation in *relative* head size is a symptom of changes in the allocation of resources to growth, where the head grows continuously, while the growth of the body depends on the predator presence and/or resource limitation. As we have argued above, predator presence may alleviate resource limitation by decreasing population densities and/or changing prey availability. To test if variation in relative head size between populations in different predation environments is driven by resource limitation, we performed a path analysis that included char presence, stickleback density (catch per unit effort), and prey biomass as explanatory variables. Because of the disparate effects of char on different zooplankton species (Figure 1), we treated the biomass of *evasive-prey* (*L. minutus)* and *non-evasive* (sum of all remaining species) as separate explanatory variables in the path model. There are three major findings from this first path analysis.

First, the biomass of *non-evasive* prey, and, to a lesser degree, *evasive* prey was negatively correlated with relative head size, such that higher prey biomass translated into individuals with smaller relative heads. This relationship between overall prey availability and head size is consistent with the idea that food limitation reduces the allocation of resources to longitudinal growth but not to the growth of the head (Olsson *et al.* 2007; Chizinski *et al.* 2010). Because char differentially affect the biomass of *evasive* and *non-evasive* prey, this result also suggests a mechanism by which char indirectly drive phenotypic variation among stickleback populations. While char-presence does not affect overall prey biomass, it shifts the biomass towards more non-evasive prey types. For a given prey biomass this shift in composition may make pelagic resources more accessible for individual stickleback and alleviate resource limitation, leading to better growth conditions for individual fish. This interpretation is also supported by a significant effect of zooplankton composition on relative head size when using a linear model (table S5)

Second, the path analysis revealed a direct strong negative effect of char presence on relative head size that was not mediated by stickleback density or prey biomass. This correlation may arise from selection on growth rates that is directly imposed by predation, e.g. to reduce mortality risk (evade gape limited predator) or increased growth rates to reach early maturity (MacColl *et al.* 2013). Such predator induced changes in life-history may have similar effects on the allometry of the head as does food limitation, i.e. fast growing individuals may allocate resources to longitudinal growth while head growth remains stable across predation regimes. Another possibility is that char directly impose selection on head size/shape, e.g. in order to reduce drag during predator escape (Langerhans 2009), though this scenario is not supported by at least one other study on stickleback (Leinonen *et al.* 2011). Alternatively, the direct statistical link may be mediated by an environmental variable that is not accounted for in our analysis. For example, our analysis is restricted to pelagic prey biomass, though Greenlandic stickleback also exploit benthic resources (e.g. chironomidae larva (Bergersen 1996)), whose interactions with char presence are currently unknown.

Third, and somewhat surprisingly, we found that stickleback density was negatively correlated with relative head size, such that more dense populations expressed smaller relative heads (i.e. a less stunted phenotype). This finding is contrary to our expectation, as we assumed that increased stickleback density will reduce per captia food availability and therefore lead to more stunted individuals (Ylikarjula *et al.* 1999). In other words, in a given environmental condition, population density and growth condition should be negatively correlated. However, across our comparative dataset environmental conditions are highly variable (chapter 3) and both pelagic resources and stickleback density may vary in magnitude due to factors such as productivity, benthic prey availability and predation intensity. Thus, while population density and growth

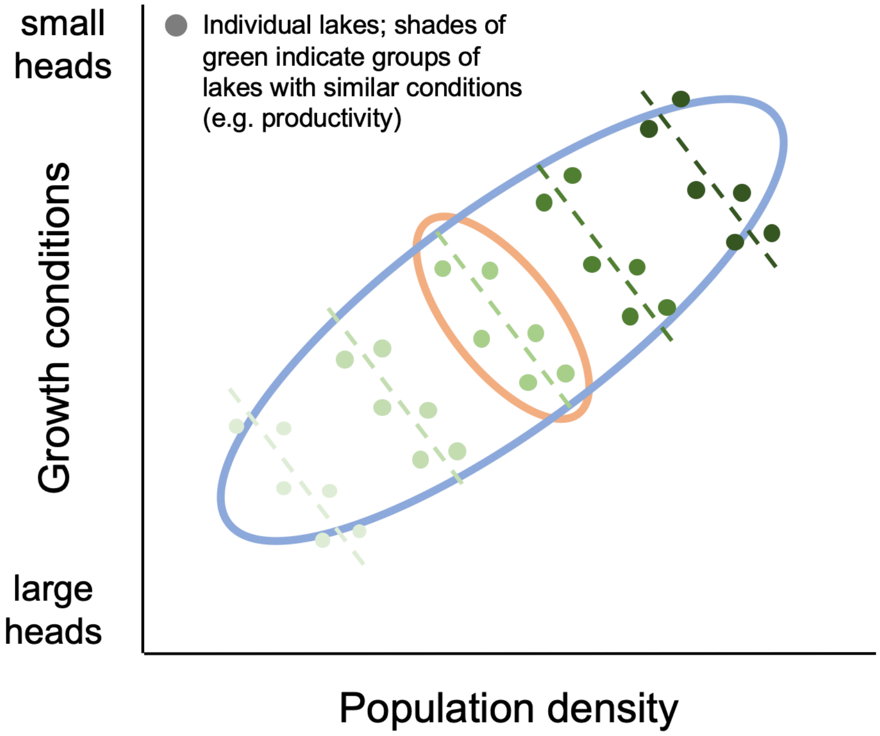

Figure 5: Potential mechanism for explaining the observed negative correlation between population density and relative head size. We hypothesized, that increasing population density negatively affects growth conditions due to increased interspecific competition, resulting larger relative heads in more dense populations. While this may be the case for lakes with similar environmental condition (indicated by lakes with the same shade of green), different ecosystems may vary in the extent to which density affects growth (lakes with different shades of green), e.g. due to variation productivity among lakes. In this case the phenotypic effects of negative correlation between population density and growth condition could only be detected if a narrow range of environmental conditions is sampled (orange oval). If the sampled lakes vary widely in condition, however, the opposite pattern may arise (blue oval). This is equivalent to the detection of resource allocation tradeoffs in natural populations, where variation in energy budgets among individuals often overrides the signal of the individual level tradeoffs (Schärer et al. 2005)

condition may be negatively correlated in any given environmental condition, they may be positively correlated when compared across systems that vary in condition (Figure 5). In this case, environments that support large populations may also provide better growth conditions. This hypothesis could be tested using manipulative experiments, where individuals are reared at different densities while controlling for resource availability or vice versa.

Phenotypic effects on other fitness-relevant traits

An important consequence of variation in head-allometry among populations is that the size of cranial traits also change relative to body size. Cranial morphology is of particular interest to understand local adaptation and ecological divergence, as variation in these traits is often linked with the foraging performance in a given habitat (McGee *et al.* 2013). Comparative studies of stickleback morphology routinely standardize cranial traits with total body size instead of total head size (McGee *et al.* 2013; Paccard *et al.* 2020). To test to what degree the observed variation in relative head size affects functional morphology, we performed a morphological PCA of 9 linear traits involved in functional trait systems. All linear traits load heavily on morphological PC1, except for epaxial width and epaxial height, which loaded on morphological PC2. To explore the environmental correlates of morphological PC1 and 2, we used simplified path analyses, with total prey biomass, char presence, stickleback density, and lake area (as a proxy for habitat distribution), and relative head size. Variation in morphological PC1 was heavily driven by head size, which was itself - consistent with the previous results - affected by prey availability, char presence, and stickleback density, but not lake area.

Individuals with larger heads also have larger cranial-traits, and although this result may seem trivial it has important implications for identifying putative causes of phenotypic evolution. Firstly, researchers often use size-correction in order to compare traits among populations or ecotypes within lakes (Lucek *et al.* 2013; McGee *et al.* 2013; Schmid *et al.* 2018; Paccard *et al.* 2020). Divergence in these size corrected traits is then interpreted based on the utility of the traits in questions. However, as we demonstrate here, this may lead to misinterpretations if changes in relative head size are not considered. For example, if we were interested in understanding the effect of char predation on gape width, we would find that predation has a negative effect on (size corrected) gape width, and we may draw conclusions about possible ecological causes (e.g. changes in prey size, habitat choice etc.). As we show here, differences in gape are primarily driven by head size (Figure 4C). However, absolute head size (and as a consequence absolute gape) does not vary in response to predation. Instead, it is the body size distribution that changes. If we include head size variation in the analysis, we thus may arrive at the opposite conclusion that gape is unaffected by predator presence, despite the potentially profound effects on other traits (such as the body).

Secondly, deviations from the covariation between linear traits and head size may have functional consequences and may provide information about alternative environmental drivers of phenotypic variation. The biomechanical functions of traits are often ratio based, and so they might not be affected if relative head size changes proportionally (Thompson *et al.* 2017). However, we observe that some traits covary with relative head size to a lesser degree than others (Figure 4B). This means that the ratio among linear traits changes, which may have functional consequences. Specifically, variation in morphological PC2 was less affected by relative-head size than morphological PC1, and was most strongly driven by lake area (Figure 4B). The two traits loading most strongly on PC2 - epaxial height and width - measure the extent of the epaxial musculature, which interacts with bony structures of the cranium to generate suction force during feeding (Wainwright *et al.* 2007). As such, epaxial width and height are not cranial traits, which may explain their relative independence of head size compared to the other traits, despite their functional connection. Variation in epaxial height has been previously associated with benthic vs. pelagic divergence in stickleback (McGee *et al.* 2013). Benthic prey items (e.g. insect larva) are typically attached to the substrate and thus require strong suction forces to detach, which requires a larger epaxial musculature (Wainwright *et al.* 2007). As lakes grow in surface area, the ratio of benthic to pelagic habitats decreases, which affects selection in traits associated with benthic and pelagic foraging (Bolnick & Lau 2008). Thus, the negative correlation between lake area and PC2 may reflect an adaptation (or a plastic response to) to smaller lakes being dominated by benthic habitats, which contain prey that require higher suction performance.

Conclusion

The multivariate-phenotype of individuals is a mosaics of traits that vary in response to a multitude of environmental factors, and can do so via different mechanisms (Felmy *et al.* 2022). To retain the functionality of the organism despite these variable responses, they may be organized into functional modules that evolve or plastically respond independently to environmental variation (Kliebenstein 2011). Our data suggest that variation in head-allometry is driven by different responses in the length of the head (less responsive) and remaining body (more responsive) to predation. This differential response might be adaptive, for example, when body growth is diminished due to resource limitation, allowing the individual to allocate energy to maintenance and reproduction. At the same time, it may be adaptive for head development to be relatively inert to such variation, retaining the functionality of the head, for example, for foraging or brain development. This then results in the typical "small-bodied-large-headed" phenotype of stunted fish (Chizinski *et al.* 2010). Our results suggest that the observed phenotypic effect of predation is, in part, mediated by changes in the zooplankton composition. This adds additional nuance to the idea that predators affect consumer phenotypes via indirect effects on resource availability (Walsh 2013; Reznick *et al.* 2019; Czorlich *et al.* 2022). Specifically, the induced shifts in pelagic species composition may make pelagic prey biomass more accessible to consumers and thereby improve growth conditions. Morphological variation arises from changes in the allometry of traits (Klingenberg & Zimmermann 1992) and understanding the environmental and genetic factors that drive the relationship between body size and traits size are thus critical to understand why populations differ in their appearance (Shingleton *et al.* 2009). In this study, we describe patterns of phenotypic variation in body size and head allometry among populations, as well as their environmental correlates. To test the mechanisms proposed in this study, future research should investigate the effects of food-limitation and predation on the growth pattern of different morphological traits, and test for their underlying genetic basis, rather than relying on cross sectional patterns of phenotypic variation.

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Supplementary material for:

Differential response of body size and head size to predator mediated resource limitation causes phenotypic variation among stickleback populations

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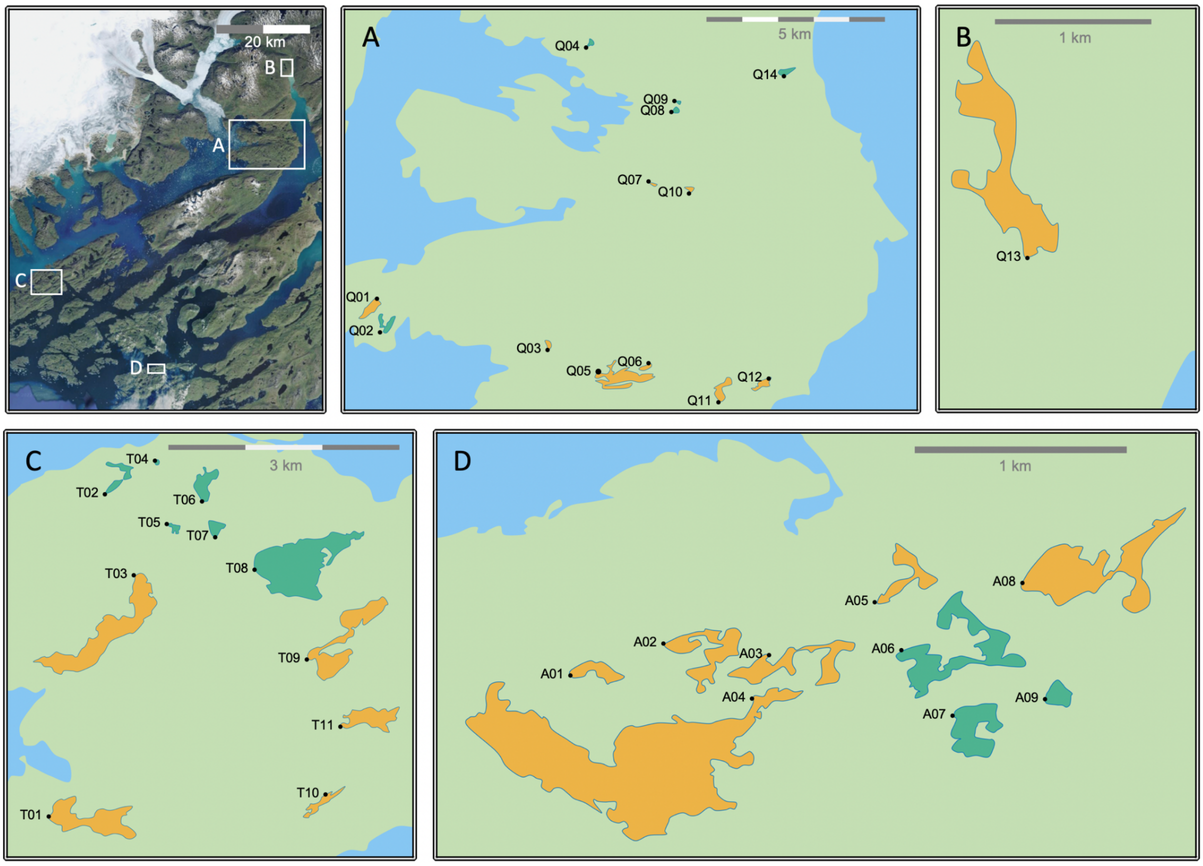

Figure S1: Overview of the sampling locations: Sampling effort was focused on three sampling regions: Peninsular mainland (A,B) and the islands Tuttutoq (C) and Akia (D). Depicted are only sampled lakes; many more lakes with varying fish communities are scattered across the landscape. All sampled lakes contained either only threespine stickleback (green), or both arctic char and threespine stickleback (orange).

Table S1: Table of sampled lakes including location, fish community composition and lake surface area.

Lake ID	Latitude	Longitude	Lake type	Area [ha]
T 01	60.824612	-46.502201	Stickleback-and-char	28.6
T 02	60.866633	-46.501537	Stickleback-only	2.5
T 03	60.850441	-46.493788	Stickleback-and-char	48.1
T 04	60.866898	-46.493249	Stickleback-only	0.3
T 05	60.859238	-46.488189	Stickleback-only	1.2
T 06	60.862552	-46.482229	Stickleback-only	7.3
T 07	60.860121	-46.481343	Stickleback-only	3
T 08	60.851511	-46.463482	Stickleback-only	64.5
T 09	60.842422	-46.455532	Stickleback-and-char	29.3
T 10	60.828433	-46.452899	Stickleback-and-char	4
T 11	60.837191	-46.448225	Stickleback-and-char	14.9
A 01	60.669143	-46.128235	Stickleback-and-char	1.5
A 02	60.669848	-46.120843	Stickleback-and-char	4.2
A 03	60.669121	-46.112315	Stickleback-and-char	3.5
A 04	60.668543	-46.111432	Stickleback-and-char	42.8
A 05	60.673742	-46.100215	Stickleback-and-char	2.1
A 06	60.672000	-46.097001	Stickleback-only	7.4
A 07	60.667246	-46.093646	Stickleback-only	3.6
A 08	60.673018	-46.091131	Stickleback-and-char	11.3
A 09	60.667962	-46.089601	Stickleback-only	1.1
Q 01	61.085355	-45.748242	Stickleback-and-char	13.4
Q 02	61.084701	-45.732472	Stickleback-only	6.02
Q 03	61.090048	-45.653901	Stickleback-and-char	3.2
Q 04	61.153176	-45.632353	Stickleback-only	2.3
Q 05	61.071582	-45.604472	Stickleback-and-char	51.8
Q 06	61.072683	-45.603635	Stickleback-and-char	3.9
Q 07	61.118979	-45.596067	Stickleback-and-char	3.3
Q 08	61.138109	-45.586845	Stickleback-only	2.5
Q 09	61.138991	-45.583631	Stickleback-only	0.7
Q 10	61.118369	-45.580845	Stickleback-and-char	2.5
Q 11	61.065108	-45.560955	Stickleback-and-char	15
Q 12	61.068634	-45.545119	Stickleback-and-char	9.5
Q 13	61.253333	-45.529141	Stickleback-and-char	26.3
Q 14	61.146044	-45.528816	Stickleback-only	7.2

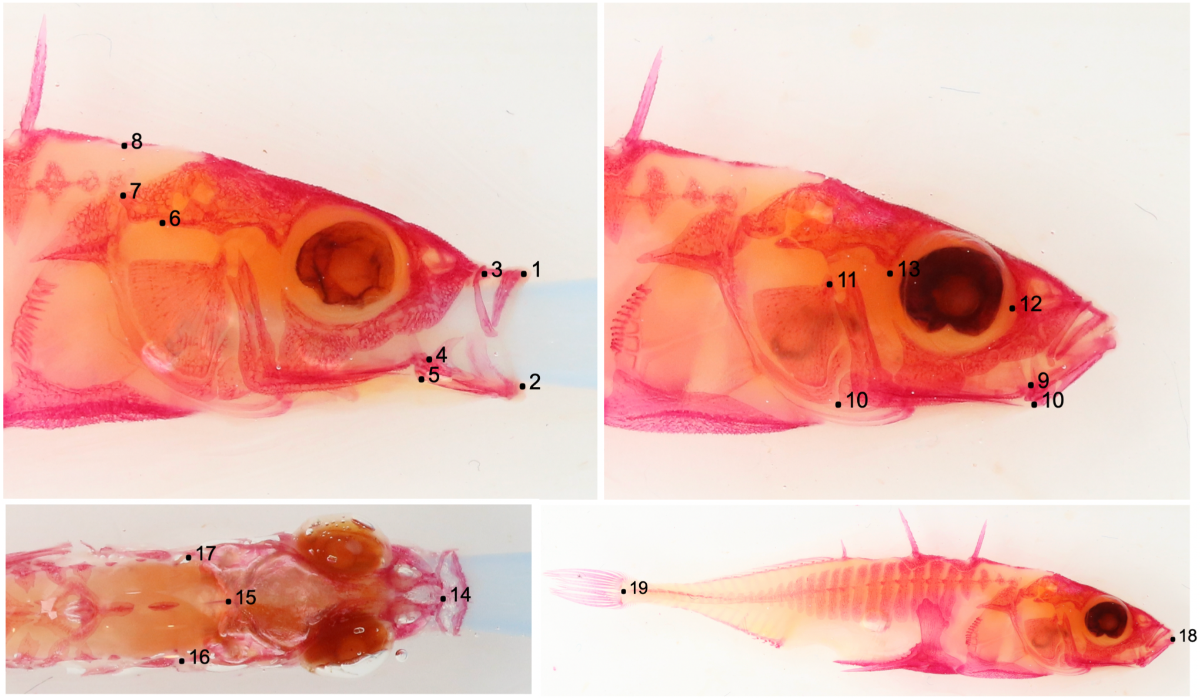

Figure S2: Landmarks used for morphological analysis (McGee, Schluter, and Wainwright 2013). 1. anteriormost extent of premaxilla; 2. anteriormost extent of dentary; 3. anteriormost extent of maxilla; 4. quadrate-articular jaw joint; 5. insertion of the interopercular-articular ligament; 6. point of articulation between the supracleithrum and post-temporal; 7. dorsalmost extent of epaxial muscle, measured dorsal to landmark 6; 8. quadrate-articular jaw joint; 9. insertion of the interopercular-articular ligament; 10. opercular joint; 11. posteroventral extent of interopercule; 12. anterior most point of orbit; 13. Posterior most point of orbit, 14. anteriodorsal extent of maxilla, 15. posterior most point of neurocranium; 16. point of articulation between the supra-cleithrum and post-temporal (left); 17. point of articulation between the supra-cleithrum and post-temporal (right); 18. anteriormost extent of dentary; 19. caudal fin start.

Table S2: Linear measurements obtained from landmarks for morphological analyses (see Figure S2)

Linear trait	Landmarks
Gape width	1,2
Jaw protrusion	1,3
Jaw inlever	4,5
Jaw outlever	2,4
Neuro. outlever	1,6
Epaxial height	6,7
Epaxial width	16,17
Coupler link	9,11
Fixed link	8,10
Input lever	10,11
Eye diameter	12,13
Head length	14,15
Standard length	18,19

Table S3: Parameters for length-weight regression used to calculate zooplankton biomasses

Taxon	A	LnA	B	Length unit	Source	Source comment
Acroperus	0.00905	-4.70	0.85	µm	Dumont (1975)	Acopercus
Alona	15.92	2.77	3.84	mm	Dumont 1975	Alona
Alonella	0.00017	-8.68	1.39	µm	Dumont 1975	Alonella
Bosmina	21.9682889	3.09	3.0935	mm	Botrell 1976	Bosmina
Ceriodaphnia	12.9656039	2.56	3.338	mm	Botrell 1976	Ceriodaphnia
Chydoridae	5.76151234	1.75	2.653	mm	Dumont 1975	Chydorus
Chydorus	89.43	4.49	3.93	mm	Dumont 1975	Chydorus
Daphnia	4.34097944	1.46	2.8292	mm	Botrell 1976	daphnia pooled
Eurycercus	5.76151234	1.75	2.653	mm	Botrell 1976	cladocera general
Holopedium	220.875678	5.40	2.0555	mm	Botrell 1976	Holopedium
Macrothrix	5.76151234	1.75	2.653	mm	Botrell 1976	Cladocera general
Polyphemus	0.00000246	-12.92	2.15	µm	Dumont 1975	Polyphenmus
Sida	5.76151234	1.75	2.653	mm	Botrell 1976	Cladocera general
Calanoid	7.04698595	1.95	2.399	mm	Botrell 1976	Copodpod pooled
Cyclopoid	7.04698595	1.95	2.399	mm	Botrell 1976	Copodpod pooled
Harpacticoida	7.04698595	1.95	2.399	mm	Botrell 1976	Copodpod pooled
Nauplius	7.04698595	1.95	2.399	mm	Botrell 1976	Copodpod pooled

Table S4: Results table of both path analyses I and II. Paths were considered significant if the 95% confidence intervals did not overlap zero.

	Response	Explanatory	Estimate	Error	95% CI
Path analysis I	CPUE	Charr (present)	-0.18	0.18	-0.54 to 0.18
	Evasive prey	Charr (present)	0	0.23	-0.46 to 0.45
	Evasive prey	CPUE	0.02	0.57	-1.13 to 1.13
	Non-evasive prey biomass	Charr (present)	0.5	0.2	0.09 to 0.89
	Non-evasive prey biomass	CPUE	0.03	0.52	-1.04 to 1.06
	Head length (stand.)	Evasive prey biomass	-0.1	0.02	-0.15 to -0.05
	Head length (stand.)	Non-evasive prey biomass	-0.14	0.03	-0.2 to -0.09
	Head length (stand.)	CPUE	-0.2	0.02	-0.23 to -0.16
	Head length (stand.)	Charr (present)	-0.4	0.02	-0.45 to -0.36
Path analysis II	Head length (stand.)	Charr (present)	-0.46	0.02	-0.5 to -0.42
	Head length (stand.)	Lake Area	0	0.02	-0.04 to 0.04
	Head length (stand.)	Prey biomass	-0.19	0.02	-0.23 to -0.16
	Head length (stand.)	CPUE	-0.21	0.02	-0.24 to -0.17
	Morphological PC1	Charr (present)	-0.29	0.03	-0.36 to -0.22
	Morphological PC1	Lake Area	-0.04	0.02	-0.08 to 0
	Morphological PC1	Prey biomass	-0.12	0.02	-0.16 to -0.07
	Morphological PC1	CPUE	0.02	0.02	-0.02 to 0.06
	Morphological PC1	Head length (stand.)	0.43	0.07	0.3 to 0.56
	Morphological PC2	Charr (present)	0.12	0.04	0.04 to 0.2
	Morphological PC2	Lake Area	-0.21	0.02	-0.25 to -0.17
	Morphological PC2	Prey biomass	0.08	0.02	0.03 to 0.12
	Morphological PC2	CPUE	0.16	0.02	0.12 to 0.21
	Morphological PC2	Head length (stand.)	-0.15	0.07	-0.28 to -0.01

Table S5: Results of linear mixed effects model to test for the effect of zooplankton community composition in relative head size

	Estimate	Std. Error	df	t	p.value	2.5 %	97.5 %
Intercept	1.023	0.005	29.96	190.363	<2e-16	1.01	1.034
log10 (TotalBM)	-0.003	0.003	29.96	-1.326	0.1948	-0.008	0.002
RDA1	0.014	0.007	29.95	2.051	0.0491	0.001	0.029
log10 (CPUE)	-0.008	0.006	29.96	-1.342	0.1896	-0.019	0.003

Synthesis: Understanding the ecological dynamics of natural selection in a community context - advances and outlook

In this thesis I have addressed issues regarding the interplay between evolutionary change and ecological dynamics in species communities. To conclude my thesis, I will now return to the overarching questions I posed in the introduction: 1) What are the relevant agents of selection in a community context, how do they interact, and how do they affect fitness? and 2) How does phenotypic variation affect selective environments? I will discuss the advances I have made in addressing these questions in the four chapters, and provide an overview about the question that remain open and how they may be addressed in future research.

1) What are the relevant agents of selection in a community context, how do they interact, and how do they affect fitness?

A useful starting point for exploring the ecological causes of natural selection are the traits of interest themselves. Understanding how traits relate to performance variation (functional analyses), allows us to derive informed hypotheses about the ecological mechanisms that link traits to fitness (MacColl 2011). However, this is not always trivial, because i) trait functions can be difficult to ascertain, ii) both trait expression and their functions can be contingent on environmental conditions (Felmy *et al.* 2022), and iii) traits can evolve due to reasons unrelated to their function, for example, as a correlated response to selection on other traits (Lande & Arnold 1983; Price & Langen 1992). Given that traits occur within a hierarchy and vary in their environment-dependent expression, it is difficult to elucidate which traits are the target of selection and which evolve as a by-product (Price & Langen 1992; Garland & Losos 1994). Furthermore, selection gradients can be shaped by indirect ecological interactions, especially in community contexts (Walsh 2013; terHorst *et al.* 2018), and so ecological pathways linking traits to fitness may be more complex than what is apparent from phenotype-environment correlations (Rodd & Reznick 1997; Reznick *et al.* 2019).

Advances and outlook

In chapter one we explored the ecological causes of trophic position evolution (Moosmann *et al.* 2021). First, we established that trophic position variation can be determined by both the food web context and the genetic make-up of populations. As such trophic position can be a heritable albeit plastic trait. Second, we explored the potential causes of selection on trophic position that arise from the community context of consumer populations. Trophic position variation is often correlated with variation in other traits (e.g. behaviors and morphology) and ultimately arises from dietary variation. As a result, trophic position can either evolve as a by-product of selection on these underlying traits (i.e. as a correlated response), or it could be the direct target of selection (Price & Langen 1992). A review of the literature revealed that various putative agents of selection can vary in consistent ways along food chains. For example, we often observe changes in the overall biomass of resources (Arim *et al.* 2007), the quality of food (Denno & Fagan 2003), the concentration of toxicants (M. Jake Vander Zanden & Rasmussen 1996), and the strength of species interactions (Cropp & Norbury 2020). This can directly link trophic position with fitness variation because an individual's trophic position will determine its interaction with these potential selective agents. While this unequal distribution of selective agents along food chains and their interactions have been identified to impact the evolution of related traits, such as diet and omnivory (Denno & Fagan, 2003), they have typically not been explicitly associated with the evolution of trophic position.

We argue that explicitly studying trophic position as an evolving phenotype could reveal new ecological mechanism by which community contexts drive micro- and macroevolutionary patterns of diversification, not only of trophic position but also of the diverse phenotypes that are associated with it (Harmon *et al.* 2019). For trophic position to be a target of natural selection, it must be i) heritable and ii) directly associated with fitness variation. Our literature review has compiled evidence for both of these conditions, however, it is currently largely unclear how commonly they are met in natural populations. Novel insights about its genetic basis could be gained from using trophic position as the trait of interest in quantitative genetics approaches, such as pedigree based animal models or GWAS (Wilson *et al.* 2010). Since the publication of chapter one, an interesting experimental study using bank voles (*Myodes glareolus*) was published, in which the authors found that artificial selection on predatory behavior can lead to increases in trophic position when individuals were released into field enclosures with natural resources (Hämäläinen *et al.* 2022). This study links trophic position to underlying behavioral variation and indirectly provides evidence for the heritability and evolvability of trophic position. Experiments such as this, where population with known heritable variation in trophic position are released into ecologically relevant common environments, provide an ideal platform to test for relationships between trophic position and fitness and for studying the ecological mechanisms that underlie such covariance.

In chapter four we used independent Greenlandic stickleback populations that are embedded in different prey and predator communities to investigate the ecological causes of phenotypic variation across the landscape. Specifically, we wanted to test how predation affects the relative head size of populations. This trait can be indicative of differential resource allocation to somatic growth among populations: Under resource limited conditions it can be adaptive to invest less into somatic growth, resulting in smaller body sizes (Ylikarjula *et al.* 1999; Chizinski *et al.* 2010). However, it may be advantageous to decouple the response of the body from the response of the head, in order to retain crucial functions of the head (e.g. brain development, foraging etc.) (Olsson *et al.* 2007; Chizinski *et al.* 2010). Previous work investigating the effects of predation on the life-history of prey species (e.g. age and size of maturity), has found that predator induced phenotypic variation often results from indirect ecological effects of predation that arise from resource limitation (e.g. prey densities, and resource availability), rather than direct selection imposed by predator induced mortality (MacColl *et al.* 2013; Reznick *et al.* 2019; Singkam & MacColl 2019; Czorlich *et al.* 2022). In Southern Greenland, lakes with and without predators (i.e. char) did not differ in stickleback population density or in total (pelagic) resource biomass. They did, however, differ in the composition of their pelagic invertebrate communities (chapter 3), with top-predator-free lakes being dominated by highly evasive resources (i.e. copepods), whereas lakes with top-predators had higher biomasses of less evasive prey species (e.g. cladocerans). Using path analysis, we present evidence that among-population variation in relative head size was strongly associated with predation regimes. This suggests differential resource allocation, where populations invest into fast somatic growth of the body, resulting in large-bodied and small headed individuals in the presence of char. By comparison, stickleback populations that do not coexist with char invest less into growth resulting in small-bodied, large headed individuals. This phenotypic effect of predation-regime was, in part, explained by the top-predator induced shift in the composition of resource biomass. Our results therefore suggest, that predation drives phenotypic variation among stickleback populations via an indirect ecological mechanism, wherein char change the composition (but not biomass) of the resource biomass, and in doing so inflict differential resource limitation onto the stickleback.

Previous studies have highlighted the role of predation in limiting resources of consumer populations and thereby shaping their body-size and life history (Ylikarjula *et al.* 1999; Reznick *et al.* 2019; Singkam & MacColl 2019; Czorlich *et al.* 2022). These studies have primarily attributed these effects to changes in prey biomass, and/or, in consumer population densities, resulting in reduced per-capita prey availability (i.e. resource limitation) (Ylikarjula *et al.* 1999). Our work shows, that these effects may also arise from predator-driven shifts in resource composition, which affects the trait distribution of resources (i.e. their evasiveness), such that in the presence of top-predators less-evasive resources are more available to individual consumers (Van Leeuwena *et al.* 2008). Furthermore, we highlight that the effects of resource limitations not only affect body size (stunting), but are also linked with changes in body shape (Olsson *et al.* 2007; Chizinski *et al.* 2010). Our comparative approach of wild population does not allow us to make strong inferences about whether the observed phenotypic variation is the product of selection or a plastic response to predation and resource limitation. This question can only be answered by rearing individuals from different genetic backgrounds in common resource/predator conditions. It is also unclear if the observed shape changes have functional implication, i.e. if the response to predation/resource limitation is adaptive. To test this idea would require measuring selection gradients of an experimental population that varies in relative head size, or in the reaction norms to resource limitation. In any case our findings have important implications for interpreting patterns of phenotypic variation in nature, as it suggests that phenotype-environment correlations can arise from mechanism that are unrelated to the apparent functions of the trait.

2) How does phenotypic change affect selective environments?

To understand the interplay between evolutionary change and community dynamics in nature, it can be useful to identify the traits by which organisms modify their environments (i.e. ecosystem-effect traits (Violle *et al.* 2007)). Knowledge about these traits and their effects is necessary to investigate how evolutionary change affects selective environments (Matthews *et al.* 2014; Best *et al.* 2017). A good starting point to identify these effect traits is functional analysis; understanding how traits relate to ecologically relevant functions (e.g. prey capture, predator evasion) can provide us with hypotheses about plausible mechanisms by which traits modify environments. In the case of stickleback, extensive work has investigated the functional relevance of phenotypic variation in foraging morphology for prey capture success in different environmental contexts (Schluter 1993; Robinson 2000; Higham *et al.* 2017; Schmid *et al.* 2018). A separate body of work has shown that lineage divergence can have community-wide effects in mesocosm environments (Harmon *et al.* 2009; Rudman *et al.* 2015; Matthews *et al.* 2016; Best *et al.* 2017), and has speculated about the functional traits that underlie such ecological effects (Schmid *et al.* 2018). This provides a basis to test the effects of phenotypic change in nature, for example, by using comparative approaches, where phenotypes of independent populations are correlated with the modifiable aspects of their environments (Post *et al.* 2008). Such correlations can be indicative of ecosystem-wide effects of traits, and provide predictions about how phenotypic change over time may modify ecosystems (i.e. space-for-time substitution, but see (Damgaard 2019)). However, the causality of such phenotype-environment correlations cannot be unambiguously interpreted as they may arise from different mechanisms, such as adaptation of organism to the prevailing ecological conditions (Kawecki & Ebert 2004), or from shared underlying causes (Schluter 2000). It is therefore necessary to combine different lines of evidence arising from experiments and comparative studies to support community-wide effects of phenotypic variation in nature.

Advances and outlook

In chapter one we have conceptualized trophic position as an individual level trait and have explored the ecological dynamics that may affect its evolution. Previous work has investigated eco-evolutionary dynamics that shape trophic structure, however, these studies have typically viewed trophic position as a by-product of evolution (Gibert & Yeakel 2019; Cropp & Norbury 2020). We provide a simple albeit important shift in perspective toward considering trophic position as an evolving trait. Trophic position is, by definition, an ecosystem-effect trait as it quantifies the sum of an individual's trophic interactions and the role it plays in the flow of energy through the food web (Levine 1980). As such, evolutionary changes in trophic position will inevitably change the ecosystem effect of the population, which may or may not affect the dynamics and the stability of natural ecosystems (Pimm SL, Lawton JH , Cohen JE 1991; McCann *et al.* 1998; Post 2002; Arim *et al.* 2007; Ingram *et al.* 2009; Rooney & McCann 2012). Thus, by explicitly studying the ecological dynamics of trophic position evolution, which we propose to do in chapter one, new insights may be gained into how evolution drives the dynamics of selective environments.

While it is undeniable that the evolutionary emergence of more complex trophic structure is an important driver of macroevolutionary patterns (Harmon *et al.* 2019; Román-Palacios *et al.* 2019), it is unclear how population-level shifts in trophic position affect the selective environment of the evolving populations and other species in the food-web. Such effects could be experimentally tested by allowing populations with variable trophic position (e.g. see above (Hämäläinen *et al.* 2022)) to modify an ecologically relevant common garden environment. This would allow to quantify the impact of trophic position evolution on ecosystems. In a second step the fitness consequences of these modifications could be tested, for example, how they affect the survival and reproduction of subsequent generations (Matthews *et al.* 2016; Best *et al.* 2017). It would be particularly interesting to see if consistent shifts in trophic position (e.g. increases in predation) lead to similar ecological outcomes and selection pressures across different systems.

As we illustrated in chapter one, a direct mechanism by which evolution can affect communities, is via changes in resource-use (whether this entails changes in trophic position or not). In chapter two, we explore the phenotypic basis of differential resource-use between stickleback lineages with divergent evolutionary histories of freshwater colonization, and differential habitat utilization in the wild. Previous mesocosm experiments using the same lineages have found differential effects on invertebrate communities, and other ecosystem properties, that likely arise from divergent resource-use (Best *et al.* 2017). In chapter two, 2e hypothesized that divergent resource use may results from phenotypic divergence in functional traits, that determine the ability of individuals to capture different prey items (Araújo *et al.* 2011). In a common garden feeding experiment, we showed that the lineages indeed had different diets when confronted with a diverse resource community. In accordance with the eco-morphological paradigm we found that at the individual level, variation in functional morphology was associated with prey-specific foraging performance. However, the traits that explained performance variation among individuals could not explain the divergence in resource-use between lineages.

This surprising result suggests that unmeasured trait variation is potentially the cause of the observed dietary differences. In particular, we did not quantify behavioral variation, for example in prey choice (which prey species are attacked vs. which prey species are ingested). Performance variation is often measured in experimental context that do not allow for behavioral variation. However, behaviors are well known to affect ecological interactions (Teckentrup *et al.* 2018), and the relationships between trait variation and performance (Garland & Losos 1994; Muñoz 2022). For future investigations of the phenotypic causes of environmental variation, it will therefore be crucial to include more detailed analyses of behavioral variation. As variation is often highly context-dependent, it would also be interesting to test how different community contexts (e.g. increasing prey community complexity or the presence of a predator; see chapter three) affects the links between behavioral variation and ecological outcomes.

In chapter three, we used a comparative analysis of Greenlandic lake ecosystems, to investigate the relative importance of phenotypic variation in stickleback foraging morphology in driving variation in zooplankton community composition. We focused on the effects of candidate traits whose (biomechanical) function in prey capture is well understood, and whose potential effects on community composition have been investigated in previous studies (Schmid *et al.* 2018). Although previous experimental works have identified community-wide effects arising from lineage divergence, and have associated some of these effects with specific traits (Matthews *et al.* 2016; Best *et al.* 2017), it is unclear how such trait variation may affect communities in nature, where other ecological mechanisms are at play. For example, previous studies in Greenlandic lake ecosystems have found that top-predator presence alters the ecological effects of stickleback on zooplankton biomass, and it is well known that abiotic gradients can have strong effects on community composition across the landscape (Jeppesen *et al.* 2017). We therefore compared the macro-zooplankton community composition of 78 independent lakes that vary in the presence and absence of stickleback and arctic char, lake morphometry and water chemistry. This approach has allowed us to assess the relative importance of phenotypic variation in driving community structure.

We show that in the absence of fish, zooplankton community structure is largely determined by a water chemistry gradient, which affects the dominance of the most abundant plankton species in the community. This water chemistry gradient loses importance in lakes with fishes, and has no effect in lakes that contain only stickleback. This is because stickleback strongly suppress species richness irrespective of abiotic conditions, resulting in very little community variation among lakes (where most lakes are dominated by the same species). Consequently, foraging morphology in these lakes could not explain community structure in those lakes. However, among lakes where stickleback and char co-occurred, some community variation was retained (a shift in the dominance of two species), and was correlated with variation in the jaw protrusion among stickleback populations. In experimental studies, jaw protrusion was associated with prey-specific foraging performance, and community changes between lineages with divergent jaw protrusion (amongst other traits) over short time scales were similar in nature to our observations (Schmid *et al.* 2018). The parallels between these experimental studies and our comparative work strongly suggest that phenotypic variation in foraging morphology may indeed affect the structure of prey communities in nature. Furthermore, our work suggests that these phenotypic effects may be dependent on ecological conditions, such as the presence of a top-predator (Catford *et al.* 2022). As such, chapter three adds to a growing body of evidence that suggests that phenotypic divergence among predator populations can drive the ecological dynamics of ecosystems in nature, and provides a rare example of such dynamics in nature (Post *et al.* 2008).

In chapter three we focused on the community-effects of phenotypic variation in threespine stickleback, however, it is likely that ecological dynamics are also affect by the phenotypic variation in the other species in the community. For example, the arctic char in the region are subject to adaptive radiations with up to five different ecomorphs present in large lakes (Doenz *et al.* 2019). While our sampling effort focused on smaller lakes, which in most cases contained one char ecotype, populations can still vary substantially – after all, char are piscivore in lakes where they co-occur with stickleback and invertivore in lakes without stickleback. Furthermore, we found that the dominant zooplankton species *Leptodiaptomus minutus* expresses variation in pigmentation that is associated with predation regimes, and likely arises from a tradeoff between radiation protection and the evasion of visual predators (Oester *et al.* 2022). Future research should aim at understanding how ecosystem dynamics are affected by phenotypic variation in multiple species. For example, it would be interesting to investigate how the ecosystem-effects of jaw protrusion are affected by variation in the cryptic pigmentation of prey species, or the presence of different char ecomorphs.

Conclusion

In this thesis I have described how community context affects the evolution and the phenotypic expression of consumer phenotypes, and how consumer trait variation affects the structure of communities. The complexity of the interplay between ecological and evolutionary dynamics poses fundamental challenges, some of which I have addressed in this thesis. As McPeek (2017) points out, "ecologists and evolutionary biologists study different perspectives on the same whole", and so interpretations of natural phenomena may depend on the perspective taken by researchers. However, to understand the feedbacks between evolutionary and community change it is crucial to being able to switch between perspectives or, in the best case, be aware of "the whole". While the latter is difficult to achieve in most cases, both evolutionary biology and ecology are deeply rooted disciplines, and so a it can be helpful to rethink ecological and evolutionary concepts from the perspective of the respective other discipline. In chapter one, for example, we explore the traditionally ecological concepts of trophic position using concepts of evolutionary biology, such as heritability, phenotypic plasticity, and fitness. This approach can allow us to bridge the gap between ecosystem processes and evolutionary dynamics without having to reinvent the wheel. A common currency between evolutionary biology and community ecology are traits (Govaert *et al.* 2021) and understanding their role as "effect traits" (the ecological perspective) and "response traits" (the evolutionarily perspective) is crucial for understanding eco-evolutionary dynamics. In chapter three, we show that some trait variation can affect the structure of communities, while other traits can be highly responsive to community contexts (chapter four). Furthermore, we discuss how the functional relevance of traits may be determined by community contexts (chapter two), and how some ecologically relevant, genetically determined traits only gain meaning in community contexts (chapter one). Eco-evolutionary dynamics are inherently complex as they involve different levels of biological organization, ranging from traits all the way to ecosystems dynamics. This means that we often cannot predict the role that eco-evolutionary phenomena play in nature based on theory or observations in simple ecological conditions. In nature, some phenomena may be overridden by other ecological mechanisms, while others may only arise in complex ecological conditions. It is therefore crucial to test eco-evolutionary mechanisms observed in controlled conditions in the wild. In chapter three, for example, use a natural experiment to describe how phenotypic effects on community structure depend on local level variation in species interactions, and regional level variation in abiotic conditions. The establishment of the Greenlandic stickleback study system is an important contribution of this thesis. What has started with me hiking from lake to lake and creating a map of the presence and absence of char and stickleback, will hopefully serve as a platform for many future observational and experimental studies, testing eco-evolutionary questions. Overall this thesis is contributing to the ongoing integration between evolutionary biology and community ecology, and their goals to understand the origins, organization and dynamics of biological diversity.

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