

Ecological Drivers and Ecosystem Consequences of Adaptive Radiation

Inauguraldissertation

der Philosophisch-naturwissenschaftlichen Fakultät

der Universität Bern

Vorgelegt von

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von Bern

Leiter der Arbeit:

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Institut für Ökologie und Evolution der Universität Bern

Abteilung Aquatische Ökologie

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Table of Contents

Chapter 1	General Introduction	7
Chapter 2	Adaptive Plasticity and Genetic Divergence in Feeding Efficiency during Parallel Adaptive Radiation of Whitefish (<i>Coregonus spp.</i>)	35
Chapter 3	Experimental Evidence for Trait Utility of Gill Raker Number in a North Temperate Fish	71
Chapter 4	Ecological Speciation and Phenotypic Plasticity in Fish Affect Ecosystems	91
Chapter 5	The Legacy of Ecosystem Effects Caused by Adaptive Radiation	119
Chapter 6	Synthesis and Future Research	147
	Acknowledgments	167
	Authentizitätserklärung	168
	Curriculum Vitae	169

Chapter 1

General Introduction

Biodiversity consists of diversity at a broad spectrum of biological scales, from genetic and trait diversity within species, over species richness, to diversity in the global distribution of biomes (Purvis & Hector, 2000; Hooper *et al.*, 2005). Experimental, theoretical and observational studies reveal that biodiversity in terms of species richness, -abundance and -distribution over time and space, as well as in terms of species functional characteristics, strongly influence ecosystem processes and properties (Hooper *et al.*, 2005). Recent studies have shown that genetic diversity within individual species can have similar effects that can be of comparable magnitude (Crutsinger *et al.*, 2006; Bailey *et al.*, 2009). Biodiversity forms the basis for human survival and economic, social and cultural well-being, because there is a link between biodiversity and the services ecosystems deliver to humans (McNeely *et al.*, 1990; Loreau *et al.*, 2001; Millenium Ecosystem Assessment, 2005; Cardinale *et al.*, 2012). Studying the mechanisms by which biological diversity evolves and persists as well as how it affects ecological processes is therefore of pertinent importance to science and society.

In this thesis I study how ecology and evolution affect each other during evolutionary diversification in a postglacial adaptive radiation of lacustrine whitefish. In a first part I investigate how phenotypic plasticity and evolutionary divergence in response to natural selection contribute to adaptation of whitefish species to different niches within a lake. In a second part I ask whether phenotypic plasticity and evolutionary divergence associated with speciation have the potential to affect the structure and function of ecosystems. The results obtained during this thesis will hopefully make a contribution towards bridging evolutionary biology and ecosystem ecology and this is urgently needed to achieve a better understanding of ecological, evolutionary and biodiversity dynamics in natural systems.

Background

In this first paragraph I give a brief (historical) overview of the most important ideas and concepts my thesis is built on. In the second part of this introduction chapter, I will revisit each major theme again in more detail.

Speciation, defined as the evolution of reproductive isolation between diverging sister populations descending from a single parent population, is the process that ultimately leads to species diversity (Rosenzweig, 1995; Coyne & Orr, 2004). More than 150 years ago, Darwin published the landmark book “The origin of species”, which set the stage for modern evolutionary biology and speciation research until now. Darwin’s biggest achievement was the identification of natural selection as the main mechanism driving phenotypic evolution in general and the origin of species in particular (Darwin, 1859). Approximately one hundred years after Darwin, during the Modern Synthesis in Evolutionary Biology, the concept of natural selection was unified with Mendel’s theory of inheritance giving rise to the field of population genetics (Fisher, 1930; Haldane, 1932). The integration of genetics into speciation research allowed researchers to focus on how reproductive isolation between species originates and their research focus lied much less on how populations diverge in their occupation of ecological niches. Speciation was thought to be nearly always allopatric (Mayr, 1942), which is when population divergence is initiated by extrinsic physical barriers

to gene flow (Mayr, 1942; Mayr, 1963). The idea of allopatric speciation was so attractive, as it overcame the difficulty of how a continuous evolutionary process can produce discrete entities which we see in nature. In allopatric speciation genetic incompatibilities between species can accumulate over time by random processes such as mutation and drift, but also through natural or sexual selection (Dobzhansky, 1937; Mayr, 1942; Mayr 1963). Such incompatibilities may later prevent successful interbreeding upon secondary contact of previously allopatric populations. The architects of the Modern Synthesis considered sympatric speciation, which is speciation from a single local population without geographical segregation (Mayr, 1942), implausible as gene flow between diverging populations would continuously homogenize emerging genetic difference and thus inhibit evolutionary divergence and the evolution of genetic incompatibilities (Dobzhansky, 1937; Mayr, 1942; Mayr, 1963). Nevertheless, it should be noted that in their seminal books both Dobzhansky (1937) and Mayr (1942) discussed at length work of contemporaries such as Woltereck (1931) and Herre (1933), who suggested that the species they studied resulted from sympatric speciation. It was not until 20 years after the completion of the Modern Synthesis that theoretical evolutionary biologists revisited the possibility that species can emerge in sympatry in the face of gene flow (Bazykin, 1965; Bazykin, 1969; Maynard-Smith, 1966; Rosenzweig, 1978; Udovic, 1980; Felsenstein, 1981). Today sympatric speciation is thought to be theoretically possible and empirically supported for at least a few cases in nature (Bolnick & Fitzpatrick, 2007). However, the classification of speciation by geographical settings (allopatric, parapatric, sympatric) turned out as rather impractical, as most of the speciation events in nature will likely be parapatric, which is anything in between sympatric and allopatric speciation with respect to opportunity for gene flow (Gavrilets, 2004). Today, speciation is more readily classified by the mechanism driving it than by its geographical mode (Schluter, 2009; Sobel *et al.*, 2009). Speciation by natural selection can be classified based on whether selection was parallel between environments or whether it was divergent (Schluter, 2009). If selection is parallel in two isolated environments, different mutations can arise and go to fixation (or allele frequencies strongly diverge) in the two populations, which can later cause incompatibilities upon secondary contact, referred to as mutation-order-speciation (Dobzhansky, 1937, Schluter, 2009). This is theoretically only plausible to happen if populations undergoing speciation are isolated through extrinsic barriers to gene flow. On the other hand speciation can, as Darwin had postulated (Darwin, 1859), be driven by ecologically-based divergent natural selection, which directly or as a by-product leads to the evolution of reproductive isolation, a class of speciation mechanisms referred to as ecological speciation (Schluter, 1996; Schluter, 2000; Schluter, 2001; Rundle & Nosil, 2005; Nosil, 2012). Ecological speciation can happen in all geographical settings, although evidence for fully sympatric ecological speciation is still scarce. The importance of ecological speciation as a source of species diversity has wide support, coming from theoretical (Kirkpatrick & Ravigné, 2002), experimental (Rice & Hostert, 1993) and empirical studies (Nosil, 2012). It should be added that not all speciation researchers agree on the usefulness of the term of ecological speciation, partially because, as they argue, “non-ecological” speciation usually includes ecological processes as well (Sobel *et al.*, 2009; Futuyma, 2012).

However, evolutionary divergence and ecological speciation is not an inevitable response to environmental heterogeneity (Rueffler *et al.*, 2006). One alternative, among others, is an organismal

response to environmental heterogeneity through phenotypic plasticity (Rueffler *et al.*, 2006). Phenotypic plasticity is the ability of a single genotype to produce more than one alternative form of morphology, physiological state, and/or behaviour in response to environmental differences (West-Eberhard, 1989; West-Eberhard, 2003). Phenotypic plasticity is an inherent property of any developing organism, but the role it can play in evolutionary diversification is maybe still underappreciated (West-Eberhard, 1989; West-Eberhard 2003). Evolutionary biologists have long tended to think that phenotypic plasticity does generally impede evolutionary change, because phenotypic plasticity allows for adaptation to the environment without underlying change in gene frequencies, what can prevent a genetic response to selection (Wright, 1931; Falconer, 1952). Another reason for the historically underappreciated role of plasticity may be that evolutionary studies dealing with phenotypic plasticity have often been mistaken as claims of Lamarckism (Lamarck, 1809; Pfennig *et al.*, 2010). But some early biologist already recognized that phenotypic plasticity can affect the course of evolution (Baldwin, 1896; Schmalhausen, 1949). Today empirical evidence as well as theory suggest that phenotypic plasticity can under some circumstances facilitate evolutionary diversification and speciation, mainly by facilitating rapid adaptation to new environments and by reducing extinction risk of a lineage in changing environments (West-Eberhard, 2003; Pfennig *et al.*, 2010).

As briefly outlined above, effects of ecology on evolution (environment to organism) have been investigated for a long time and are understood reasonably well, at least in general terms (Darwin, 1859; Schluter, 2000; Futuyma, 2009; Nosil, 2012). Also the inversed causal pathway evolution to ecology (organism to environment) has a long scientific history. Again, already Darwin described how earthworms may through their burrowing activities change both physical and the chemical properties of soils (Darwin, 1881; Lee, 1985). Today ecologists refer to organisms, which modify their physical surrounding, including physical habitat structure and physical ecosystem metrics such as pH or light environment, as ecosystem engineers (Jones *et al.*, 1994). Among the classic examples of ecosystem engineers are beavers building dams (Wright *et al.*, 2002) and various organisms extracting calcium from the water and by doing so contribute to the formation of reefs in the Sea (Sheppard *et al.*, 2009). A related ecological concept is the keystone species concept, while keystone species are those that have a particularly large effect on other organisms in a community (Paine, 1995). The modifications ecosystem engineers and keystone species bring to their environment are quite frequently investigated in ecological studies, but are more rarely considered in evolutionary analysis (reviewed in Odling-Smee *et al.*, 2003). This is a gap in our understanding of evolutionary processes and how these interact with ecological dynamics, because such strong organism-mediated effects on the environment are likely to affect selection pressures of other organisms in the community. If a population of organisms, through any of its activities, affects adaptive landscapes experienced by any other population of organisms in the environment and if there is an evolutionary response to such organism-mediated environmental modifications, it is considered a niche-constructor (Odling-Smee *et al.*, 1996; Odling-Smee *et al.* 2003). Ecosystem engineering is one mechanism of niche construction, but niche construction can also happen through organismal modification of chemical (i. e. resource acquisition in plants) or biotic components (i. e. through consumption or competition) of the environment (reviewed in Matthews *et al.*, in revision). A very

striking example of niche construction is the emergence of photosynthetic cyanobacteria, which have started to oxygenate the earth's atmosphere approximately 2.4 billions of years before present and have dramatically changed oxygen availability on earth (ecosystem engineering) and by doing so affected subsequent evolution of many organisms distributed over the entire planet (niche construction; Stal, 2000; Odling-Smee *et al.*, 2003). A special case of niche construction is when ongoing evolution affects the evolving population's own selective landscape and not only that of other species in the community (Udovic, 1980; Geritz *et al.*, 1998; Odling-Smee *et al.*, 2003; Yoshida *et al.*, 2003; Dieckmann *et al.*, 2004; Post & Palkovacs, 2009). The idea that organisms continuously modify their fitness landscape as evolution proceeds is at the core of adaptive dynamics theory and adaptive speciation research (Geritz *et al.*, 1998; for review see Dieckmann *et al.*, 2004). The focus of adaptive speciation and adaptive dynamics theory lies on intraspecific negative frequency dependent biological interactions, meaning that the fitness of a particular phenotype mostly depends on the phenotypic composition of its own population. A very much related concept, in the sense that evolution and the environment are dynamically linked through cyclical feedback loops, is referred to as eco-evolutionary feedbacks (Post & Palkovacs, 2009). Eco-evolutionary feedbacks have been defined as the cyclical reciprocal interaction between evolution and ecology, such that an organism affects its environment, which in turn affects evolution in the organism, what then modifies its effects on the environment; and so on and so forth (Post & Palkovacs, 2009). The concepts of adaptive speciation and eco-evolutionary feedbacks have in common that a population's adaptive landscape is dynamically changing as it is evolving. However, in adaptive dynamics theory the time-scale between ecological and evolutionary processes is not fully congruent as opposed to the fully congruent time-scales of eco-evolutionary dynamics (Fussmann *et al.*, 2007): In adaptive dynamics modeling, evolutionary dynamics are assumed to happen at a slower timescale than ecological dynamics, evolution is only allowed to happen when ecological dynamics reached equilibrium, and the two processes are analytically separated (Fussmann *et al.*, 2007). Further, in adaptive dynamics theory, which was developed by theoretical evolutionary biologists, the dynamic environment, which affects fitness of evolving organisms consists most often of the frequency of phenotypes of conspecifics and negative-frequency dependent selection is at the core of adaptive dynamics theory (Dieckmann *et al.*, 2004). In the eco-evolutionary feedback concept, which was developed by community and ecosystem ecologists, the dynamic environment is often broader and includes community composition and ecosystem structure and function and negative frequency dependent intraspecific interactions do not necessarily play a central role as source of selection (Post & Palkovacs, 2009). Currently we know little about how often evolutionary diversification is accompanied by eco-evolutionary feedbacks and how common adaptive speciation is (Dieckmann *et al.*, 2004; Erwin, 2008; Losos, 2010; Yoder *et al.*, 2010) and we are just beginning to understand how evolutionary processes and ecological dynamics interact during evolutionary diversification and speciation (Grant & Grant, 2006; Post *et al.*, 2008; Harmon *et al.*, 2009; Bassar *et al.*, 2010).

Adaptive radiations and ecological speciation

It was proposed that a large fraction of world's biodiversity might have arisen through the process of adaptive radiation (Simpson, 1953; Schluter, 2000; Gavrillets & Losos, 2009). Adaptive radiation has been defined as the evolution of phenotypic and ecological diversity in a rapidly multiplying lineage

in response to ecological or ecologically based sexual selection (Schluter, 2000). The most famous examples of adaptive radiations include Darwin finches, Hawaiian silverswords, lacustrine cichlid fish and Greater Antillean Anolis lizards (Schluter, 2000). Adaptive radiation can be identified based on four criteria (Schluter, 2000): The first is common ancestry of component species. Tests of common ancestry are relatively straightforward using molecular phylogenetic trees, however, genetic relationships of radiation member species can be complex (Seehausen, 2004; Glor, 2010; for an example see Hudson *et al.*, 2011). The second is a significant association between the phenotype of a species and the environment it inhabits, referred to as phenotype-environment correlation (PE). Evidence for PE typically comes from field observations and PEs are a first indication of an adaptive fit between a trait or phenotype and the environment (for an example from whitefish see Harrod *et al.*, 2010). The third criterion is trait utility, meaning that traits underlying PEs indeed enhance fitness in the environment that they are associated with. Reciprocal transplant experiments are classic tests for trait utility of the n-dimensional phenotype and they provide evidence for selection-driven divergence in response to the environment (for an example see Via *et al.*, 2000). To make a strong argument for the functional significance of a particular trait as opposed to the entire phenotype, a trait's effect on performance or fitness needs to be measured independent of effects of the rest of the phenotype (for an example see Wainwright, 1994). The fourth defining criterion is fast speciation or increased rate of evolution of substantial reproductive isolation. This is usually inferred from phylogenies and is indicated by bursts in diversification rate early in the evolutionary history of a species flock (for an example see Harmon *et al.*, 2003).

The process of adaptive radiation is thought to be driven by ecological speciation, where reproductive isolation arises as a consequence of divergent natural or ecologically-based sexual selection (Schluter, 2000). Ecological speciation needs a source of divergent selection, a form of reproductive isolation and a genetic mechanism linking the two (Nosil, 2012). Divergent selection can be directly caused by differences in the environment organisms inhabit (Schluter, 1996). Strong support for this type of divergent selection to be relevant to speciation comes from direct selection measurements in the wild (Benkman, 2003) and from experimental work measuring fitness or performance of divergently adapted individuals in different environments (Schluter, 1995; Nosil & Crespi, 2006; Michel *et al.*, 2010). Second, divergent selection can result from resource competition. One aspect of competition is ecological opportunity, where competitor and predator free niche space relaxes selection such that a diversity of genotypes can accumulate and a diversity in phenotypes can evolve. When carrying capacity is approached, competition between individuals intensifies and drives them to exploit distinct resources, potentially leading to relaxed selection and ecological character displacement between closely related sympatric populations (Schluter, 1996). Evidence for competition as driver of divergence can be observational, most commonly based on exaggerated trait divergence in sympatry (Grant, 1986; Schluter & McPhail, 1992; Schluter, 2000b) or directly derived from experiments (Kirschel *et al.*, 2009; Calsbeek & Cox, 2010). Evidence for the importance of ecological opportunity in facilitating adaptive radiation indirectly comes from the observation that examples of young adaptive radiations often happened on remote archipelagos or isolated lakes with depauperate species communities (Losos, 2010). More direct evidence for example comes from a recent analysis of cichlid fish species diversity across 46 African lakes, which

showed that cichlids radiated more readily when physical environmental structure provided much ecological opportunity (Wagner *et al.*, 2012).

In ecological speciation, reproductive isolation between species can either be pre- or postzygotic. Prezygotic isolation includes divergent habitat preferences, temporal isolation, immigrant inviability, divergent mating preferences and divergent gamete recognition. Postzygotic isolation includes ecologically based natural or sexual selection against hybrids and intrinsic genetic incompatibilities that may be driven by ecological selection (Nosil, 2012), antagonistic coevolution of the sexes (Presgraves, 2010) or by genetic drift. The final aspect of ecological speciation is a genetic mechanism linking divergent selection to reproductive isolation (Nosil, 2012). One way to achieve this is through a non-random association of loci under divergent selection and loci causing assortative mating. Under this scenario, genes under divergent selection and those responsible for assortative mating are physically different and they need to become coupled to each other through linkage disequilibrium. This mechanism has been shown to theoretically be possible, although conditions are restrictive (Felsenstein, 1981): While divergent selection keeps pushing diverging populations apart, only limited amounts of gene flow will, through recombination, continuously break down emerging linkage disequilibrium between the loci under selection and those affecting reproductive isolation. Therefore, either very strong selection or factors reducing recombination are needed to prevent the breaking down of linkage disequilibrium (reviewed in Nosil, 2012). A particularly powerful mechanism, because it is not sensitive to recombination, is when the allele affecting assortative mating is one and the same in the diverging populations (referred to as one-allele-mechanism of speciation, Felsenstein, 1981). This can for example be a gene that causes individuals to mate with another individual phenotypically similar to themselves. A second and simpler scenario how divergent selection and reproductive isolation can be linked is when loci under divergent selection and those responsible for assortative mating are identical, meaning that they pleiotropically affect a trait under divergent selection and reproductive isolation (Kirkpatrick & Barton, 1997). If for example a gene for preference to the native habitat is under selection and if mating occurs in or close to the preferred habitat, this gene under natural selection will directly affect reproductive isolation (Bush, 1969; Funk *et al.*, 2002). This model of speciation has sometimes been referred to as “magic trait model of speciation”, “magic” because it overcomes the selection-recombination antagonism that can constrain divergence in the face of gene flow (Gavrilets, 2004; Maan & Seehausen, 2011).

Phenotypic plasticity

The fact that many of the influential books that were published about speciation in recent years do not include (extended) discussions about phenotypic plasticity’s role in speciation (e. g. Schluter, 2000; Coyne & Orr, 2004; Nosil, 2012), may indicate that phenotypic plasticity is not yet a fully inherent part of speciation research. This may limit our understanding of evolutionary diversification, because plasticity is i) a ubiquitous feature of life and ii) it is an evolving trait with a genetic basis that is shaped by natural selection (West-Eberhard, 2003; Pigliucci, 2005). On one hand plasticity is seen as a process acting during development and from this perspective the most important question for an evolutionary biologist is probably how plasticity affects development and by doing so shapes evolution (West-Eberhard, 2003; De Jong, 2005). On the other hand plasticity is seen a quantitative

trait under natural selection and from this perspective the most important question for an evolutionary biologist is how plasticity itself evolves and is shaped by selection (Via & Lande, 1985; Stearns & Koella, 1986; Schlichting & Pigliucci, 1998; De Jong, 2005; Hutchings, 2011). Below I try to combine elements of both views in order to get a clearer picture on how plasticity affects evolutionary divergence.

Phenotypic plasticity can impede or facilitate evolutionary divergence (Pfennig *et al.*, 2010). It can facilitate occupation of and persistence in new environments, for example by facilitating exploitation of underutilized niches through adaptive peak shifts (Pfennig *et al.*, 2006). Further, plasticity can allow a population to persist under changing conditions, even if it is genetically not well adapted. Such persistence then allows for genetic variation to arise over time (through recombination, gene flow or mutation) and for natural selection to increase the adaptive fit to the environment through changes in allele frequencies. If the environment is stable, selection may favor a decrease of plasticity (Day *et al.*, 1994; Pigliucci *et al.*, 2006; Suzuki & Nijhout, 2006). This decrease in a trait's plasticity over the course of evolution is called genetic assimilation (West-Eberhard, 2003). Genetic assimilation happens because the ability to display an optimal trait through plasticity often brings along a fitness cost to the organism compared to being optimally adapted through fixed development (Pigliucci, 2005). Further, when the reaction norm of a plastic response is shaped by natural selection, selection will optimize the reaction norm across various environments and this will not necessarily result in optimal phenotypes in each environment, indicating another type of cost of plasticity (De Jong, 2005). The colonization of new niches through plasticity can additionally expose other, non-plastic, traits to new selection pressures and by doing so drive genetic differentiation in these uniplastic traits, a process referred to as genetic accommodation (Baldwin, 1896; Price *et al.*, 2003; West-Eberhard, 2003; Price *et al.*, 2008). On the other hand, genetically induced changes in the phenotype are also accommodated through plasticity in the developmental system (referred to as phenotypic accommodation). The most famous example of phenotypic accommodation are observations of two-legged goats first described by Slijper in the 1940ies, which developed special peculiarities through phenotypically plastic changes during development and which are even able to hop around on two legs (West-Eberhard, 2003). Although having only two legs is obviously not beneficial for a goat, the goat examples impressively illustrate the power of phenotypic accommodation in facilitating adaptation through genetic change (West-Eberhard, 2003).

On the other hand, plasticity can also impede evolutionary change. It does so by dampening effects of natural selection (Schlichting, 2004), for two different reasons: First, plasticity enables organisms to increase their fitness without changes in allele frequencies and second, it allows organism with different genotypes to display similar traits and by doing so hides unfavorable genetic variation from selection.

There is no clear answer yet to the question whether plasticity generally rather constrains or facilitates differentiation, it can do both and whether it does the former or the latter depends on various things (De Jong, 2005; Pfennig *et al.*, 2010): Among others, it depends on the cost of plasticity, which affects the degree of plasticity expressed. Moderate levels of plasticity are most

favorable for diversification (Price *et al.*, 2003), because if plasticity is too low, it does not contribute to survival in and adaptation to novel environments. If it is too high, plasticity alone allows for shifts too close to an adaptive peak so that selection is weak or absent and genetic divergence inhibited. It also depends on how plasticity affects gene flow between diverging populations and how gene flow affects divergence. Plasticity has been shown to increase gene flow by increasing survival of dispersers (Crispo, 2008) or by making dispersers assume the local mating phenotype (Maan & Seehausen, 2011). Gene flow will usually rather constrain population divergence, but it can also facilitate population divergence through two very different processes, reinforcement (Slatkin, 1987; Servedio & Noor, 2003) or matching habitat choice leading to directed and adaptive gene flow (Edelaar *et al.*, 2008; Bolnick *et al.*, 2009).

Although many taxa undergoing adaptive radiation display considerable levels of plasticity in traits characterizing the radiation (Grant, 1986; Day *et al.*, 1994; Losos *et al.*, 2000; Bouton *et al.*, 2002), the ecological theory of adaptive radiation does not directly deal with plasticity (Schluter, 2000). The flexible stem model of adaptive radiation on the other hand suggests that the nature of developmental plasticity in the ancestor will affect the course of evolution during adaptive radiation (West-Eberhard, 2003). Under this hypothesis variation that allowed for adaptation to new environments was achieved through ancestral plasticity, while phenotypic differences have subsequently been genetically assimilated and phenotypes have further been modified by selection over the course of the radiation (West-Eberhard, 2003). Direct evidence for the flexible stem hypothesis is derived from the observation that diet-induced plasticity in marine stickleback populations, the ancestor of stickleback adaptive radiations in freshwaters, produced similar phenotypes as the genetic differences that evolved in benthic limnetic stickleback species pair in the freshwater (Wund *et al.*, 2008). Similar findings in the adaptive radiation of spadefoot toads (Gomez-Mestre & Buchholz, 2006) indicate that effects of ancestral plasticity on the course of adaptive radiations may not be exceptional. Examples from fish show that rearing a benthic specialist in a limnetic environment can induce trait values that resemble those seen in a limnetic specialist (or vice versa), a pattern consistent with the flexible stem model of adaptive radiation (Day *et al.*, 1994; Wimberger, 1994; Skúlason *et al.*, 1999; West-Eberhard, 2003). Finally, it was shown that clades with the ability to display strong plasticity are more species rich than closely related clades with limited ability for plasticity, suggesting that plasticity facilitates evolutionary diversification (Pfennig & McGee, 2010). However, such patterns can result from other reasons than increased speciation rates due to plasticity, for example through reduced extinction risk of more plastic clades in changing environments (Sol *et al.*, 2005; West-Eberhard, 2003; Charmantier *et al.*, 2008; Chevin *et al.*, 2010). Nevertheless, such findings suggest that phenotypic plasticity promotes diversity.

Evolutionary divergence can affect ecosystems

In recent years evidence that short term evolutionary change can proceed rapidly has accumulated (Hendry & Kinnison, 1999; Carroll *et al.*, 2007) and it was at least partially this recognition that stimulated a new, still ongoing synthesis about the interplay of evolution and ecology (Schoener, 2011). The observation that evolution can be a substantial determinant of ecosystem structure and function and by doing so affect the course of further evolution (Odling-Smee *et al.*, 2003; Schoener,

2011), indicates that an integration of these disciplines is urgently needed to achieve a better understanding of ecological and evolutionary dynamics in natural systems (Schoener, 2011). A considerable body of research within this emerging synthesis deals with how phenotypic differentiation between recently diverged populations affects ecosystems (i.e. Post *et al.*, 2008; Bassar *et al.*, 2010). For example, differences in temporal dynamics of predation pressures between lakes harbouring landlocked and lakes harbouring anadromous populations of alewife fish resulted in strong contrasting effects on the zooplankton prey community and food-web structure (Post *et al.*, 2008). Another example stems from guppies where populations adapted to either high- or low-predation environments have strongly contrasting effects on ecosystem structure and function (Palkovacs *et al.*, 2009; Bassar *et al.*, 2010). Such findings indicate that local adaptation can substantially change ecosystems and it has even been shown that divergent local adaptation as an ecosystem determinant can compete in strength with classic ecological determinants such as species invasions (Palkovacs *et al.*, 2009). However, almost nothing is known about how phenotypic differentiation that evolves during adaptive radiation affects ecosystems. So far, only one study looked at how diversification and speciation in a stickleback adaptive radiation affects ecosystems by comparing ecosystem effects of generalist and specialist stickleback species from Canadian coastal lakes (Harmon *et al.*, 2009). They found that both, speciation and adaptive diversification of stickleback fish had strong effects on how ecosystems function (Harmon *et al.*, 2009). Almost all studies that yet looked at how phenotypic differentiation causes divergence in ecosystems either used wild-caught individuals in experimental ecosystems or studied populations in the wild. These studies can therefore not distinguish between contrasting ecosystem effects caused by genetic differentiation and such resulting from phenotypic plasticity, although this distinction is important (Seehausen, 2009). Plasticity has been shown to have a wide-range of effects on ecological processes (Miner *et al.*, 2005) and if contrasting ecosystem effects of different phenotypes do often result from environmentally induced non-heritable differences, then evolutionary divergence, such as local adaptation, speciation or adaptive radiation is not required to explain variation in ecosystem effects between populations.

Contrasting ecosystem effects of recently diverged species or populations can have ecological consequences, but can also result in differences in niche construction, meaning that there might be evolutionary consequences of organism-mediated environmental change (Odling-Smee *et al.*, 2003). For ecosystem effects to have evolutionary consequences ecosystem modification and evolutionary response to it need to happen at overlapping time-scales, indicating that the likelihood for evolutionary consequence increases with the persistence time of contrasting ecosystem effects and the potential rate of evolution. Persistency of contrasting ecosystem effects can be achieved through continuous environmental modification over time. It can also be achieved through persistency of previous environmental modifications. The latter may be particularly important outside the tropics, where many organisms are either migratory, hibernating or display other changes in behavior and activity over the seasons.

Evidence for a role of niche construction (including eco-evolutionary feedbacks) in contributing to evolutionary divergence between species or populations is rare, but available: By comparing systems

with the potential for strong versus weak feedbacks, Palkovacs & Post (2008) inferred eco-evolutionary feedbacks to be an important engine shaping spatial patterns of evolutionary and ecological diversity and divergence in alewife populations (*Alosa pseudoheringus*). There is also empirical and theoretical support for the idea that speciation in adaptive radiation can be driven by negatively frequency dependent disruptive selection, which is a case of niche construction and eco-evolutionary feedback (Dieckmann & Doebeli, 1999; Benkman, 2003; Ito & Dieckmann, 2007). As adaptive radiation is often characterized by high standing genetic variation in the ancestral population and high rates of phenotypic evolution and as the functional link between the phenotype of an organism and the environment it inhabits is particularly strong among organisms of adaptive radiations (Schluter, 2000; Barret & Schluter, 2008), eco-evolutionary feedbacks may be important in many adaptive radiations (Dieckmann *et al.*, 2004; Pelletier *et al.*, 2009). Because of the potential importance of eco-evolutionary feedbacks during adaptive radiation, ecosystem and evolutionary consequences of adaptive radiation are an area of research that warrants additional exploration.

Adaptive radiation in north-temperate postglacial fishes

Using his famous metaphor of the “tape of life”, Gould (1989) claimed that if this “tape of life” was rewound and allowed to be played again, the evolutionary outcome would be fundamentally different than what we observe now, suggesting that evolution is rather more stochastic than deterministic. Adaptive radiations seem to challenge this claim at least to some degree, as they have a tendency to proceed along a non-random sequence of stages (Schluter, 2000; Streelman & Danley, 2003) and as they frequently result in parallel outcomes (Rundle *et al.*, 2000; Schluter, 2000). Some of the most striking patterns of parallel radiations are probably found in north-temperate fish, which emerged in a maximum of 15'000 years, following the retreat of the ice after the last glacial maxima (Schluter, 1996; Taylor, 1999; Schluter, 2000; Ostbye *et al.*, 2006; Hudson *et al.*, 2007). Because of their recent origins, the repeated co-existence of multiple species in sympatry and their replicated patterns of evolution these fish have gained considerable attention of evolutionary biologist and became model organisms for ecological speciation and adaptive radiation research.

In many cases the diversifying clades first split along the benthic and limnetic habitat axis. Taxa displaying this benthic-limnetic-split are most frequently found within the order of the Salmoniformes, including brown trout, arctic charr, rainbow smelt, *Prosopium* and European whitefish (Schluter, 1996; Schluter, 2000; Hudson *et al.*, 2007; Hynes *et al.*, 1996), but also in Non-Salmoniformes such as three-spine stickleback (Rundle *et al.*, 2000) and in pumpkinseed sunfish and perch albeit apparently there without speciation (Svanbäck & Eklöv, 2003; Robinson *et al.*, 1993). Limnetic species and ecotypes usually have more gill rakers (gill rakers are small bony or cartilaginous structures that project from the branchial gill arch), lower growth rates, a more slender body and rely more strongly on zooplankton as prey than benthic species, suggesting that trophic adaptation was involved during diversification of these fish (Schluter & McPhail, 1992; Malmquist *et al.*, 1992; Schluter, 2000; Amundsen *et al.*, 2004; Kottelat & Freyhof, 2007; Harrod *et al.*, 2010). The functional prediction for a higher number of gill rakers is increased retention capability after capture of small zooplankton in the limnetic habitat through increased filtering efficacy of the gill apparatus (Link & Hoff, 1998). Computational fluid dynamics and video endoscopy of suspension-feeding fish indeed

found that gill rakers serve as cross-flow filters transporting particles from the oral cavity towards the oesophagus (Sanderson *et al.*, 2001). Growth patterns and body shape differences are likely shaped by the interaction of various ecological selection pressures and phenotypic plasticity. Slower growth in the limnetic habitat may be associated with high bioenergetic costs of living in this habitat, with small prey distributed over a large volume of water (Kahilainen *et al.*, 2003; Trudel *et al.*, 2001; Kahilainen *et al.*, 2007) or it may be adaptive with regard to the limnetic feeding niche. Deeper bodies and faster growth in the littoral benthic habitat constitutes a different predator escape strategy, namely through accelerated growth to reach a size above the predation window of gape limited piscivorous fish instead of adaptations in predator avoidance through swimming behavior (Brönmark & Miner, 1992; Kahilainen & Lehtonen, 2002; Reznick *et al.*, 2001; Rogers *et al.*, 2002). Deeper bodies do also increase maneuverability and fast acceleration in spatially more complex benthic habitats, whereas streamlined bodies reduce drag during sustained cruising through the pelagic habitat (Walker, 1997). Such multidimensional phenotypic differentiation between benthic-limnetic species has been experimentally shown to affect feeding performance in the two extreme habitats in several fish taxa and in directions consistent with functional morphology predictions (Bentzen & MacPhail, 1984; Robinson, 2000; Adams & Huntingford, 2002). Such repeated parallel patterns of phenotypic divergence in adaptive radiations are a strong indication for the action of natural selection during species formation (Rundle *et al.*, 2000; Schluter, 2000; Landry *et al.*, 2007). However, phenotypic plasticity probably also played an important role in initiating some of these radiations, indicated by direct evidence for the flexible stem hypothesis in stickleback (Wund *et al.*, 2008) and by observations of high degrees of adaptive plasticity in morphology and behavior in experiments with benthic limnetic species pairs of stickleback and char (Wimberger, 1994; Day & McPhail, 1996; Skúlason *et al.*, 1999; Robinson & Parson, 2002; Adams & Huntingford, 2004; Lucek *et al.*, in prep.).

The Coregonus model system

The family Coregonidae belongs to the order of the Salmoniformes and it consists of three genera, *Stenodus*, *Prosopium* and *Coregonus* (Bernatchez, 2004). Especially fish belonging to the genus *Coregonus* have fascinated evolutionary biologists for a long time, because this genus is so species rich and because closely related species can often coexist within a single lake in geographical sympatry (Bernatchez, 2004). Taxonomic relationships between the species within this genus are complex and systematics has traditionally been considered as a nightmare (Svärdson, 1949; Kottelat & Freyhof, 2007). According to Kottelat & Freyhof (2007), the genus *Coregonus* can be further divided into two subgenera, the *Coregonus sensu stricto* group (from here onwards referred to as whitefish) and the *Coregonus albula* species group (ciscoes and vendace). Whitefish are found in large parts of Scandinavia, northern Eurasia, the northern Alpine region of south-central Europe and northern North America (Bernatchez, 2004; Kottelat & Freyhof, 2007). In northern North America the whitefish species complex has often been referred to as Lake whitefish or *C. clupeaformis* species complex (Bernatchez, 2004), while in Europe the whitefish species complex has often been referred to as European whitefish or *C. lavaretus* species complex (Kottelat & Freyhof, 2007). As the use of the name *C. lavaretus* differs between different authors, I here use the term European whitefish (species complex) when referring to European whitefish species and the term Alpine whitefish (species

complex) when specifically referring to whitefish species from the Alpine region in central Europe. Alpine whitefish are a monophyletic radiation of hybridogenic origin within the European whitefish species complex (Hudson *et al.*, 2011).

Similar to other north temperate fish, a split along the benthic limnetic habitat axis is commonly observed in whitefish (Taylor 1999; Harrod *et al.*, 2010). Co-existing species are phenotypically most strongly differentiated in traits linked to trophic ecology such as gill raker counts, growth rates and body shape (Landry *et al.*, 2007; Lu & Bernatchez, 1999; Vonlanthen *et al.*, 2009; Siwertsson *et al.*, 2010; Vonlanthen *et al.*, 2012). But the number of species per lake frequently exceeds two and additional axes of divergence, such as spawning depth and spawning time, are involved in whitefish adaptive radiation (Vonlanthen *et al.*, 2009; Siwertsson *et al.*, 2010; Vonlanthen *et al.*, 2012; Lundsgaard-Hansen *et al.*, in prep.). European whitefish might well fulfill the four criteria characterizing adaptive radiations: Common ancestry and rapid intralacustrine speciation has been shown for the Alpine whitefish species flock overall and for individual lake flocks (Douglas *et al.*, 1999; Hudson *et al.*, 2011) and phenotype-environment correlations have been shown for Scandinavian whitefish species (Amundsen *et al.*, 2004; Harrod *et al.*, 2010; Kahilainen *et al.*, 2011). However, trait utility has not been experimentally demonstrated for any whitefish radiation.

The Alpine whitefish radiation, which is outstanding amongst post-glacial radiations due to its large ecological and species diversity, has suffered from a loss of around one third of its species in just a few decades (Vonlanthen *et al.*, 2012). This diversity loss was driven by human-mediated eutrophication occurring in the last century and is associated with speciation reversal, as it also happened in Lake Victoria cichlids (Seehausen *et al.*, 1997; Vonlanthen *et al.*, 2012). This highlights how ecologically fragile young radiations are. It also shows that processes acting during evolutionary diversification and those acting during biodiversity loss can be analog (Seehausen *et al.*, 1997; Seehausen *et al.*, 2008; Vonlanthen *et al.*, 2012). It thus exemplifies how an understanding of processes driving evolutionary diversification may help to prevent biodiversity loss in the future.

Study system

In Swiss Lakes, whitefish have radiated into more than 30 different species after the last glacial maxima, with species diversity per lake varying from one to at least five (Steinmann, 1950; Vonlanthen *et al.*, 2012). In this thesis, I used whitefish species from two of the most species-rich lakes, Lake Thun and Lake Lucerne. In Lake Lucerne five or six different whitefish species have been documented based on phenotypic and genetic information (Steinmann, 1950; Svarvar & Müller, 1982; Lundsgaard-Hansen *et al.*, in prep.): The fast growing, sparsely-rakered and winter-spawning Balchen (*C. sp.* “Bodenbalchen”); the slow growing, densely-rakered and summer- to winter spawning Albeli (*C. zugensis*); the in growth and gill raker numbers intermediate, winter-spawning Schwebbalchen (*C. sp.* “Schwebbalchen”); the intermediate growing, intermediate to densely-rakered and summer spawning Edelfisch (*C. nobilis*), the intermediate growing and winter-spawning Alpnacherfelchen (*C. sp.* “Alpnacherfelchen”), and potentially an additional species of intermediate gill raker numbers and growth which might spawn in the pelagic (Lundsgaard-Hansen *et al.*, in prep.). Also in Lake Thun five different whitefish species have been identified (Steinmann, 1950; Bittner,

2009; Vonlanthen *et al.*, 2012): The fast growing, sparsely-rakered and winter-spawning Balchen (*C. sp.* “Balchen”); the intermediate growing, intermediately-rakered and winter-spawning Albock (*C. fatioti*); the slow growing, intermediately-rakered and winter-spawning Tiefenalbock or Felchen (*C. sp.* “Felchen”); the slow growing, densely-rakered and summer to winter-spawning Brienzlig (*C. albellus*) and the slow growing, sparsely-rakered and summer-spawning Kropfer (*C. alpinus*).

Of these species I here used *C. zugensis* and *C. sp.* “Bodenbalchen” from Lake Lucerne and *C. albellus* and *C. sp.* “Balchen” from Lake Thun. I chose to focus on these species because they phenotypically correspond well to the benthic-limnetic split commonly observed in north temperate fish (Schluter, 2000). *C. sp.* “Bodenbalchen” and *C. sp.* “Balchen” correspond to the benthic phenotypes, they grow fast and reach maximum sizes of 600 and 450 mm, respectively, their mean gill raker number is 29.4 (ranging from 22-34) and 30.5 (ranging from 22-33) and both of them spawn in shallow water of approximately 2-5 m depth (Steinmann, 1950; Vonlanthen *et al.*, 2012). *C. zugensis* and *C. albellus* correspond to the limnetic phenotype, they grow slowly and reach a maximum size of only 300 mm, their mean gill raker number is 38.8 (ranging from 34-43) and 38.1 (ranging from 35-44) respectively and both spawn in 25 m depth and deeper (Steinmann, 1950; Vonlanthen *et al.*, 2012). The sympatric species are genetically clearly differentiated from each other in both cases (Vonlanthen *et al.*, 2012) but clearly more closely related to each other than to the corresponding ecotype from the other lake (Hudson *et al.*, 2011). Gut content analysis of Lake Lucerne species showed that *C. zugensis* feeds almost exclusively on zooplankton, while the diet of *C. sp.* “Bodenbalchen” is more benthic (Michel, 1996; Mookerji *et al.*, 1998). Functional morphology considerations and comparison with other whitefish species with known feeding ecology (i.e. Bernatchez *et al.*, 1999; Sanderson *et al.*, 2001; Amundsen *et al.*, 2004; Harrod *et al.*, 2010) suggest that the studied whitefish species from Lake Thun also differ in resource use in nature.

Goals and methods

In this thesis I want to make a step towards the integration of ecosystem ecology into evolutionary biology and evolutionary biology into ecosystem ecology. On the one hand I study which ecological driving forces are involved in diversification and speciation during adaptive radiation of whitefish and what role natural selection and phenotypic plasticity play in the diversification process. On the other hand I investigate whether phenotypic differentiation during these adaptive radiations affects ecosystems and how ecological consequences of adaptive radiation result from genetic differentiation associated with speciation and from environmentally induced phenotypic plasticity associated with having been exposed to different feeding niches.

To address these questions, I did feeding efficiency and mesocosm experiments. To do so we bred and raised multiple families of *C. sp.* “Bodenbalchen”, *C. sp.* “Balchen”, *C. zugensis* and *C. albellus* in the lab under common garden conditions. Lake Lucerne fish (*C. sp.* “Bodenbalchen”; *C. zugensis*) were raised with reciprocal food treatments in both species. We used red mosquito larvae to simulate a benthic foraging environment and we used zooplankton to simulate a limnetic feeding environment. This resulted in four different groups: Genetically benthic fish raised on benthic food (BB), genetically benthic fish raised on limnetic food (BL), genetically limnetic fish raised on benthic

food (LB) and genetically limnetic fish raised on limnetic food (LL). Fish from Lake Thun (*C. sp.* “Balchen” and *C. albellus*) were only raised on benthic food, resulting in two groups (BB and LB). These fish were then used in feeding efficiency and mesocosm experiments. For Lake Lucerne fish, the rearing regime allows us to partition the variation in feeding efficiency and in ecosystem effects into genetically heritable and phenotypically plastic components. All chapters presented in this thesis are based on experiments using fish from this common garden experiment.

Evidence that adaptation to different trophic niches is involved in adaptive radiation of north temperate fish is available from field studies as well as from experimental work (Schluter, 1996; Harrod *et al.*, 2010). However, the mechanisms of inheritance of traits involved in resource acquisition, in particular the relative importance of phenotypic plasticity and genetic predisposition in foraging behavior, are not fully understood (but see Day & McPhail, 1996). Furthermore, no published tests of feeding efficiency differentiation between benthic and limnetic whitefish species were available. In the first chapter, I therefore experimentally test for adaptation to different trophic niches between the species, and hence whether such divergent adaptation may indeed be the important ecological driving force in whitefish adaptive radiation that it is often assumed to be. I want to make a contribution to a better understanding what role natural selection and phenotypic plasticity played during species formation in these adaptive radiations. To do so I measured various feeding efficiency variables using benthic food and the fish from the common garden experiment described above. Further, this experiment can be considered as a test of trait utility of the n-dimensional phenotype for benthic feeding and it thus also represents the first available test whether the Alpine whitefish radiation fulfills the four defining criteria for adaptive radiation.

In adaptive radiations of north temperate fish, the same traits often diverged in a replicated fashion in different lakes and across different taxa (Rundle *et al.*, 2000; Landry *et al.*, 2007). Between species differentiation among limnetic versus benthic sister species is maybe most strongly replicated in the number of gill rakers, which are thought to have functional importance in benthic versus limnetic feeding (Robinson, 2000; Sanderson *et al.*, 2001). However, although the assumption of an adaptive value of variation in gill raker numbers is so central to ecological speciation research in fish, direct experimental evidence for their functional importance is still very scarce and restricted to a single study on Threespine stickleback (Robinson, 2000). Within the Salmoniformes, which are responsible for most of the species diversity seen in adaptive radiations of north temperate post-glacial fish, evidence for a functional role of gill rakers in feeding on zooplankton was not available. In the second chapter I therefore tested limnetic feeding efficiency of the two Lake Thun species reared in the common garden experiment using zooplankton as prey to experimentally test for trait utility of variation in gill raker number for zooplankton feeding. Second, by combining the findings of chapter two and three, I test for functional trade-offs between adaptations to benthic and limnetic environments.

Overall, evidence that evolutionary processes matter for ecosystems is increasing (Schoener, 2011). But we are still just beginning to understand what consequences local adaptation has for ecosystems (Post *et al.*, 2008; Bassar *et al.*, 2010) and almost nothing is known about ecosystem consequences of

speciation and adaptive radiation. In a seminal study Harmon *et al.* (2009) showed that phenotypic differentiation between closely related stickleback species can have strong effects on a wide variety of ecosystem properties. However, whether the contrasting ecosystem effects of these species result from evolutionary differentiation or from phenotypic plasticity remained unknown (Seehausen, 2009). In the third chapter, I therefore conducted a mesocosm experiment using the fish from Lake Lucerne that we raised in a common garden/reciprocal food treatment setup to test whether closely related whitefish species that evolved in a young adaptive radiation have divergent ecosystem effects. Second, I test whether these effects do result from genetic differences between the species or from phenotypic plasticity.

How important evolution is as a determinant of ecological dynamics and how likely niche construction or eco-evolutionary feedbacks are to affect evolutionary divergence, may also depend on how persistent ecosystem effects of evolutionary divergence are (Odling-Smee *et al.*, 2003; Post & Palkovacs, 2009). Yet the time-scale of persistence of contrasting ecosystem effects of phenotypic differentiation had never been experimentally investigated. Such investigations are needed to improve our understanding of adaptive radiation, where eco-evolutionary feedbacks are likely to be important (Dieckmann *et al.*, 2004; Pelletier *et al.*, 2009). In the fourth chapter I therefore did a mesocosm experiment, which was divided into two phases. In the first phase whitefish from different species and/or rearing environments were put in mesocosm ecosystems to induce ecosystem divergence. In a second phase the fish were removed, while the measurement of ecosystem metrics of interest was continued. By doing so I investigated the persistency of contrasting ecosystem effects resulting from evolutionary divergence, and also those resulting from phenotypic plasticity. Further, I included tanks without any fish in order to compare effect strength of evolution and plasticity to that of fish presence versus absence.

My goal with this thesis is to make a contribution towards a better understanding of how ecology and evolution interact. An integration of ecosystems ecology and evolutionary biology is timely and is urgently required to achieve a deeper understanding of ecological dynamics and evolutionary processes in natural systems. This understanding will be needed to better inform attempts to protect and restore biodiversity at all its scales. As Earth is currently facing the sixth and probably fastest ever mass extinction (Barnosky *et al.*, 2011) and as biodiversity matters for human well-being (Hooper *et al.*, 2005), conserving biodiversity may today be more urgent than ever before (Barnosky *et al.*, 2011).

References

- Adams, C.E. & Huntingford, F.A. 2002. The functional significance of inherited differences in feeding morphology in a sympatric polymorphic population of Arctic charr. *Evol. Ecol.* 16: 15-25.
- Adams, C.E. & Huntingford, F.A. 2004. Incipient speciation driven by phenotypic plasticity? Evidence from sympatric populations of Arctic charr. *Biol. J. Linn. Soc.* 81: 611-618.
- Amundsen, P.A., Knudsen, R., Klemetsen, A. & Kristoffersen, R. 2004. Resource competition and interactive segregation between sympatric whitefish morphs. *Ann. Zool. Fenn.* 41: 301-307.
- Bailey, J.K., Schweitzer, J.A., Ubeda, F., Koricheva, J., Le Roy, C. J., Madritch, M.D., Rehil, B.J., Bangert, R.K., Fischer, D.G., Allan, G.J. & Whitman, T.G. 2009. From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Philos. T. Roy. Soc. B.* 364: 1607-1616.
- Baldwin, J. M. 1896. A new factor in evolution. *Am. Nat.* 30: 441-451.
- Barnosky, A.D., Matzke, N., Tomiya, S., Wogan, G.O.U., Swartz, B., Quental, T.B., Marshall, C., McGuire, J.L., Lindsey, E.L., Maguire, K.C., Mersey, B. & Ferrer, E.A. 2011. Has the Earth's sixth mass extinction already arrived? *Nature* 471: 51-57.
- Barret, R.D. & Schluter, D. 2008. Adaptation from standing genetic variation. *Trends Ecol. Evol.* 23: 38-44.
- Bassar, R.D., Marshall, M.C., Lopez-Sepulcre, A., Zandon, E., Auer, S.K., Travis, J., Pringle, C.M., Flecker, A.S., Thomas, S.A., Fraser, D.F. & Reznick, D.N. 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. *P. Natl. Acad. Sci. USA* 107: 3616-3621.
- Bazykin, A.D. 1965. On the possibility of sympatric species formation (in Russian). Byulleten Moskovskogo Obshchestva Ispytateley Pirody. *Otdel Biologicheskiiy* 70: 161-165.
- Bazykin, A.D. 1969. Hypothetical mechanism of speciation. *Evolution* 23: 685-687.
- Benkman, C.W. 2003. Divergent selection drives the adaptive radiation of crossbills. *Evolution* 57: 1176-1190.
- Bentzen, P. & McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): specialization for alternative trophic niches in the Enos Lake species pair. *Can. J. Zoolog.* 62: 2280-2286.
- Bernatchez, L. 2004. Ecological Theory of Adaptive Radiation. An empirical Assessment from Coregonine Fishes (Salmoniformes). In: *Evolution Illuminated* (Hendry, A.P. & Stearns, S.C., eds.). pp. 175-207. Oxford University Press, Oxford.
- Bernatchez, L., Chouinard, A. & Lu, G.Q. 1999. Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, *Coregonus sp.*, as a case study. *Biol. J. Linn. Soc.* 68: 173-194.
- Bittner, D. 2009. Gonad deformations in whitefish (*Coregonus spp.*) from Lake Thun, Switzerland - A population genetic and transcriptomic approach. In: *CMPG*, Vol. PhD. pp. University of Bern, Bern.
- Bolnick, D.I. & Fitzpatrick, B.M. 2007. Sympatric speciation: Models and empirical evidence. *Annu. Rev. Ecol. Evol. S.* 38: 459-487.

-
- Bolnick, D.I., Snowberg, L.K., Patenia, C., Stutz, W.E., Ingram, T. & Lau, O.L. 2009. Phenotype-dependent native habitat preference facilitates divergence between parapatric lake and stream stickleback. *Evolution* 63: 2004-2016.
- Bouton, N., Witte, F. & Van Alphen, J.J.M. 2002. Experimental evidence for adaptive phenotypic plasticity in a rock-dwelling cichlid fish from Lake Victoria. *Biol. J. Linn. Soc.* 77: 185-192.
- Brönmark, C. & Miner, J.G. 1992. Predator-induced phenotypical change in body morphology in crucian carp. *Science* 258: 1348-1350.
- Bush, G. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). *Evolution* 23: 237-251.
- Calsbeek, R. & Cox, R.M. 2010. Experimentally assessing the relative importance of predation and competition as agents of selection. *Nature* 465: 613-616.
- Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Sirvastava, D.S. & Naeem, S. 2012. Biodiversity loss and its impact on humanity. *Nature* 486: 59-67.
- Carroll, S.P., Hendry, A.P., Reznick, D.N. & Fox, C.W. 2007. Evolution on ecological time-scales. *Funct. Ecol.* 21: 387-393.
- Charmantier, A., McCleery, R.H., Cole, L.R., Perrins, C., Kruuk, L.E.B. & Sheldon, B.C. 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* 320: 800-803.
- Chevin, L.-M., Lande, R. & Mace, G.M. 2010. Adaptation, plasticity and extinction in a changing environment. *Plos Biol.* 8, e1000357.
- Coyne, J.A. & Orr, H.A. 2004. Speciation. Sinauer Associates, Sunderland.
- Crispo, E. 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. *J. Evolution. Biol.* 21: 1460-1469.
- Crutsinger, G.M., Collins, M.D., Fordyce, J.A., Gompert, Z., Nice, C.C. & Sanders, N.J. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science* 313: 966-968.
- Darwin, C. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London.
- Darwin, C. 1881. The formation of vegetable mold through the action of worms, with observation on their habits. John Murray, London.
- Day, T. & McPhail, J.D. 1996. The effect of behavioural and morphological plasticity on foraging efficiency in the threespine stickleback (*Gasterosteus* sp.). *Oecologia* 108: 380-388.
- Day, T., Pritchard, J. & Schluter, D. 1994. A comparison of two sticklebacks. *Evolution* 48: 1723-1734.
- De Jong, G. 2005. Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. *New Phytol.* 166: 101-117.
- Dieckmann, U. & Doebeli, M. 1999. On the origin of species by sympatric speciation. *Nature* 400: 354-357.

Dieckmann, U., Doebeli, M., Metz, A.J. & Tautz, D. eds. 2004. Adaptive Speciation. Cambridge University Press, Cambridge.

Dobzhansky, T. 1937. Genetics and the origin of species. Columbia University Press, New York.

Douglas, M.R., Brunner, P.C. & Bernatchez, L. 1999. Do assemblages of *Coregonus* (Teleostei: Salmoniformes) in the Central Alpine region of Europe represent species flocks? 1999. *Mol. Ecol.* 8: 589-603.

Edelaar, P., Siepielski, A.M. & Clobert, J. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. *Evolution* 62: 2462-2472.

Erwin, D.H. 2008. Macroevolution of ecosystem engineering, niche construction and diversity. *Trends Ecol. Evol.* 23: 304–310.

Falconer, D.S. 1952. The problem of environment and selection. *Am. Nat.* 86: 293-298.

Felsenstein, J. 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution* 35: 124-138.

Fisher, R.A. 1930. The genetical theory of natural selection. Clarendon.

Funk, D.J., Filchak, K.E. & Feder, J.L. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. *Genetica* 116: 251-267.

Fussmann, G. F., Loreau, M & Abrams, P. A. 2007. Eco-evolutionary dynamics of communities and ecosystems. *Funct. Ecol.* 21: 465-477.

Futuyma, D.J. 2009. Evolution, 2nd edition. Sinauer Associates, Sunderland.

Futuyma, D.J. 2012. The origin of species by means of ecological selection. *Curr. Biol.* 23: R217-R219.

Gavrilets, S. 2004. Fitness landscapes and the origin of species. Princeton University Press, Princeton.

Gavrilets, S. & Losos, J. 2009. Adaptive radiation: Contrasting theory with data. *Science* 323: 732-737.

Geritz, S.A.H., Kidsi, E., Meszena, G. & Metz, J.A.J. 1998. Evolutionary singular strategies and the adaptive growth and branching of the evolutionary tree. *Evol. Ecol.* 12: 35-57.

Glor, R.E. 2010. Phylogenetic insights on adaptive radiation. *Annu. Rev. Ecol. Evol. S.* 41: 251-270.

Gomez-Mestre, I. & Buchholz, D.R. 2006. Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *P. Natl. Acad. Sci. USA* 103: 19021-19026.

Gould, S.J. 1989. Wonderful life; the Burgess shale and the nature of history. W.W. Norton, New York.

Grant, P.R. 1986. Ecology and evolution of Darwin's finches. Princeton University Press, Princeton.

Grant, P.R. & Grant, B.R. 2006. Evolution of character displacement in Darwin's finches. *Science* 313: 224–226.

Haldane, J.B.S. 1932. The causes of evolution. Princeton University Press, Princeton.

Harmon, L.J., Matthews, B., Des Roches, S., Chase, J.M., Shurin, J.B. & Schluter, D. 2009. Evolutionary diversification in stickleback affects ecosystem functioning. *Nature* 458: 1167–1170.

-
- Harmon, L.J., Schulte, J.A., Larson, A. & Losos, J.B. 2003. Tempo and mode of evolutionary radiation in iguanian lizards. *Science* 301: 961-964.
- Harrod, C., Mallela, J. & Kahilainen, K. 2010. Phenotype-environment correlations in a putative whitefish adaptive radiation. *J. Anim. Ecol.* 79: 1057-1068.
- Hendry, A.P. & Kinnison, M.T. 1999. The pace of modern life: Measuring rates of contemporary microevolution. *Evolution* 53: 1637-1653.
- Herre, A.W.C.T. 1933. The fishes of Lake Lanao: a problem in evolution. *Am. Nat.* 67: 154-162.
- Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J. & Wardle, D.A. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecol. Monogr.* 75: 3-35.
- Hudson, A.G., Vonlanthen, P., Müller, R. & Seehausen, O. 2007. Review: The geography of speciation and adaptive radiation in coregonines. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol.* 60: 111-146.
- Hudson, A.G., Vonlanthen, P. & Seehausen, O. 2011. Rapid parallel adaptive radiations from a single hybridogenic ancestral population. *P. R. Soc. B.* 278: 58-66.
- Hutchings, J.A. 2011. Old wine in new bottles: reaction norms in salmonid fishes. *Heredity* 106: 421-437.
- Hynes, R.A., Ferguson, A. & McCann, M.A. 1996. Variation in mitochondrial DNA and post-glacial colonization of north western Europe by brown trout. *J. Fish Biol.* 48: 54-67.
- Ito, H.C. & Dieckmann, U. 2007. A new mechanism for recurrent adaptive radiations. *Am. Nat.* 170: E96-E111.
- Jones, C.G., Lawton, J.H. & Shachak, M. 1994. Organisms as ecosystem engineers. *Oikos* 69: 373-386.
- Kahilainen, K.K. & Lehtonen, H. 2002. Brown trout (*Salmo trutta* (L.)) and Arctic charr (*Salvelinus alpinus* (L.)) as predators of three sympatric whitefish (*Coregonus lavaretus* (L.)) in the subarctic Lake Muddusjärvi. *Ecol. Freshw. Fish* 11: 158-167.
- Kahilainen, K.K., Lehtonen, H. & Könönen, K. 2003. Consequences of habitat segregation to growth rate of two sparsely rakered whitefish (*Coregonus lavaretus* (L.)) in a subarctic lake. *Ecol. Freshw. Fish* 12: 275-285.
- Kahilainen, K.K., Malinen, T., Tuomaala, A., Alajärvi, E., Tolonen, A. & Lehtonen, A. 2007. Empirical evaluation of phenotype-environment correlation and trait utility with allopatric and sympatric whitefish (*Coregonus lavaretus* (L.)) in subarctic lakes. *Biol. J. Linn. Soc.* 92: 561-572.
- Kahilainen, K.K., Siwertsson, A., Gjelland, K.O., Knudsen, R., Bohn, T. & Amundsen, P.-A. 2011. The role of gill raker variability in adaptive radiation of coregonid fish. *Evol. Ecol.* 25: 573-588.
- Kirkpatrick, M. & Barton, N.H. 1997. The strength of indirect selection on female mating preferences. *P. Natl. Acad. Sci. USA* 94: 1282-1286.
- Kirkpatrick, M. & Ravigné, V. 2002. Speciation by natural and sexual selection: Models and experiments. *Am. Nat.* 159: S22-S35.

- Kirschel, A.N.G., Blumstein, D.T. & Smith, T.B. 2009. Character displacement of song and morphology in African tinkerbirds. *P. Natl. Acad. Sci. USA* 106: 8256-8261.
- Kottelat, M. & Freyhof, J. 2007. Handbook of European freshwater fishes. Kottelat, Cornol, Switzerland; and Freyhof, Berlin.
- Lamarck, J.-B. 1809. Philosophie zoologique, ou, exposition des considérations relative à l'histoire naturelle des animaux. Paris.
- Landry, L., Vincent, W.F. & Bernatchez, L. 2007. Parallelism between limnological features and phenotypic evolution of lake whitefish dwarf ecotypes. *J. Evolution. Biol.* 20: 971-984.
- Lee, K.E. 1985. Earthworms: Their ecology and relation with soil and land use. London: Academic.
- Link, J. & Hoff, M.H. 1998. Relationship of Lake Herring (*Coregonus artedii*) gill raker characteristics to retention probabilities of zooplankton prey. *J. Freshw. Ecol.* 13: 55-65.
- Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D. & Wardle, D.A. 2001. Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science* 294: 804-808.
- Losos, J.B. 2010. Adaptive radiation, ecological opportunity and evolutionary determinism. *Am. Nat.* 175: 623-639.
- Losos, J.B., Creer, D.A., Glossip, D., Goellner, R., Hampton, A., Roberts, G., Haskell, N., Taylor, P. & Ettling, J. 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. *Evolution* 54: 301-305.
- Lu, G. & Bernatchez, L. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. *Evolution* 53: 1491-1505.
- Lucek, K., Sivasundar, A. & Seehausen, O. Distentangling the role of phenotypic plasticity and genetic divergence in contemporary ecotype formation during a biological invasion. In review in *Evolution*.
- Lundsgaard-Hansen, B., Vonlanthen, P., Hudson, A.G., Lucek, K. & Seehausen, O. Ecological speciation on a water depth gradient in Alpine whitefish. In preparation.
- Maan, M.E. & Seehausen, O. 2011. Ecology, sexual selection and speciation. *Ecol. Lett.* 14: 591-602.
- Malmquist, H.J., Snorrason, S.S., Skúlason, S., Jonsson, B., Sandlund, O.T. & Jonasson, P.M. 1992. Diet differentiation in polymorphic Arctic charr in Thingvallavatn, Iceland. *J. Anim. Ecol.* 61: 21-35.
- Matthews, B., De Meester, L., Jones, C., Ibelings, B., Bouma, T., Nuutinen, V., van de Koppel, J. & Odling-Smee, J. Making niche construction operational in the context of eco-evolutionary dynamics. In revision.
- Maynard-Smith, J. 1966. Sympatric speciation. *Am. Nat.* 109: 659-676.
- Mayr, E. 1942. Systematics and the origin of species from the viewpoint of a zoologist. Harvard University Press, Cambridge.
- Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge.

- McNeely, J.A., Miller, K.R., Reid, W.V., Mittermeier, R.A. & Werner, T.B. 1990. Conserving the World's Biological Diversity. IUCN, World Resources Institute, Conservation International, WWF-US and the World Bank: Washington, DC.
- Michel, M. 1996. Untersuchungen zur Nahrungsökologie von Grossfelchen im Vierwaldstättersee während des Sommerhalbjahres 1996. Master thesis EAWAG.
- Michel, A.P., Sim, S., Powell, T.H.Q., Taylor, M.S., Nosil, P. & Feder, J.F. 2010. Widespread genomic divergence during sympatric speciation. *P. Natl. Acad. Sci. USA* 121: 9724-9729.
- Millenium Ecosystem Assessment. 2005. Ecosystems and Human Well-being: Biodiversity synthesis. World resources institute.
- Miner, B.G., Sultan, S.E., Morgan, S.G., Padilla, D.K. & Relyea, R.A. 2005. Ecological consequences of phenotypic plasticity. *Trends Ecol. Evol.* 20: 687-692.
- Mookerji, N., Heller, C., Meng, H.J., Bürgi, R. & Müller, R. 1998. Diel and seasonal patterns of food uptake and prey selection by *Coregonus* sp. in re-oligotrophicated Lake Lucerne, Switzerland. *J. Fish Biol.* 52: 443-457.
- Nosil, P. 2012. Ecological speciation. Oxford University Press, Oxford.
- Nosil, P. & Crespi, B.J. 2006. Experimental evidence that predation promotes divergence in adaptive radiation. *P. Natl. Acad. Sci. USA* 103: 9090-9095.
- Odling-Smee, F.J., Laland, K.N. & Feldmann, M.W. 1996. Niche construction. *Am. Nat.* 147: 641-648.
- Odling-Smee, F.J., Laland, K.N. & Feldmann, M.W. 2003. Niche construction – the neglected process in evolution. Princeton University Press, Princeton.
- Ostbye, K., Amundsen, P.A., Bernatchez, L., Klemetsen, A., Knudsen, R., Kristoffersen, R., Naesje, T.F. & Hindar K. 2006. Parallel evolution of ecomorphological traits in the European whitefish *Coregonus lavaretus* (L.) species complex during postglacial times. *Mol. Ecol.* 15: 3983-4001.
- Paine, R.T. 1995. A conversation on refining the concept of keystone species. *Conserv. Biol.* 9: 962-964.
- Palkovacs, E.P., Marshall, M.C., Lamphere, B.A., Lynch, B.R., Weese, D.J., Fraser, D.F., Reznick, D.N., Pringle, C.M. & Kinnison, M.T. 2009. Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Philos. T. Roy. Soc. B.* 364: 1617–1629.
- Palkovacs, E.P. & Post, D.M. 2008. Eco-evolutionary interactions between predators and prey: can predator-induced changes to prey communities feedback to shape predator foraging traits? *Evol. Ecol. Res.* 10: 699-720.
- Pelletier, F., Garant, D. & Hendry, A.P. 2009. Eco-evlutionary dynamics. *Philos. T. Roy. Soc. B.* 364: 1483-1489.
- Pfennig, D.W. & McGee, M. 2010. Resource polyphenism increases species richness: a test of the hypothesis. *Philos. T. Roy. Soc. B.* 365: 577-591.
- Pfennig, D.W., Rice, A.M. & Martin, R.A. 2006. Ecological opportunity and phenotypic plasticity interact to promote character displacement and species coexistence. *Ecology* 87: 769-779.

-
- Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., Cruickshank, T., Schlichting, C.D. & Mosczek, A.P. 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol. Evol.* 25: 459-467.
- Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? *Trends Ecol. Evol.* 20: 481-486.
- Pigliucci, M., Murren, C.J. & Schlichting, C.D. 2006. Phenotypic plasticity and evolution by genetic assimilation. *J. Exp. Biol.* 209: 2362-2367.
- Post, D.M. & Palkovacs, E.P. 2009. Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. *Philos. T. Roy. Soc. B.* 364: 1629-1640.
- Post, D.M., Palkovacs, E.P., Schielke, E.G. & Dalton, S.I. 2008. Intraspecific phenotypic variation in a predator affects community structure and cascading trophic interactions. *Ecology* 89: 2019-2032.
- Presgraves, D.C. 2010. The molecular evolutionary basis of species formation. *Nat. Rev. Genet.* 11: 175-180.
- Price, T.D., Qvarnström, A. & Irwin, D.E. 2003. The role of phenotypic plasticity in driving genetic evolution. *P. R. Soc. B.* 270: 1433-1440.
- Price, T.D., Yeh, P.J. & Harr, B. 2008. Phenotypic plasticity and the evolution of a socially selected trait following colonization of a novel environment. *Am. Nat.* 172: S49-S62.
- Purvis, A. & Hector, A. 2000. Getting the measure of biodiversity. *Nature* 405: 212-219.
- Reznick, D., Butler IV, M.J. & Rodd, H. 2001. Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *Am. Nat.* 157: 126-140.
- Rice, W.R. & Hostert, E.E. 1993. Laboratory experiments on speciation – what have we learned in 40 years? *Evolution* 47: 1637-1653.
- Robinson, B.W. 2000. Trade offs in habitat-specific foraging efficiency and the nascent adaptive divergence of sticklebacks in lakes. *Behaviour* 137: 865-888.
- Robinson, B.W. & Parsons, K.J. 2002. Changing times, spaces and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Can. J. Fish. Aquat. Sci.* 59: 1819-1833.
- Robinson, B.W., Wilson, D.S., Margosian, A.S. & Lotito, P. 1993. Ecological and morphological polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). *Evol. Ecol.* 10: 1-10.
- Rogers, S.M., Gagnon, V. & Bernatchez, L. 2002. Genetically based phenotype-environment association for swimming behavior in lake whitefish ecotypes (*Coregonus clupeaformis* Mitchill). *Evolution* 56: 2322-2329.
- Rosenzweig, M.L. 1978. Competitive speciation. *Biol. J. Linn. Soc.* 10: 275-289.
- Rosenzweig, M.L. 1995. Species diversity in space and time. Cambridge University Press, Cambridge.
- Rueffler, C., Van Dooren, T.J.M., Leimar, O. & Abrams, P.A. 2006. Disruptive selection and then what? *Trends Ecol. Evol.* 21: 452-466.

-
- Rundle, H.D., Nagel, L., Boughmann, J.W. & Schluter, D. 2000. Natural selection and parallel speciation in sympatric sticklebacks. *Science* 287: 306-308.
- Rundle, H.D. & Nosil, P. 2005. Ecological speciation. *Ecol. Lett.* 8: 336-352.
- Sanderson, S.L., Cheer, A.Y., Goodrich, J.S., Graziano, J.D. & Callan, W.T. 2001. Crossflow filtration in suspension-feeding fishes. *Nature* 412: 439-441.
- Schlichting, C.D. 2004. The role of phenotypic plasticity in diversification. In Phenotypic plasticity: Functional and conceptual approaches (DeWitt, T. J. and Scheiner, S. M., eds.), pp. 191-200. Oxford University Press, Oxford.
- Schlichting, C.D. & Pigliucci, M. 1998. Phenotypic evolution – a reaction norm perspective. Sinauer Associates, Sunderland.
- Schluter, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology* 76: 82-90.
- Schluter, D. 1996. Ecological speciation in postglacial fishes. *Philos. T. Roy. Soc. B.* 351: 807-814.
- Schluter, D. 2000. The Ecology of Adaptive Radiation. Oxford University Press, Oxford.
- Schluter, D. 2000b. Ecological character displacement in adaptive radiation. *Am. Nat.* 156: S4-S16.
- Schluter, D. 2001. Ecology and the origin of species. *Trends Ecol. Evol.* 16: 372-380.
- Schluter, D. 2009. Evidence for ecological speciation and its alternative. *Science* 323: 737-741.
- Schluter, D. & McPhail, J.D. 1992. Ecological character displacement and speciation in sticklebacks. *Am. Nat.* 140: 85-108.
- Schmalhausen, I. 1949. Factors of Evolution. Blakiston, Philadelphia.
- Schoener, T.W. 2011. The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science* 331: 426-429.
- Seehausen, O. 2004. Hybridization and adaptive radiation. *Trends Ecol. Evol.* 19: 198-207.
- Seehausen, O. 2009. Speciation affects ecosystems. *Nature* 458: 1122-1123.
- Seehausen, O., Takimoto, G., Roy, D. & Jokela, J. 2008. Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Mol. Ecol.* 17: 30-44.
- Seehausen, O., van Alphen, J.J.M. & Witte, F. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* 277: 1808-1811.
- Servedio, M.R. & Noor, M.A.F. 2003. The role of reinforcement in speciation: Theory and data. *Annu. Rev. Ecol. Evol. S.* 34: 339-364.
- Sheppard, C.R.C., Davy, S.K. & Piling, G.M. 2009. The biology of coral reefs. Oxford University Press, Oxford.
- Simpson, G.G. 1953. The major features of evolution. Columbia University Press, New York.

- Siwertsson, A., Knudsen, R., Kahilainen, K., Praebel, K., Primicerio, R. & Amundsen, P.A. 2010. Sympatric diversification as influenced by ecological opportunity and historical contingency in a young species lineage of whitefish. *Evol. Ecol. Res.* 12: 929-948.
- Skulason, S., Snorrason, S.S. & Jonsson, B. 1999. Sympatric morphs, populations and speciation in freshwater fish with emphasis on Arctic charr. In: Magurran, A. E., May, R., eds. *Evolution of biological diversity*, pp. 70-92. Oxford University Press, Oxford.
- Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science* 236: 787-792.
- Sobel, J.M., Chen, C.F., Watt, L.R. & Schemske, D.W. 2009. The biology of speciation. *Evolution* 64: 295-315.
- Sol, D., Duncan, R.P., Blackburn, T.M., Cassey, P. & Lefebvre, L. 2005. Big brains, enhanced cognition, and response of birds to novel environments. *P. Natl. Acad. Sci. USA* 102: 5460-5465.
- Stal, L.J. 2000. Cyanobacterial mats and stromatolites. In Whitton BA, Potts M, eds. *The Ecology of Cyanobacteria: Their Diversity in Time and Space*. Kluwer Academic, London.
- Stearns, S.C. & Koella, J. 1986. The evolution of phenotypic plasticity in life-history traits: predictions for norm of reaction for age- and size-at-maturity. *Evolution* 40: 893-913.
- Steinmann, P. 1950. Monographie der schweizerischen Koregonen. Beitrag zum Problem der Entstehung neuer Arten. Spezieller Teil. *Schweiz. Z. Hydrol.* 12: 340-491.
- Streelman, J.T. & Danley, P. D. 2003. The stages of vertebrate evolutionary radiation. *Trends Ecol. Evol.* 18: 126-131.
- Suzuki, Y. & Nijhout, H.F. 2006. Evolution of a polyphenism by genetic accommodation. *Science* 311: 650-652.
- Svanbäck, R. & Eklöv, P. 2003. Morphology dependent foraging efficiency in perch: a trade-off for ecological specialization? *Oikos* 102: 273-284.
- Svärdson, G. 1949. The *Coregonus* problem. Rep. Inst. Freshw. Res. Dottingholm. 29: 89-101.
- Svarvar, P.O. & Müller, R. 1982. Die Felchen des Alpnersees. *Schweiz. Z. Hydrol.* 44: 295-314.
- Taylor, E.B. 1999. Species pairs of north temperate freshwater fishes: evolution, taxonomy, and conservation. *Rev. Fish Biol. Fisher.* 9: 299-324
- Trudel, M., Tremblay, A., Schetagne, R. & Rasmussen, J.B. 2001. Why are dwarf fish so small? An energetic analysis of polymorphism in lake whitefish (*Coregonus clupeaformis*). *Can. J. Fish. Aquat. Sci.* 58: 394-405.
- Udovic, D. 1980. Frequency-dependent selection, disruptive selection, and the evolution of reproductive isolation. *Am. Nat.* 116: 621-641.
- Via, S., Bouck, A.C. & Skillman, S. 2000. Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environment. *Evolution* 54: 1626-1637.
- Via, S. & Lande, R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39: 505-522.

- Vonlanthen, P., Bittner, D., Hudson, A.G., Young, K.A., Müller, R., Lundsgaard-Hansen, B., Roy, D., Di Piazza, S., Largiadèr, C.R. & Seehausen, O. 2012. Anthropogenic eutrophication drives extinction by speciation reversal in parallel adaptive radiations. *Nature* 482: 357-363.
- Vonlanthen, P., Roy, D., Hudson, A.G., Largiadèr, C.R., Bittner, D. & Seehausen, O. 2009. Divergence along a steep ecological gradient in lake whitefish (*Coregonus sp.*). *J. Evolution. Biol.* 22: 498-514.
- Wagner, C.E., Harmon, L.J. & Seehausen, O. 2012. Ecological opportunity and sexual selection together predict adaptive radiation. *Nature* 487: 366-370.
- Wainwright, P.C. 1994. Functional morphology as a tool in ecological research. In *Ecological morphology* (ed. P. C. Wainwright and S. M. Reilly), pp. 42-59. Chicago University Press, Chicago.
- Walker, J. A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasteroidae) body shape. *Biol. J. Linn. Soc.* 61: 3-50.
- West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20: 249-278.
- West-Eberhard, M.J. 2003. Developmental plasticity and evolution. Oxford University Press, Oxford.
- Wimberger, P.H. 1994. Trophic polymorphisms, plasticity, and speciation in vertebrates. In *Theory and application in fish feeding ecology* (eds D. J. Stouder, K. L. Fresh & R. J. Feller). University of South Carolina Press, Columbia.
- Woltereck, R. 1931. Wie entsteht eine endemische Rasse oder Art? *Biol. Zbl.* 51: 231-253.
- Wright, S. 1931. Evolution in mendelian populations. *Genetics* 16: 97-159.
- Wright, J.P., Jones, C.G. & Flecker, A.S. 2002. An ecosystem engineer, the beaver, increases species richness at the landscape scale. *Oecologia* 132: 96-101.
- Wund, M.A., Baker, J.A., Clancy, B., Golub, J.L. & Foster, S.A. 2008. A test of the flexible stem model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. *Am. Nat.* 172: 449-462.
- Yoder, J.B., Clancey, E., Des Roches, S., Eastman, J.M., Gentry, L., Godsoe, W., Hagey, T.J., Jochimsen, D., Oswald, B.P., Roberston, J., Sarver, B.A.J., Schenk, J.J., Saer, S.F. & Harmon, L.J. 2010. Ecological opportunity and the origin of adaptive radiations. *J. Evolution. Biol.* 23: 1581-1596.
- Yoshida, T., Jones, L. E., Ellner, S. P., Fussmann, G. F. & Hairston, N. G. 2003. Rapid evolution drives ecological dynamics in a predator-prey system. *Nature* 424: 303-306.

Chapter 2

Adaptive plasticity and genetic divergence in feeding efficiency during parallel adaptive radiation of whitefish (*Coregonus spp.*)

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Abstract

Parallel phenotypic divergence in replicated adaptive radiations could either result from parallel genetic divergence in response to similar divergent selection regimes, or from equivalent phenotypically plastic response to the repeated occurrence of contrasting environments. In postglacial fish replicated divergence in phenotypes along the benthic-limnetic habitat axis is commonly observed. Here we use two benthic-limnetic species pairs of whitefish from two Swiss lakes, raised in a common garden design, with reciprocal food treatments in one species pair, to experimentally measure whether feeding efficiency on benthic prey has a genetic basis or whether it underlies phenotypic plasticity (or both). To do so we offered experimental fish mosquito larvae, partially buried in sand, and measured multiple feeding efficiency variables. Our results reveal both, genetic divergence as well as phenotypically plastic divergence in feeding efficiency, with the phenotypically benthic species raised on benthic food being the most efficient forager on benthic prey. This indicates that both, divergent natural selection on genetically heritable traits and adaptive phenotypic plasticity, are likely important mechanisms driving phenotypic divergence in adaptive radiation.

Introduction

Parallel adaptive radiations of closely related taxa often exhibit a repeated occurrence of similar ecotypes in similar niches (Schluter, 2000). Such parallel ecotypic differentiation is often attributed to similar evolutionary responses to divergent selection between contrasting environments (Schluter & Nagel, 1995; Schluter, 2000; Barrett & Schluter, 2008), which assumes a genetically heritable basis of the traits characterizing the adaptive radiation. On the other hand, adaptive radiation in general and replicated radiation in particular can be facilitated by phenotypic plasticity (Pfenning et al., 2010). The evolution of similar solutions to the same problems (the repeated evolution of similar phenotypes in different radiations) can be explained by ancestral developmental plasticity (Pfenning et al., 2010). Importantly, the ancestral plasticity hypothesis does not negate the importance of natural selection for the fixation of phenotypic differences, rather it proposes that plasticity explains the origin of those differences (West-Eberhard 2003). Neither are adaptation through divergent evolution and adaptation through phenotypic plasticity mutually exclusive and both could act in concert during the origins of adaptive radiations.

There is growing evidence for fitness trade-offs between differentiated morphs or species inhabiting distinct ecological environments across such a broad range of taxa as plants, snails, insects and fish (Boulding & Van Alstyne, 1993; Schluter, 1995; Schluter, 2000; Via et al., 2000; Rundle, 2002; Nosil, 2004). Such trade-offs suggest that the genetically heritable divergence is a result of divergent natural selection and support the idea that natural selection plays an important role in species formation (Schluter, 2000; Via et al., 2000; Rundle, 2002; Nosil, 2004). But some of these reciprocal transplant experiments were not designed to determine whether genetically heritable or phenotypically plastic divergence in early development caused differential fitness in contrasting environments (Boulding & Van Alstyne, 1993; Schluter, 1995). Indeed, there is considerable empirical support for the importance of phenotypic plasticity in diversification of various taxa: Adaptive radiations such as that of Darwin's finches, cichlid fish, stickleback and *Anolis* lizards all display

variable levels of phenotypic plasticity in traits characterizing these radiations (Grant, 1986; Day et al., 1994; Losos et al., 2000; Bouton et al., 2002; West-Eberhard, 2003; Wund et al., 2008). Furthermore it has been suggested that phenotypic plasticity increases species richness of a clade, most likely by facilitating adaptive diversification and by reducing the risk of extinction (Pfennig & McGee, 2010).

Northern postglacial fish provide striking examples of adaptive radiations, but the mechanisms of inheritance in these radiations, in particular the relative importance of phenotypic plasticity and genetic predisposition in behavior, are not fully understood. Adaptation to alternative trophic niches has been repeatedly observed in these taxa and has been proposed to be an important driver in their diversification (Skúlason & Smith, 1995; Schluter, 2000). Typically, a split along the benthic (lake bottom) to limnetic (open water) habitat axis is observed, which is accompanied by divergence in morphology and trophic ecology: Limnetic morphs/species are usually planktivorous, rather slender, smaller, with a narrower mouth and longer and more numerous gill rakers, whereas benthic morphs/species are more benthivorous, more deep bodied, larger, with a larger mouth and fewer and shorter gill rakers (Robinson & Wilson 1994; Smith & Skúlason, 1996; Schluter, 2000). Taxa displaying this benthic-limnetic-split can be found e.g. in threespine stickleback, rainbow smelt, brown trout, Arctic charr, *Prosopium* and *Coregonus* (Smith & Skúlason, 1996; Taylor, 1999; Schluter, 2000). A genetic basis for shape divergence and differences in feeding efficiency and swimming behavior has been shown in some of these morphs/species (Robinson, 2000; Adams & Huntingford, 2002; Rogers et al. 2002; Klemetsen et al. 2006). In other cases it has been shown that plasticity can affect morphological divergence (Robinson & Parsons 2002), but plasticity in feeding behavior and efficiency have only rarely been measured (but see Day & McPhail, 1996). Experiments specifically designed to measure phenotypically plastic and genetically heritable components in morphology of benthic vs. limnetic ecotypes, found evidence for the presence of both (Day et al., 1994; Adams & Huntingford, 2004; Proulx & Magnan, 2004). However, to our knowledge no study has yet measured the effects of plasticity and of genetic divergence on morphology as well as on feeding behavior in one and the same experiment, although this is important to identify the traits that affect feeding efficiency.

Whitefish species complexes might fulfill the four criteria that define an adaptive radiation (Schluter 2000), i.e. common ancestry (Bernatchez & Dodson 1994; Pigeon et al. 1997 ; Ostbye et al. 2005a ; Hudson et al. 2011), fast speciation (Bernatchez et al. 1999; Ostbye et al. 2006, Hudson et al. 2011), phenotype-environment correlation (Harrod et al. 2010) and trait utility (Bernatchez 2004; Kahilainen et al. 2007; 2011); and thus represent a good model system to study mechanisms of diversification in adaptive radiations. Sympatric whitefish morphs/species (we adopt species hereafter) are morphologically most strongly divergent in number of gill rakers and in adult body size (Steinmann, 1950; Svårdson 1979; Lindsey 1981; Vonlanthen et al. 2012)), traits likely involved in foraging, which have also been shown to probably be under divergent selection (Bernatchez, 2004; Ostbye et al., 2005b; Rogers & Bernatchez, 2007). Speciation involves divergence along the benthic-limnetic habitat axis as described above (Bernatchez et al., 1996; Lu & Bernatchez, 1999; Ostbye et al., 2006; Landry et al., 2007), but adaptive radiations with more than two species frequently occurred in

European whitefish (Steinmann 1950; Svärdson 1979; Hudson et al., 2007; Siwertsson et al. 2010; Vonlanthen et al. 2012). Phenotype-environment correlations between traits involved in foraging and niche utilization in sympatric whitefish has been well documented and suggests an important role of trophic adaptation in the commonly observed benthic-limnetic split of these fish (Bernatchez et al. 1999; Amundsen et al. 2004; Harrod et al. 2010). Similarly, suggestive evidence for trait utility has been observed in sympatric whitefish, indicating that a higher number of gill rakers likely facilitates feeding on smaller zooplankton (Kahilainen et al. 2007; 201). However, experimental evidence for divergence in feeding efficiency between whitefish species as well as for trait utility is lacking and remains to be tested.

In the large subalpine lakes of Switzerland, multiple whitefish radiations constituting more than 40 different species originated after the last glacial maximum 15000 years ago (Steinmann, 1950; Hudson et al., 2011; Vonlanthen et al. 2012). This impressive whitefish species diversity consists of at least five different adaptive radiations that evolved in parallel (Hudson et al., 2011). Such young and replicated radiations offer excellent opportunities to test for the importance of driving forces and mechanisms of diversification. Here, we raised two species pair of benthic-limnetic whitefish from two Swiss lakes in a common garden design, with reciprocal food treatment in one species pair, to experimentally measure whether feeding efficiency divergence between them has a genetic basis, if phenotypic plasticity can modify feeding efficiency and how feeding efficiency is affected by variation in phenotypes (fish body size and shape). If variation in feeding efficiency was entirely genetically determined we expected to not find any differentiation between the same species raised on different food, but differentiation between the different species independent of the food they were raised on (Figure 1a). If, on the other hand, variation in feeding efficiency was entirely the result of phenotypic plasticity we expected to find no differentiation between the different species when raised on the same food but differentiation between the same species raised on different food (Figure 1b). If feeding efficiency was affected by both, genetic divergence and phenotypic plasticity, we expected to find the strongest difference between the benthic species raised on benthic food and the limnetic species raised on limnetic food, while the other treatments would be expected to be intermediate (Figure 1c). In scenario 1b and 1c we assumed plasticity to be adaptive such that feeding efficiency on benthic food would be higher for fish raised on benthic food than for fish raised on limnetic food. For all hypothetical scenarios outlined above, we for simplicity further assumed that the strength of plasticity does not differ between species. Based on the above outlined existence of empirical evidence for the importance of both, plasticity and genetic divergence in morphology and feeding efficiency of north temperate fish, we predicted that variation in feeding efficiency would have both, a genetic and an environmentally induced component.

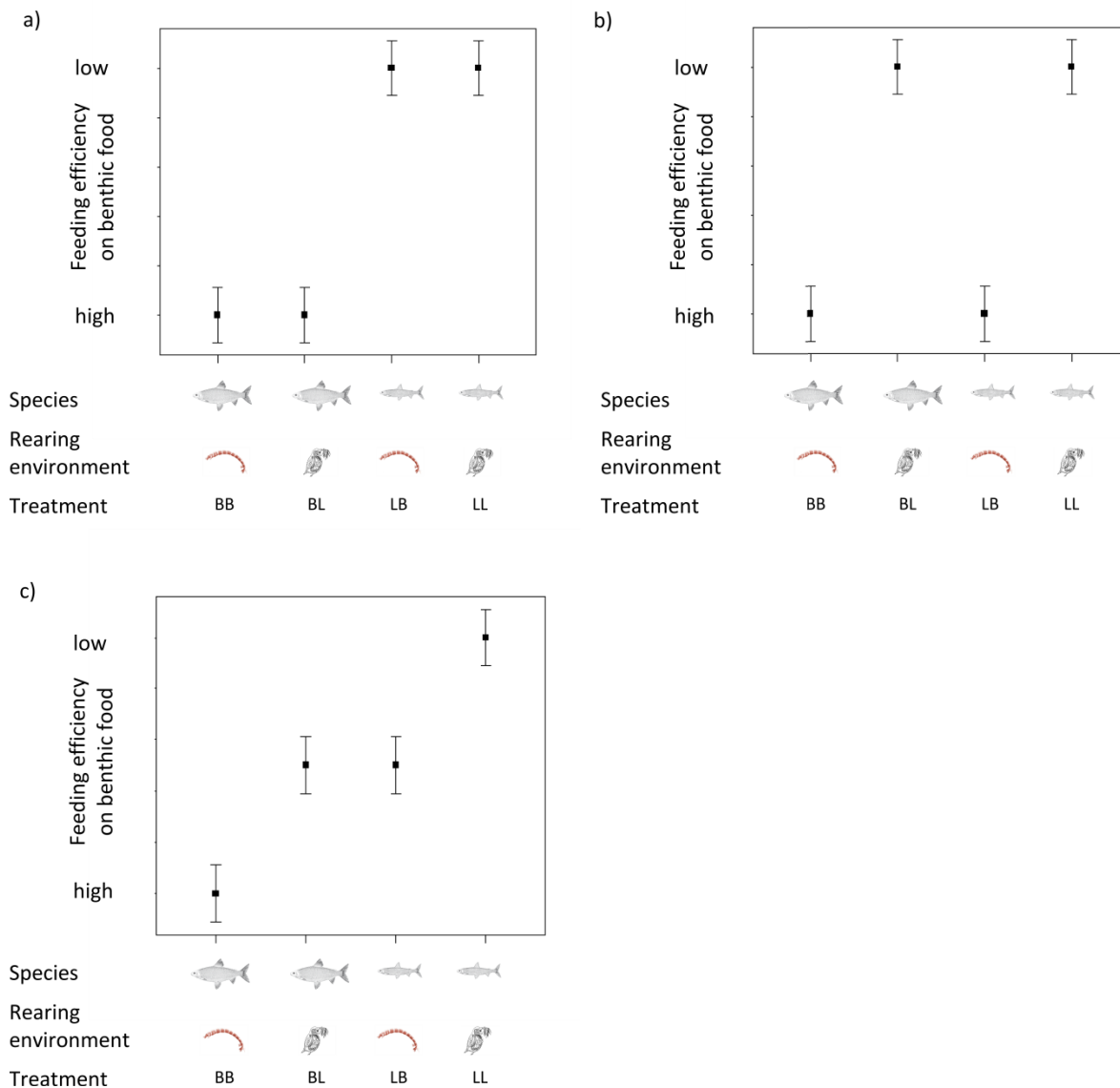


Figure 1: Hypothetical scenarios for a) variation in feeding efficiency, if it was entirely under genetic control, b) variation in feeding efficiency, if it was entirely the results of phenotypic plasticity, c) variation in feeding efficiency, if it would have similar genetic and plastic components. Plasticity effects in panel b) and c) are adaptive. In all panels, plasticity is assumed to be equally strong in both species. Shown are the treatments on the x-axis and a hypothetical feeding efficiency value on the y-axis. Error bars are hypothetical standard deviations. In the top line of the legend to the x-axis a large fish corresponds to the benthic species and a small fish to the limnetic species; in the line below a mosquito larvae corresponds to a benthic raising environment and a zooplankton item to a limnetic raising environment; in the lowest line the first letter stands for the species (B=benthic, L=limnetic) and the second letter for the raising environment (B=benthic, L=limnetic).

Materials & Methods

Study species

Whitefish from two subalpine lakes, Lake Thun and Lake Lucerne, were used. In each of these lakes at least five different whitefish species have been documented based on phenotypic and genetic data (Svarvar & Müller 1982; Bittner, 2009; Vonlanthen et al. 2012), and these represent two independently evolved radiations (Hudson et al. 2011). We studied two species, a benthic and a limnetic ecotype, from each of the lakes, namely *C. sp. "Bodenbalchen"* and *C. zugensis* from Lake Lucerne and *C. sp. „Balchen"* and *C. albellus* from Lake Thun. We chose to focus on these species, because they phenotypically correspond to the commonly observed benthic-limnetic split of north temperate fish (Schluter 2000). *C. sp. "Bodenbalchen"* and *C. sp. "Balchen"* correspond to the benthic phenotypes, they grow fast and reach maximum sizes of 600 and 450 mm, respectively, their mean gill raker number is 29.4 (22-34) and 30.5 (22-33) and both of them spawn in very shallow water of approximately 2-5 m depth (Steinmann 1950; Vonlanthen et al. 2012). *C. zugensis* and *C. albellus* correspond to the limnetic phenotype, they grow slow and reach a maximum size of 300 mm, their mean gill raker number is 38.8 (34-43) and 38.1 (35-44) respectively and both spawn in 25 m and deeper (Steinmann 1950; Vonlanthen et al. 2012). Both species pairs are genetically clearly differentiated from each other (Vonlanthen et al. 2012). Gut content analysis of Lake Lucerne species showed that *C. zugensis* almost exclusively feeds on zooplankton, while the diet of *C. sp. "Bodenbalchen"* is more benthic (Michel, 1996; Mookerji et al. 1998). Although gut content data for fish from Lake Thun is lacking, evidence for divergence in resource use in between whitefish species with different gill raker numbers is abundant (i. e. Bernatchez et al. 1999; Amundsen et al. 2004; Harrod et al. 2010). This suggests that the studied whitefish species from Lake Thun also differ in resource use in nature.

Breeding and raising of fish

Parental fish were caught in winter 2006, during their spawning time on their respective spawning grounds, to breed experimental fish. The benthic species from the two lakes were caught in approximately 2-5 m depth with gill nets having 38-45 mm mesh sizes. The limnetic species were caught in 30-50 m depth using gill nets of 25-28 mm mesh sizes. By doing target fishing on the extreme ends of whitefish spawning depth gradients and by visual inspection of the catches, we made sure that pure individuals belonging to a particular species and no hybrids were caught, although hybridization has not been uncommon during eutrophication of Swiss lakes (Bittner et al. 2010; Vonlanthen et al. 2012). From the catches five females and five males were randomly selected from each species. Eggs and sperm were striped in the lab and eggs of all five females were mixed. The eggs were fertilized simultaneously with sperm from the five males, ideally resulting in 25 half-sib families per species. All fish were fed ad libidum once a day, except on Sundays. All juvenile fish were fed with zooplankton for approximately one year. Zooplankton was collected daily from Lake Lucerne by trawling a plankton-net with a mesh size of 250 µm in a depth of around 8 m. Most common zooplankton taxa were *Daphnia*, Copepods, *Chydorus* and *Bosmina*, which ranged from a size of 250 µm to approximately 5 mm. As soon as fish were large enough to be fed with mosquito larvae (*Chironomus plumosus*), food of all juveniles from Lake Thun was switched to mosquito larvae and the juveniles from Lake Lucerne were subsequently raised in a split family design with reciprocal

food treatments. Frozen mosquito larvae were used to simulate a benthic feeding environment and zooplankton was used to simulate a limnetic feeding environment. This resulted in four different treatments for fish from Lake Lucerne: Fish belonging to the benthic species raised on benthic food *BB* and raised on limnetic food *BL*; fish belonging to the limnetic species raised on benthic food *LB* and raised on limnetic food *LL*; and two treatments for Lake Thun, *BB* and *LB*. Each treatment was distributed over two raising aquaria, each with a volume of 120*71*50 cm for fish from Lake Lucerne and of 120*142*50 cm for fish from Lake Thun. A flow through system (~ 2.5 l/min) with lake water was used. Water temperature during raising varied over the seasons and ranged from 6 to 15 ° C (temperature fluctuations were much less pronounced in experiments, as no experiments were done in winter, see below). Illumination was provided with a Cool White T 8 light tube with 5200 LM and with 12 h day and 12 h night rhythm. Initially each aquarium contained 100 individuals. One raising aquarium of the *LB* treatment from Lake Thun was lost due to a technical accident. As a consequence, the limnetic species of Lake Thun was raised in one aquarium only. Mortalities in aquaria of Lake Thun fish were: $BB_{AQ1}=0.03$; $BB_{AQ2}=0.07$; $LB_{AQ1}=0.1$. In aquaria of Lake Lucerne fish they were: $BB_{AQ1}=0.06$; $BB_{AQ2}=0.07$; $BL_{AQ1}=0.06$; $BL_{AQ2}=0.2$; $LB_{AQ1}=0.05$; $LB_{AQ2}=0.06$; $LL_{AQ1}=0.02$; $LL_{AQ2}=0.09$. When densities of fish diverged through time between raising aquaria, food provisioning was adjusted by eye. At the end of the raising time, fish from the same treatment, which were raised in different aquaria, were consequently never significantly different in size (t-test: the smallest observed p-value = 0.07 for Lake Lucerne fish of the *LL* treatment).

Two months before the trials started and for the duration of the trials we switched the food environments in the holding tanks once every week to allow all fish to familiarize with both food-types and avoid food recognition or other short-term learning effects to affect our results. The switching of food was paused from October 2009 to May 2010, as no experiments were done in this time period.

Experimental set-up

Experimental aquaria, each with a size of 55*142*40 cm, were divided lengthwise into two compartments using a Plexiglas wall, resulting in one compartment with a size of 33*142*40 cm and the other compartment with a size of 22*142*40 cm. Water temperature varied between 12 – 15 ° C over the entire experimental phase, and was similar between experimental aquaria at each day. The water flow in the aquaria was paused from the moment the fish was introduced into the tank until the experiment was finished. Illumination was the same as during rearing of the fish. The front window of the aquaria was covered with a reflecting mirror foil to prevent fish from seeing the observer, to avoid observer-induced behavioral changes. The bottom of the aquaria was covered with a layer of quartz sand. The trial was conducted in the larger front compartment, where one fish was tested at one time. Additionally two fish were put in the smaller back compartment and were left there for the entire duration of the trials to stimulate natural behavior of the single experimental fish in the front compartment (single isolated individuals did not display natural behavior).

Trials were performed from July 2009 to August 2010. Which treatment was tested at which day was randomized for all fish tested in 2009 and for all fish from Lake Thun. Additionally a low number of

fish from Lake Lucerne (7 individuals from the LB and 1 from the BL treatment, see Table 1) was tested in 2010 to increase the sample size in these treatments. Despite this, the effect of time was unlikely to bias our findings, as time (in days after the first trial was done) was overall not different between any treatment comparison in any of the lakes. To make sure time did not affect our results, we also included the factor year in generalized linear model analysis to control for potential time effects.

Table 1: Sample sizes and body size variation per treatment. Four treatments were available for fish from Lake Lucerne and two treatments were available for fish from Lake Thun. The first letter of the treatment refers to genetic background of the fish and the second letter of the treatment refers to their food during raising. B stands for benthic and L stands for limnetic. The first number corresponds to fish tested in 2009 and the second number to fish tested in 2010. N_{total} includes all fish. These fish were used to test for divergence between Treatments in length. N_{shape} includes all fish for which shape data was available. These fish were used to test for divergence between treatments in shape. N_{ph} (ph= post hoc) includes all fish that started feeding, including those for which shape data was missing. These fish were used in post-hoc tests for associations of feeding efficiency with each of the explanatory variables except shape. N_{GLM} includes all fish that started feeding and for which shape data was available. These fish were used in the GLMs. Fish without shape data had to be excluded from the GLMs even when no shape variable was kept for the most likely model, because AIC is only comparable between models with the same number of observations. In the last column we report mean length of fish (mm) from a particular treatment with the respective standard deviations (with years separated using “/”).

Lake	Treatment	Genetics	Environment	Ntotal	Nshape	Nnp	NGLM	Mean length
Lucerne	BB	Benthic	Benthic	23/0	22/0	23/0	22/0	160 (13)
	BL	Benthic	Limnetic	21/1	20/1	21/1	20/1	151 (17)/186 (0)
	LB	Limnetic	Benthic	17/7	17/7	16/7	16/7	141 (14)/139(24)
	LL	Limnetic	Limnetic	30/0	28/0	26/0	24/0	133 (12)
	Total			91/8	87/8	86/8	82/8	
Thun	BB	Benthic	Benthic	10/7	10/7	10/7	10/7	144 (21)/154(14)
	LB	Limnetic	Benthic	10/7	10/7	10/7	10/7	139 (17)/135(9)
	Total			20/14	20/14	20/14	20/14	
Lake	Treatment	Genetics	Environment	N_{total}	N_{shape}	N_{np}	N_{GLM}	Mean length
Lucerne	BB	Benthic	Benthic	23/0	22/0	23/0	22/0	160 (13)
	BL	Benthic	Limnetic	21/1	20/1	21/1	20/1	151 (17)/186 (0)
	LB	Limnetic	Benthic	17/7	17/7	16/7	16/7	141 (14)/139(24)
	LL	Limnetic	Limnetic	30/0	28/0	26/0	24/0	133 (12)
	Total			91/8	87/8	86/8	82/8	
Thun	BB	Benthic	Benthic	10/7	10/7	10/7	10/7	144 (21)/154(14)
	LB	Limnetic	Benthic	10/7	10/7	10/7	10/7	139 (17)/135(9)
	Total			20/14	20/14	20/14	20/14	

Approximately 48 hours before a trial, the experimental fish was introduced into the experimental aquarium and was not fed until the trial started, to increase its motivation to feed. When an experiment started, two petri dishes filled with quartz sand, each containing 10 partially buried but well visible mosquito larvae, were deposited on the bottom using threads to let them down. As soon as the petri dishes were placed on the bottom, the experimental fish was videotaped until all the 20 mosquito larvae were eaten. Fish that did not start feeding within an hour were removed and were not re-used in this experiment (in total 5, all from the limnetic species from Lake Lucerne: 4 LL and 1 LB). All fish that started feeding ate all the larvae within less than one hour after first feeding. After each trial the fish was removed from the experimental aquaria, was anesthetized, total length and weight were measured and a picture from the left side of the body was taken for shape analysis. Photos were not available for four fish used in the experiments due to a technical problem with a storage device (see Table 1).

Behavioral measurements

Three variables related to feeding efficiency were measured from the video tapes, time to first feeding, time to food depletion, and the number of unsuccessful attacks. Time to first feeding was the time until a fish started feeding after the petri dishes were placed at the bottom of the experimental aquaria. Time to food depletion was the time a fish needed to eat all twenty larvae, measured from the moment it started feeding. As all fish that started feeding ate all larvae, time to food depletion was equivalent to a feeding rate. The number of unsuccessful attacks was the number of targeted attacks a fish made that did not yield a mosquito larva (because it couldn't grab it/lost it immediately after grabbing it). Because all fish were given the same number of larvae, this measure was equivalent to food capture efficiency. Time to first feeding was related to the ability to detect food, the motivation to feed on it and maybe also searching efficiency, while time to food depletion was related to a combination of searching efficiency, food capture efficiency and handling time and the number of unsuccessful attacks represents food capture efficiency. For all feeding efficiency variables, a lower value indicates a higher efficiency.

Shape measurement

Overall body shape variation was quantified using geometric morphometrics methods (Bookstein, 1991). Fourteen homologous landmarks distributed over the whole fish body, were selected based on standard landmark description and previous analysis of *Coregonus* body shape variation (Zelditch et al., 2004; Vonlanthen et al., 2009). Landmarks were set using the software TPSDIG (Rohlf; 2006). Nonshape variation, such as variation in location and orientation, was removed using Generalized Procrustes superimposition (Rohlf & Slice, 1990). Shape variables (x-y-coordinates of individual landmarks) for each individual were then generated using the thin-plate-spline equation (Bookstein, 1991).

Size correction was done by regression of each shape variable against fish size to remove variation due to allometry (Loy et al., 1998). Residuals were then used for further analysis. As the allometric relationships differed between lakes but not between treatments within lakes, size correction and further analysis of morphometric data was done separately for the two lakes, but pooled for the

treatments within lakes. A Principal Component Analysis was performed to display the major axes of shape variation. All morphometric analyses, including size corrections, were performed as implemented in MORPHOJ v.1.02H (Klingenberg, 2011).

Data analysis

Differentiation in Shape and Growth

To test whether size or shape differed significantly between two treatments a Wilcoxon Rank Sum Test was used, because traits were not always normally distributed (Lehmann, 1975). If four treatments were compared, a Kruskal Wallis ANOVA was used (Kruskal & Wallis, 1952). Additionally fish length was compared between treatments and years in two ANOVAs (one per lake) including treatment and year as explanatory variables (residuals of the ANOVAs were normally distributed indicating that assumptions were met). These statistical tests were performed using R v. 2.13.0 (R Development Core Team 2010).

Differentiation in feeding efficiency

Generalized linear models were used to test for associations of feeding efficiency variables with species identity of a fish (referred to as species), with food environment (referred to as environment), PC1 and PC2 of body shape (referred to as PC1 and PC2, respectively), total length of a fish (referred to as length) and the year the experiment was performed (referred to as year). One GLM was calculated for each lake and for each response variable. The error distribution with the best structural fit of the data to the model was chosen (Burnham & Anderson, 1998), which was a gaussian distribution for time to food depletion (after a log transformation for Lake Lucerne and a square root transformation for Lake Thun), a negative binomial distribution for time to first feeding and a quasi poisson distribution for number of unsuccessful attacks. For gaussian error distribution we used the identity link function, for the quasi poisson distribution we used the log link function and for negative binomial error distribution we used the logit link function as implemented in R (Bolker et al. 2008; R Development Core Team 2010). The initial model included all potential explanatory variables as well as an interaction of species and environment. A backward elimination model selection approach based on AIC was then used to find the model that best explained the variance in the data (Burnham & Anderson, 1998), while always retaining the main effects (species and environment). If necessary, an AICc instead of an AIC was calculated to correct for low sample sizes ($n < 40$, Burnham & Anderson, 1998). QAIC, which is an approximation to AIC, was calculated, when a quasi poisson error distribution was used, because AIC cannot be calculated when using this error distribution (see Table 2) (Lebreton, 1992; Burnham & Anderson, 1998). We compared models using AIC, Akaike Weights (w_i) and evidence ratios (L ratio) (Burnham & Anderson, 1998). In the final model the relationship between residuals and the fitted values was visually checked to ensure normal residuals and similar variance over the fitted values (Zuur et al., 2009). As differences in AIC between the most likely and the second most likely model were sometimes small (< 2 , Burnham & Anderson, 1998), the results of the second most likely model were also examined, but they are not reported as these models yielded similar results and interpretation.

In multivariate analysis two or more explanatory variables influence each other, when they share information. This can lead to different estimated effects of a variable depending on which co-variables are included. Thus univariate post hoc comparisons of the feeding efficiency measure and the variables retained in the final models were performed, if the most likely model contained more than one explanatory variable. As Post hoc tests for species, environment and year we calculated Wilcoxon Rank Sum Tests, while Spearman Rank Tests were calculated as post hoc tests for PC2 and length (Lehmann, 1975; Lehmann & D'Abrera, 1998). For post hoc tests for species and environment for Lake Lucerne we applied a random sampling approach, because to compare the two species without confounding the comparison by effects of the raising environment (or vice versa), it was necessary that both species contained the same numbers of fish raised in each of the two environments. Therefore an equal number of fish from all four treatments was needed for these comparisons (N_{ph} ranges from 22 to 26, see Table 1). Equal numbers per treatment were achieved by randomly sub-sampling the number of fish in a particular treatment 1000 times to the same sample size in the treatment with the smallest sample size. Subsequently fish from the same species but different rearing environment were pooled to calculate differences between species (or vice versa). For each pooled random sample, Wilcoxon Rank Sum Tests were performed and test statistics were averaged.

All generalized linear model statistics and post hoc comparisons were performed using R v. 2.13.0 (R Development Core Team, 2010). Analyses based on a negative binomial distribution were performed using the packages MASS in R v. 2.13.0 (Venables & Ripley, 2002). All graphs visualizing the models were created using the package Gplots in R v. 2.13.0.

Results

We compared fish feeding efficiency (time to first feeding, time to food depletion and the number of unsuccessful attacks) and fish morphology (length and shape) between different raising aquaria within treatment. As only one out of 24 comparisons was significant (less than expected by chance) and it was further no more significant after Bonferroni correction (the lowest p-value=0.014; critical p-value after Bonferroni correction = 0.002), we pooled aquaria of the same treatments for all analyses.

Differentiation in size and shape

Individual fish sizes ranged from 95 mm to 186 mm for Lake Lucerne: The BB fish were largest, the BL fish second largest, the LB were second smallest and the LL fish were smallest (Table 1). These between treatment differences in size were significant in an ANOVA including treatment ($n=99$; F-ratio=15.9, $p<0.001$) and year ($n=99$, F-ratio=0.39, $p=0.53$) as explanatory variables. In Lake Thun fish sizes ranged from 112 to 187 and there was a trend for increased size of the benthic species ($n=34$, F-ratio=2.89, $p=0.09$), while fish from the different years did not differ significantly in size ($n=34$, F-ratio=0.23, $p=0.64$). Pairwise post-hoc tests for size differences between the treatments reveal plasticity and heritable differences in size (Supplementary Table 1a). PC1 of shape accounted for 31 % of shape variation in Lake Lucerne and for 38% in Lake Thun. PC2 accounted for 24% of shape variation in Lake Lucerne and 15% in Lake Thun. Other PC scores are not included as they neither

differentiated between treatments nor were associated with any measured feeding efficiency variable, and the percentage of explained variance was rather low ($< 11\%$ in Lake Thun, $< 8\%$ in Lake Lucerne). PC1 did not differ between treatments in either of the lakes (not shown). But the four treatments of Lake Lucerne fish did significantly differ in PC2 (Kruskal-Wallis chi-squared=8.7, d.f.=3, $p=0.03$). Pairwise post hoc tests between treatments indicate that shape divergence mainly arises as a consequence of genetic differences between species and not as a result of phenotypic plasticity (Supplementary Table 1b). PC2 was lower in the benthic species, corresponding to more sub-terminal mouths in this species (see Figure 4d). In Lake Thun the two species did not differ in PC2 (Wilcoxon Rank Sum Test: $n=34$, $W=135$, $p=0.76$, Supplementary Table 1b).

Differentiation in Feeding Efficiency

i) Lake Lucerne

All three measures of feeding efficiency revealed that the BB fish were most efficient and the LL fish were least efficient in feeding on benthic insect larvae (Figure 2). The BL and the LB fish were intermediate between fish from the BB and LL treatments in all feeding efficiency variables. Results from Kruskal-Wallis ANOVA show that these between treatment differences were significant for time to first feeding and time to food depletion; while there was a trend for the number of unsuccessful attacks (Supplementary Table 2). Pairwise post-hoc tests for feeding efficiency differences between treatments indicate both, a genetic basis as well as phenotypic plasticity, in feeding efficiency divergence (Supplementary Table 2). All feeding efficiency variables were negatively correlated with fish length (Figure 2), indicating that larger fish were generally more efficient. However, these correlations were only significant over all four treatments and except from one exception not significant within treatment (Foraging Time in the BB treatment, Supplementary Table 3).

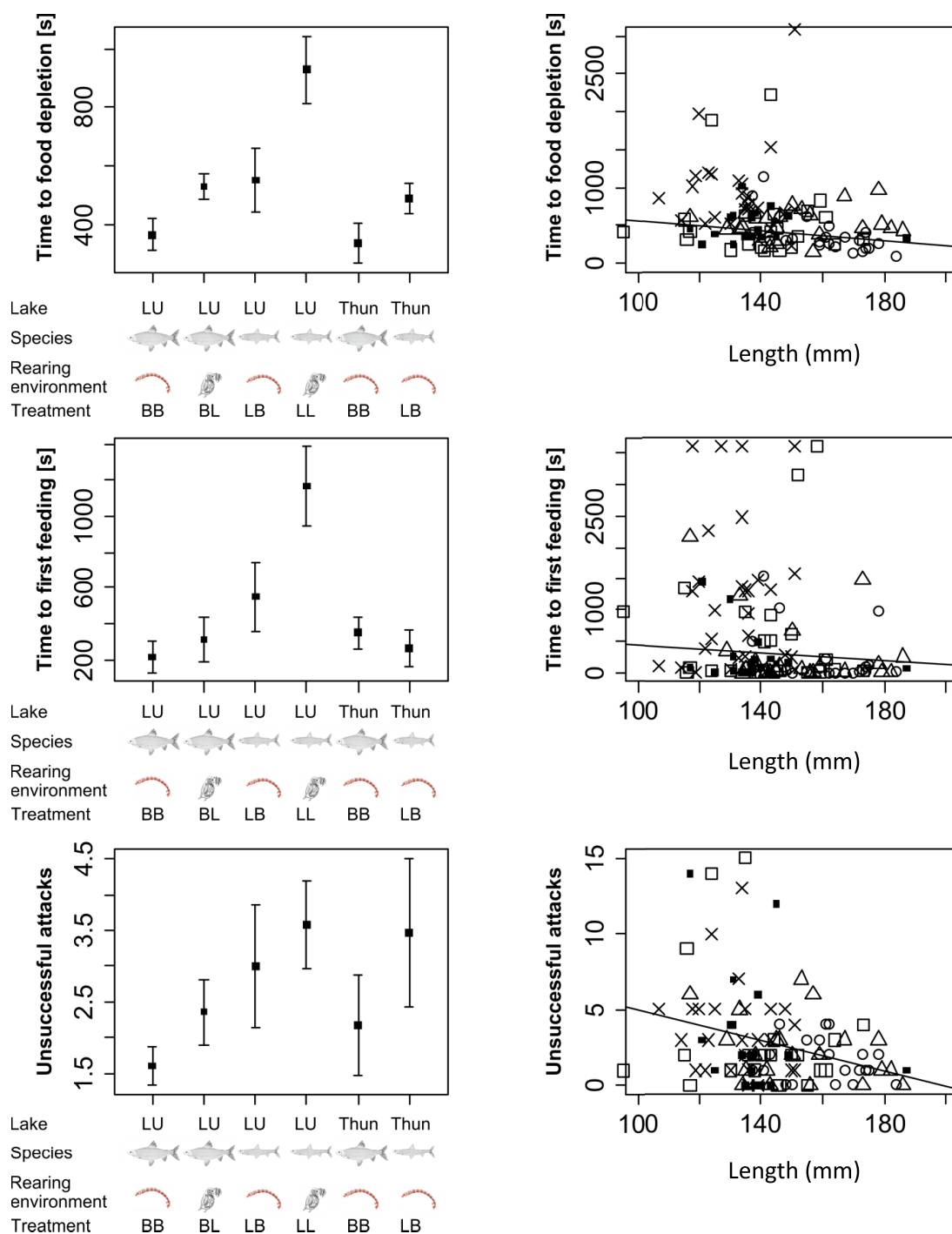


Figure 2: Feeding efficiency vs. treatments and feeding efficiency vs. length. Figures on the left show the treatments (for both lakes separately) on the x-axis (see legend of Figure 1 for more detail), and figures on the right show total fish length on the x-axis. The y-axis shows time to food depletion [sec] in figures a) and b), time to first feeding [sec] in figures c) and d), and the number of unsuccessful attacks in figures e) and f). Error bars are the treatment specific standard deviations. In the figures on the left, LU stands for Lake Lucerne and Thun stands for Lake Thun. In the figures on the right, empty dots represent fish from Lake Lucerne (circles=BB; triangles=BL; squares=LB; crosses=LL) and filled dots represent fish from Lake Thun (circles=BB, squares=LB). Solid lines correspond to a linear regression line for Lake Lucerne and dashed lines to a linear regression line for Lake Thun.

The observation of plasticity and species divergence in feeding efficiency as well as in fish length (Supplementary Table 1 and 2), combined with the observation of effects of fish length on feeding efficiency (Supplementary Table 3), suggest that species and plasticity effects on feeding efficiency can be twofold: We referred to *direct* species /environmental effects on feeding efficiency in subsequent paragraphs, if length is included in a model as a co-variable and the measured species/environmental effect is therefore independent of effects of length on feeding efficiency. Additionally the effects of length on feeding efficiency can be considered as *indirect* plasticity or species effects, because the more benthic a treatment is the larger its fish are and the larger fish are, the more efficient they feed on benthic food.

Using generalized linear modeling, time to food depletion in Lake Lucerne was best explained by a model including species, environment and length (Table 2). The effects of the environment and of species were both significant and there was a trend for an effect of length (Table 3). If we controlled for the effect of length on time to food depletion, fish raised on benthic food and those from the benthic species were more efficient than fish raised on limnetic food and belonging to the limnetic species (Figure 3a). If we controlled for the effects of species and of the environment on time to food depletion, larger fish depleted food in less time (Figure 4a). Post hoc tests revealed that all of the variables retained in the most likely model (Length, species, environment) were significantly associated with time to food depletion (Supplementary Table 2 and 3). The differences in significance levels between multivariate modeling and univariate post hoc tests arose as a result of shared information between different explanatory variables affecting their significance levels in the GLM.

Time to first feeding was best explained by a model including species, environment, length and year (Table 2), whereas only the effect of the environment was significant and there was a trend for the effect of length (Table 3). If we controlled for the effect of length and year on time to first feeding, fish from the benthic species and raised on benthic food were more efficient than fish from the limnetic species and raised on limnetic food (Figure 3b). Plasticity effects seemed to be stronger in the limnetic species, although there was no statistical support for this, as the interaction between genetics and environment was not significant. If we controlled for the effects of species, the environment and year, larger fish had a lower time to first feeding than smaller fish (Figure 4b). Univariate post hoc tests revealed that there was a significant association of time to first feeding with the environment, with species and with length, but not with year (Supplementary Table 2 and 3).

The number of unsuccessful attacks was best explained by a model including species, environment, length, PC2 and year (Table 2), whereas length was the only variable with a significant effect (Table 3). Larger fish displayed fewer unsuccessful attacks, independent of the effects of species, of the environment and other co-variables retained in the most likely model (Figure 4c). There was a trend for the effect of year, with fish tested in the second year failing less often in grabbing larvae. PC2 was non-significant, but there might be a weak trend. Controlling for species, the environment, length and year, illustrated that fish with a more sub-terminal mouth tended to display less unsuccessful attacks (Figure 4d). Species and the environment were non-significant, but the benthic species seemed to be slightly more efficient than the limnetic species, when controlling for the effects of

length, PC2 and year (Figure 3c). Univariate post hoc tests revealed a similar pattern as the GLM and were only significant for length (Supplementary Table 2 and 3; and PC2: $S=11972$, $\rho=0.07$, $p=0.46$).

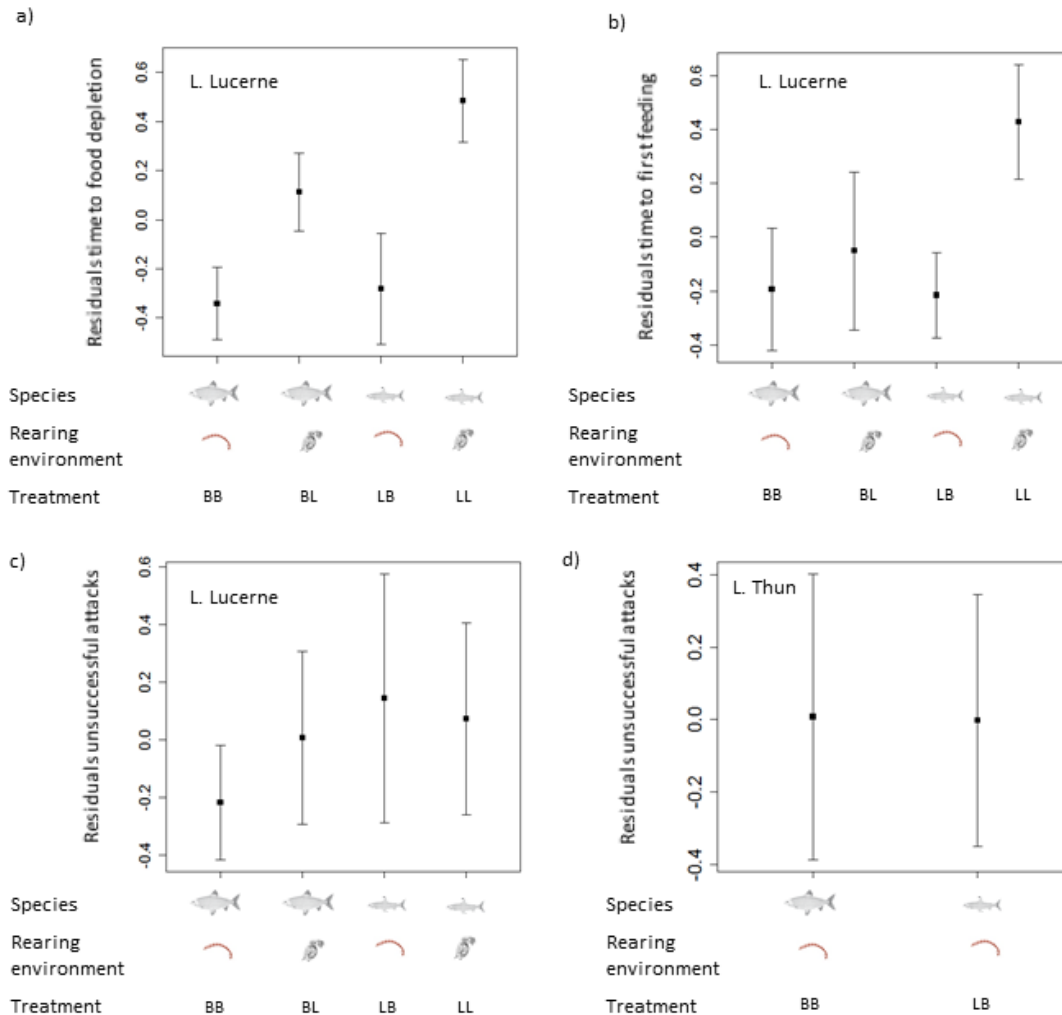


Figure 3: Effects of species and the environment on feeding efficiency. Shown are the treatments on the x-axis (see legend of Figure 1 for more detail) and the residuals of the most likely model excluding species (and in Lake Lucerne also the environment) from that model. This illustrates the effects of species and of the environment corrected for the effects of co-variables in the most likely model (residuals). Positive residuals indicate lower efficiency than predicted based on co-variables alone, while negative residuals predict higher efficiency than predicted based on co-variables alone. a) Time to food depletion of fish from lake Lucerne: Residuals of the model "Time to food depletion = length" on the y-axis. b) Time to first feeding of fish from Lake Lucerne: Residuals of the model "Time to first feeding = length + year" on the y-axis. c) Number of unsuccessful attacks of fish from Lake Lucerne: Residuals of the model "Number of unsuccessful attacks = length + PC2 + year" on the y-axis. d) Number of unsuccessful attacks of fish from Lake Thun: Residuals of the model "Number of unsuccessful attacks = length + PC2 + year" on the y-axis. Error bars are the standard deviations of the residuals per treatment. As species was the only variable retained in the most likely model of Time to food depletion and Time to first feeding of fish from Lake Thun, no residuals could be generated and plotted against species (but see Figure 2).

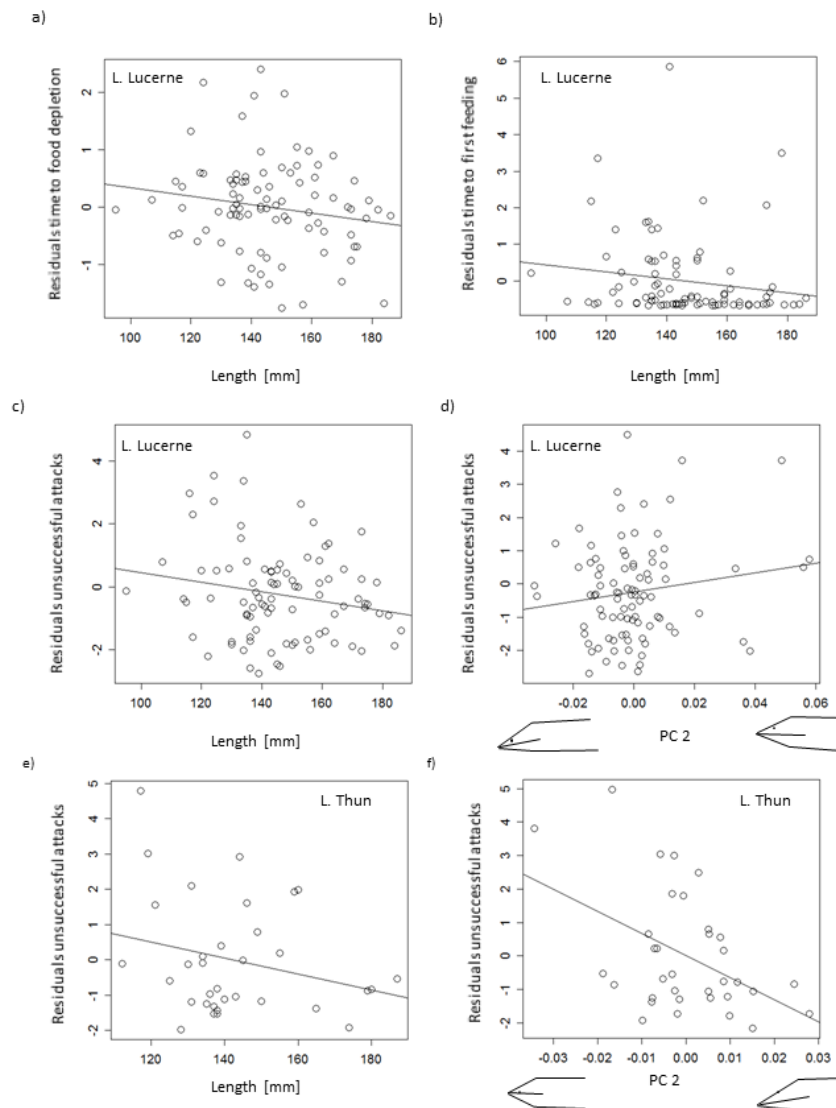


Figure 4: Effects of fish length and shape on feeding efficiency. Shown are either length (panel a-c and e) or PC2 (panel d and f) on the x-axis and the residuals of the corresponding most likely model excluding either length or PC2. This illustrates the effect of length and PC2 corrected for the effects of their co-variables in the most likely model (residuals). Positive residuals indicate lower efficiency than predicted based on co-variables, while negative residuals predict higher efficiency than predicted based on co-variables. a) Time to food depletion of fish from Lake Lucerne: Residuals of the model “Time to food depletion = species + environment” on the y-axis. b) Time to first feeding of fish from Lake Lucerne: Residuals of the model “Time to first feeding = species + environment + year” on the y-axis. c) Number of unsuccessful attacks of fish from Lake Lucerne: Residuals of the model “Number of unsuccessful attacks = species + environment + PC2 + year” on the y-axis. d) Number of unsuccessful attacks of fish from Lake Lucerne: Residuals of the model “Number of unsuccessful attacks = species + environment + length + year” on the y-axis. e) Number of unsuccessful attacks of fish from Lake Thun: Residuals of the model “Number of unsuccessful attacks = species + PC2 + year” on the y-axis. f) Number of unsuccessful attacks of fish from Lake Thun: Residuals of the model “Number of unsuccessful attacks = species + length + year” on the y-axis. Fish head shapes (drawn from a subset of landmarks) in panel d) and f) correspond to head shapes at the extremes of the PC2 axis and differences are threefold overdrawn. As the most likely models of time to food depletion and time to first feeding of fish from Lake Thun did not contain length or a shape PC, morphological effects on these efficiency measures are not illustrated.

Table 2: Generalized linear model selection. Models of Lake Lucerne are reported first, models of Lake Thun are reported below. Given for each model are its AIC, delta AIC to the most likely model (Delta i), the likelihood of each model (Likelihood), Akaike weights (w_i) and the evidence ratio (L ratio). The evidence ratio indicates how much less likely a particular model is compared to the most likely model. The model likelihood decreases for each model from the top to the bottom and the most likely model is highlighted in bold. $N_{\text{unsuccessful}}$ = number of unsuccessful attacks. SP = species, ENV = raising environment, L = length, PC1/PC2=principal components fish body shape variation and Y = year.

Backward model selection Lucerne	AIC	Delta i	Likelihood	w_i	L ratio
Time to food depletion = (SP×ENV)+SP+ENV+L+PC1+PC2+Y	232.67	6.39	0.04	0.02	24.41
Time to food depletion = SP+ENV+L+PC2+Y	230.68	4.4	0.11	0.06	9.03
Time to food depletion = SP+ENV+L+PC2+Y	228.88	2.6	0.27	0.14	3.67
Time to food depletion = SP+ENV+L+Y	227.44	1.16	0.56	0.28	1.79
Time to food depletion = SP+ENV+L	226.28	0	1	0.5	
Time to first feeding = (SP×ENV)+SP+ENV+L+PC1+PC2+Y	1206.6	4.4	0.11	0.07	9.03
Time to first feeding = SP+ENV+L+PC1+PC2+Y	1206.1	3.9	0.14	0.09	7.03
Time to first feeding = SP+ENV+L+PC1+Y	1204.1	1.9	0.39	0.24	2.59
Time to first feeding = SP+ENV+L+Y	1202.2	0	1	0.61	
$N_{\text{unsuccessful}}$ =(SP×ENV)+SP+ENV+L+PC1+PC2+Y	181.79	3.52	0.17	0.11	5.81
$N_{\text{unsuccessful}}$ =SP+ENV+L+PC1+PC2+Y	180.26	1.99	0.37	0.24	2.7
$N_{\text{unsuccessful}}$ =SP+ENV+L+PC2+Y	178.27	0	1	0.65	
Backward model selection Lake Thun	AIC	Delta i	Likelihood	Weights	L ratio
Time to food depletion = SP+L+PC1+PC2+Y	223.13	4.48	0.11	0.04	9.39
Time to food depletion = SP+L+PC1+PC2	221.18	2.53	0.28	0.11	3.54
Time to food depletion = SP+PC1+PC2	220.07	1.42	0.49	0.19	2.03
Time to food depletion = SP+PC1	219.35	0.7	0.7	0.27	1.42
Time to food depletion = SP	218.65	0	1	0.39	
Time to first feeding = SP+ L+ PC1+PC2+Y	458.19	6.87	0.03	0.02	31.03
Time to first feeding = SP+L+ PC2+Y	456.2	4.88	0.09	0.04	11.47
Time to first feeding = SP+L+Y	454.22	2.9	0.23	0.12	4.26
Time to first feeding = SP+L	452.39	1.07	0.59	0.3	1.71
Time to first feeding = SP	451.32	0	1	0.52	
$N_{\text{unsuccessful}}$ = SP+L +PC1+PC2+Y	73.2	2.97	0.23	0.18	4.41
$N_{\text{unsuccessful}}$ = SP+L +PC2+Y	70.23	0	1	0.82	

ii) Lake Thun

In Lake Thun, the benthic species was more efficient than the limnetic species by means of time to food depletion and the number of unsuccessful attacks (Figure 2). Time to first feeding on the other hand was lower in limnetic than in benthic fish (Figure 2). Wilcoxon Rank Sum Tests show that species differences in time to food depletion were significant, while other efficiency variables were not significantly different between species (Supplementary Table 2). Feeding efficiency was generally higher for larger fish (Figure 2), however these correlations were neither significant over both species nor within species (Supplementary Table 3).

Using generalized linear modeling, time to food depletion was best explained by a model including species only (Table 2), where the benthic species depleted the food in significantly shorter time (Table 3, Figure 2). Time to first feeding was also best explained by a model including species only (Table 2), but in this case the effect of species was non-significant (Table 3, Figure 2). The number of unsuccessful attacks was best explained by a model including species, length, PC2 and year (Table 2), where PC2 was the only variable with a significant effect (Table 3). Fish having a more sub-terminal mouth failed less often in grabbing larvae independent of their species identity, of their length and of the year they were tested (Figure 4f). There was a trend for length; as larger fish, independent of their genetic background, their shape (PC2), and the year when they were tested, displayed fewer unsuccessful attacks (Figure 4e). And there was a trend for year, with fish tested in the second year failing to grab larvae less often. The effect of species was not significant, but the benthic species had fewer failed attacks than the limnetic species (Figure 2e). However, this difference between the two species disappeared when we controlled for the effects of length, PC2 and year on the number of unsuccessful attacks (Figure 3d). Univariate post hoc tests were non-significant for an association of species and length with the number of unsuccessful attacks, while they were significant for PC2 and year (Supplementary Table 2 and 3; PC2: $n=34$, $\rho=-0.4$, $p=0.02$).

Fish tested in the second year generally tended to be slightly more efficient than fish tested in the first year, although the effect of year was never significant in any model (see results above). Size differences between the years cannot explain this pattern, because effects of year remained similar if one controlled for the effects of size on efficiency by including it as a co-variable and fish tested in the second year were not generally larger. We lack a testable explanation for this observation. But independent of the reason, the effect of time was unlikely to bias our findings, as treatments were generally randomly assigned to experimental days and time (in days after the first trial was done) was not different between treatments in neither of the lakes. Further the inclusion of year in the GLM analysis controls for year effects and the effects inferred from the GLMs are thus independent of potentially confounding year effects.

Table 3: Generalized linear model coefficients of the most likely models. The different models are listed in rows, the different variables are listed in columns. Abbreviations are as in Table 2. Given are the estimated model coefficients (Coef), their error (error) and the p-value (p, significant values highlighted in bold). A positive model coefficient indicates a positive relationship. For species and environment this relationship goes from benthic to limnetic. A positive model coefficient thus means that limnetic fish have a higher value than benthic fish (indicating a lower efficiency) in the response variable and vice versa. For year a positive model coefficient thus means that fish in the second year were less efficient. Environmentally induced effects could not be measured for Thun, which is indicated by the term na.

		SP	ENV	L	PC2	Y
Time to food depletion Lucerne	Coef/error	0.41/0.2	0.7/0.17	0.01/0.005	-	-
	P	0.048	<0.001	0.065	-	-
Time to first attack Lucerne	Coef/error	0.42/0.37	0.71/0.33	-0.02/0.01	-	0.93/0.57
	P	0.26	0.03	0.08	-	0.11
N _{unsuccessful} Lucerne	Coef/error	0.2/0.25	0.05/0.21	-	9.78/6.12	-
	P	0.42	0.81	0.01	0.11	0.076
Time to food depletion Thun	Coef/error	4.55/1.95	na	-	-	-
	P	0.03	na	-	-	-
Time to first attack Thun	Coef/error	-	na	-	-	-
	P	0.56	na	-	-	-
N _{unsuccessful} Thun	Coef/error	0.03/0.36	na	-0.02/0.01	-	-39.95/13.48
	P	0.92	Na	0.07	>0.01	0.06

Discussion

Our results show that the sympatric benthic-limnetic species pairs of whitefish differ in their feeding efficiency on benthic food, with the benthic species being more efficient than the limnetic species when raised on the same food in both lakes, suggesting a genetic basis of feeding efficiency divergence. These results are in agreement with field studies reporting that sympatric whitefish species often exhibit differences in resource use along the between benthic-limnetic resource axis (Bernatchez et al., 1999; Amundsen et al., 2004; Harrod et al., 2010) and they add more evidence that adaptation to different trophic niches is likely involved in diversification of north temperate fish. In Lake Lucerne, we further found effects of phenotypic plasticity on feeding efficiency, while we did not quantify plasticity effects in Lake Thun. These findings of a genetic basis and of phenotypic plasticity in feeding efficiency are consistent with the suggested importance of both divergent natural selection on heritable traits as well as adaptive phenotypic plasticity in the evolutionary diversification of traits related to trophic ecology in whitefish (Wimberger, 1994; Rogers & Bernatchez, 2007), and more generally in the build-up of diversity in adaptive radiation (Schluter, 2000; Pfennig et al., 2010).

Species divergence in growth

Independent of the food the fish were raised on, the benthic species grew bigger than the limnetic species, indicating heritable species divergence in growth. This was found for both lakes albeit it was marginally non-significant in fish from Lake Thun. These finding with faster growth in the benthic species (*C. sp. "Balchen"* and *C. sp. "Bodenbalchen"*) are in the same direction as species divergence in nature (Vonlanthen et al. 2012) and are consistent with previous work reporting a genetic basis in species divergence in growth of various fishes, including many salmonids and whitefish (Hatfield, 1997; Garant et al., 2003; Rogers & Bernatchez, 2007). Among fish from Lake Lucerne, we also observed effects of the rearing environment on growth. Fish raised on benthic food generally grew larger than fish raised on limnetic food. This may be explained by differences in energetic profitabilities between our food treatments (zooplankton vs. mosquito larvae), which were not standardized to equal energetic content.

The observed heritable species divergence in growth might have accumulated as a result of divergent selection favoring different growth patterns in the benthic and the limnetic habitat. Slower growth in the limnetic habitat is probably associated with high bioenergetic costs of living in this habitat, with small, spatially widely distributed prey (Mookerji et al. 1998; Trudel et al., 2001; Kahilainen et al. 2007). The benthic habitat with larger and more spatially clustered prey requires less swimming effort and attacks, what allows faster growth (Kahilainen et al. 2003). In this experiment we showed that increased size is associated with increased feeding efficiency on benthic food and might constitute an adaptation to exploit benthic resources. It might additionally constitute a different predator escape strategy, namely through accelerated growth to reach a size above the predation window of piscivore fish instead of adaptations in predator avoidance through swimming behavior (Kahilainen and Lehtonen 2002; Roger et al., 2002). Because the studied species are young, having emerged after the last glacial maximum (Hudson et al., 2011), our findings of heritable growth divergence between species are consistent with a role of divergent selection on growth early in the

speciation process, as it has been shown for other whitefish systems (Rogers and Bernatchez 2007). Taken together evidence for divergent selection on growth and the predominant role of size as a mate-choice signal in fish (Foote and Larkin 1988; Sigurjonsdottir and Gunnarsson 1989; McKinnon et al. 2004), indicates that size might potentially be a magic trait of speciation in whitefish (magic-trait model of speciation: Gavrillets 2004).

In Lake Lucerne, our results further show weak but significant species divergence in shape: The benthic species has a more sub-terminal mouth. In Lake Thun, the two species were not significantly divergent in the shape components we measured. The measured shape components were non-labile in respect to our divergent raising environments for Lake Lucerne fish, indicated by the lack of plasticity effects on shape. Many studies have reported critical effects of the timing of environmental induction on the strength of the plastic response to it (West-Eberhard, 2003). In our experiment all fish had to be raised on zooplankton in the first year (whitefish larvae cannot effectively be raised on benthic food), which could explain why we did not find strong plasticity in morphology induced by divergent feeding regimes while other authors, studying other fish taxa, did find such effects (Day & McPhail, 1996; Bouton et al., 2002; Robinson & Parson, 2002; Muschick et al., 2011). Alternatively it could reflect real differences in canalization of morphology between whitefish and other fish species, however, other studies reported strong plasticity in whitefish morphology (Lindsey 1981).

Evidence for inherited species differences and phenotypic plasticity in feeding efficiency

We found that both benthic species were generally more efficient in foraging on benthic food than their limnetic sister species, suggesting heritable divergence in feeding efficiency. A genetic component of feeding efficiency between benthic-limnetic sister species is consistent with previous experiments using north temperate fish (Robinson, 2000; Adams & Huntingford, 2002). GLM analyses indicate that the effects of this feeding efficiency divergence between species are twofold. On one hand they are manifested as direct behavioral effects, independent of morphological differences (fish length and shape) between species. On the other hand they can be manifested as indirect effects due to inherited differences in length and shape, which themselves influences feeding efficiency. In our experiment fish of the benthic species from both lakes grew larger, and larger fish were generally more efficient foragers on benthic food, independent of their genetic background. This observation of increased efficiency with increasing size is consistent with empirical observation that the more benthic species are usually larger (Schluter 2000; Vonlanthen et al. 2012) and it is not inconsistent with ontogenetic diet shifts to more benthic prey with increasing size in whitefish (Sandlund et al. 1992; Pothoven & Nalepa 2006). Further, in Lake Lucerne we found the benthic species to have a more sub-terminal mouth than the limnetic species and individuals with a sub-terminal mouth displayed fewer failed attacks in our experiments than those with a more terminal mouth. This is consistent predictions from functional morphology and with the empirical observation on many fish taxa, including whitefish, that the position of the mouth relative to the body is associated with benthic vs. limnetic feeding, with benthic feeders having a more sub-terminal mouth (Steinmann, 1950; McCart, 1970; Caldecutt & Adams, 1998; Bernatchez 1999; Clabaut et al., 2007; Harrod et al. 2010).

The observed heritable divergence in feeding behavior between the benthic and limnetic whitefish species is consistent with a role for divergent natural selection favoring different trophic strategies in contrasting foraging environments in north temperate fish (Schluter 1995; Rogers et al., 2002; Klemetsen et al. 2006). Speciation in Lake Thun and Lake Lucerne whitefish was proposed to be intra-lacustrine (Hudson et al., 2011), therefore ecological character displacement after allopatric speciation and secondary contact seems very unlikely. It remains uncertain whether species divergence in feeding efficiency was a driving force of speciation at the very beginning of the process, or whether it could have occurred as a by-product after speciation was initialized (speciation could have been initialized e.g. by physiological adaptation to different thermal regimes in the contrasting environments and divergence in feeding efficiency would have accumulated afterwards). The species differences in feeding efficiency could also have evolved through genetic assimilation of initially plastic differences in feeding efficiency between whitefish growing up in different habitats (West-Eberhard, 2003).

Our results of species differences in exploiting benthic resources can be considered as evidence for trait utility (Schluter 2000). Trait utility means that a trait associated with a particular environment enhances performance there (Schluter 2000). This feature of adaptive radiations has so far not experimentally been demonstrated for whitefish (Bernatchez 2004), though indirect evidence from comparative approaches suggest that a high number of gill rakers increases fitness in the limnetic environment (Kahilainen et al. 2007; 2011). We showed that the overall phenotype (including behavior), which can be seen as a multi-dimensional trait, of the benthic species increases its ability to exploit the benthic environment. Our results also show that increased size increases feeding efficiency on benthic prey independent of a fish's genetic background. And this is consistent with trait utility of size in regard to fitness in the benthic niche. However, it remains to be tested whether increased size would also increase feeding efficiency on limnetic prey, before we want to draw strong conclusions about trait utility of size in the benthic niche. Other potential traits are, among others, the number of gill rakers (which was not quantified here, as fish were kept alive), the position of the mouth as well as behavioral traits (for example swimming behavior).

Consistent with earlier work (Day and McPhail, 1996), we also observed significant effects of environmental plasticity on feeding efficiency. Fish raised on benthic food became more efficient foragers on benthic food than fish raised on zooplankton, indicating that the observed plasticity in feeding efficiency was adaptive. This is consistent with the suggested importance of phenotypic plasticity for species diversification in adaptive radiation (West-Eberhard, 1989, 2003; Pfennig et al. 2010). We have two lines of evidence that the effects of plasticity are not simply due to plastic components of size and shape, but primarily the result of plasticity in feeding behavior itself. First, fish size (length) was included in general linear models with significant environmental effects, indicating that plasticity effects are not just due to plasticity in length. Second, there was no plasticity in shape. Earlier work on sticklebacks suggested that behavioral plasticity mainly influenced searching efficiency (Day & McPhail 1996). Consistent with this, the two efficiency variables that showed plasticity in our experiments, time to food depletion and time to first feeding, are more related to detection ability and searching efficiency; whereas the number of unsuccessful attacks,

which did not reveal plasticity, is more related to prey capture efficiency. Phenotypic plasticity was suggested to explain why some taxa are more diverse than others, with plasticity increasing species diversity (Pfennig & McGee, 2010). Whitefish and Arctic charr are of the most diverse taxa within the order of the Salmoniformes (Kottelat & Freyhof 2007). Maybe their ability to display strong phenotypic plasticity in feeding behavior and morphology might be one explanation for their high species diversity.

Conclusions

Natural selection is thought to be the most important mechanism behind the diversification of species in adaptive radiations (Schluter, 2000). Our findings of heritable feeding efficiency differences between whitefish species of two parallel adaptive radiations are consistent with this. Additionally, our observation of strong phenotypic plasticity in feeding efficiency indicates an important role of adaptive phenotypic plasticity in diversification of north temperate fish. In conclusion, our data suggest that both, phenotypic plasticity and evolutionary divergence resulting from divergent natural selection, are likely important mechanisms of adaptive radiation.

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References

- Adams, C. E. & Huntingford, F. A. 2002. The functional significance of inherited differences in feeding morphology in a sympatric polymorphic population of Arctic charr. *Evol. Ecol.* 16: 15-25.
- Adams, C. E. & Huntingford, F. A. 2004. Incipient speciation driven by phenotypic plasticity? Evidence from sympatric populations of Arctic charr. *Biol. J. Linn. Soc.* 81: 611-618.
- Amundsen, P. A., Knudsen, R., Klemetsen, A. & Kristoffersen, R. 2004. Resource competition and interactive segregation between sympatric whitefish morphs. *Ann. Zool. Fenn.* 41: 301-307.
- Barrett, R. D. H. & Schluter, D. 2008. Adaptation from standing genetic variation. *Trends Ecol. Evol.* 23: 38-44.
- Bernatchez, L. 2004. Ecological Theory of Adaptive Radiation: An Empirical Assessment from Coregonine Fishes (Salmoniformes). In: *Evolution Illuminated* (Hendry, A. P. & Stearns, S. C., eds.). pp. 175-207. Oxford University Press, Oxford.
- Bernatchez, L., Chouinard, A. & Lu, G. Q. 1999. Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, *Coregonus sp.*, as a case study. *Biol. J. Linn. Soc.* 68: 173-194.
- Bernatchez, L., & Dodson, J. J. 1994. Phylogenetic relationships among palearctic and nearctic whitefish (*Coregonus sp.*) populations as revealed by mitochondrial DNA variation. *Can. J. Fish. Aquat. Sci.* 51: 240-251.
- Bernatchez, L., Vuorinen, J. A., Bodaly, R. A & Dodson, J. J. 1996. Genetic evidence for reproductive isolation and multiple origins of sympatric trophic ecotypes of whitefish (*Coregonus*). *Evolution* 50: 624-635.
- Bittner, D. 2009. Gonad deformations in whitefish (*Coregonus spp.*) from Lake Thun, Switzerland - A population genetic and transcriptomic approach. In: CMPG, Vol. PhD. pp. University of Bern, Bern.
- Bittner, D., Excoffier, L. & Largiadèr, C. R. 2010. Patterns of morphological changes and hybridization between sympatric whitefish morphs (*Coregonus spp.*) in a Swiss lake: a role for eutrophication? *Mol. Ecol.* 19: 2152-2167.
- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., White, J.-S. S. 2008. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24: 127-135.
- Bookstein, F.L. 1991. *Morphometric Tools for Landmark Data, Geometry and Biology*. Cambridge University Press, Cambridge.
- Boulding, E. G. & Van Alstyne, K. L. 1993. Mechanisms of differential survival and growth of two species of *Littorina* on wave-exposed and on protected shores. *J. Exp. Mar. Biol. Ecol.* 169: 139-166.
- Bouton, N., Witte, F. & Van Alphen, J.J.M. 2002. Experimental evidence for adaptive phenotypic plasticity in a rock-dwelling cichlid fish from Lake Victoria. *Biol. J. Linn. Soc.* 77: 185-192.
- Burnham, K. P. & Anderson, D. R. 1998. *Model selection and multimodel inference, a practical information-theoretic approach*. Second edition. Springer, New York.
- Caldecutt, W. J. & Adams, D. C. 1998. Morphometrics of trophic osteology in the threespine stickleback, *Gasterosteus aculeatus*. *Copeia* 4: 827-838.

- Clabaut, C., Bunje, P. M. E., Salzburger, W. & Meyer, A. 2007. Geometric morphometric analyses provide evidence for the adaptive character of the tanganyikan cichlid fish radiations. *Evolution* 61: 560-518.
- Day, T. & McPhail, J. D. 1996. The effect of behavioural and morphological plasticity on foraging efficiency in the threespine stickleback (*Gasterosteus sp.*). *Oecologia* 108: 380–388.
- Day, T., Pritchard, J. & Schluter, D. 1994. A comparison of two stickleback. *Evolution* 48: 1723-1734.
- Foote, C.J., & Larkin P. A. 1988. The role of male choice in the assortative mating of anadromous and non-anadromous sockeye salmon, *Oncorhynchus nerka*. *Behaviour* 106: 43-62.
- Garant, D., Dodson, J.J. & Bernatchez, L. 2003. Differential reproductive success and heritability of alternative reproductive tactics in wild Atlantic Salmon (*Salmo salar L.*). *Evolution* 57: 1133–1141.
- Gavrilets, S. 2004. *Fitness Landscapes and the Origin of Species*. Princeton Univ. Press, Princeton.
- Grant, P. R. 1986. *Ecology and evolution of Darwin's finches*. Princeton University Press, Princeton.
- Harrod, C., Mallela, J. & Kahilainen, K. 2010. Phenotype-environment correlations in a putative whitefish adaptive radiation. *J. Anim. Ecol.* 79: 1057-1068.
- Hatfield, T. 1997. Genetic divergence in adaptive characters between sympatric species of stickleback. *Am. Nat.* 149: 1009-1029.
- Hudson, A. G., Vonlanthen, P. & Seehausen, O. 2011. Rapid parallel adaptive radiations from a single hybridogenic ancestral population. *P. R. Soc. B* 278: 58-66.
- Hudson, A. G., Vonlanthen, P., Müller, R., & Seehausen, O. 2007. Review: The geography of speciation and adaptive radiation in coregonines. *Adv. Limnol.* 60: 111-146.
- Kahilainen, K. K., & Lehtonen, H. 2002. Brown trout (*Salmo trutta* (L)) and Arctic charr (*Salvelinus alpinus* (L)) as predators of three sympatric whitefish (*Coregonus lavaretus* (L)) in the subarctic Lake Muddusjärvi. *Ecol. Freshw. Fish* 11: 158-167.
- Kahilainen, K. K., Lehtonen, H., & Könönen, K. 2003. Consequences of habitat segregation to growth rate of two sparsely rakered whitefish (*Coregonus lavaretus* (L.)) in a subarctic lake. *Ecol. Freshw. Fish* 12: 275-285.
- Kahilainen, K. K., Malinen, T., Tuomaala, A., Alajärvi, E., Tolonen, A., & Lehtonen, A. 2007. Empirical evaluation of phenotype-environment correlation and trait utility with allopatric and sympatric whitefish (*Coregonus lavaretus* (L.)) in subarctic lakes. *Biol. J. Linn. Soc.* 92: 561-572.
- Kahilainen, K. K., Siwertsson, A., Gjelland, K. O., Knudsen, R., Bohn, T., & Amundsen, P.-A. 2011. The role of gill raker variability in adaptive radiation of coregonid fish. *Evol. Ecol.* 25: 573-588.
- Klemetsen, A., Knudsen, R., Primicerio, R. & Amundsen, P. A. 2006. Divergent, genetically based feeding behaviour of two sympatric Arctic charr, *Salvelinus alpinus* (L.), morphs. *Ecol. Freshw. Fish* 15: 350-355.
- Klingenberg, C. P. 2011. MORPHOJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* 11: 353-357.
- Kottelat, M. & Freyhof, J. 2007. *Handbook of European freshwater fishes*. Kottelat, Cornol, Switzerland and Freyhof, Berlin, Germany.

- Kruskal, W. & Wallis, W. A. 1952. Use of ranks in one-criterion variance analysis. *J. Am. Stat. Assoc.* 47: 583–621.
- Landry, L., Vincent, W. F. & Bernatchez, L. 2007. Parallelism between limnological features and phenotypic evolution of lake whitefish dwarf ecotypes. *J. Evolution Biol.* 20: 971-984.
- Lindsey, C. C. 1981. Stocks are chameleons: Plasticity in gill rakers of coregonid fishes. *Can. J. Fish. Aquat. Sci.* 38: 1497-1506.
- Lebreton, J.D., Burnham, K. P., Clobert, J. & Anderson, D. R. 1992. Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecol. Monogr.* 62: 67-118.
- Lehmann, E. L. 1975. Nonparametric statistical methods based on ranks. McGraw-Hill, New York.
- Lehmann, E. L. & D'Abrera, H. J. M. 2006. Nonparametrics: Statistical methods based on ranks. Springer, New York.
- Losos, J. B., Douglas, A. C., Glossip, D., Goellner, R., Hampton, A., Roberts, G. et al. 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. *Evolution* 54: 301-305.
- Loy, A., Mariani, L., Bertelletti, M. & Tunesi, L. 1998. Visualizing allometry: geometric morphometrics in the study of shape changes in the early stages of the two-banded sea bream, *Diplodus vulgaris* (Perciformes, Sparidae). *J. Morphol.* 237: 137-146.
- Lu, G. & Bernatchez, L. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. *Evolution* 53: 1491-1505.
- McCart, P. 1970. Evidence for the existence of sibling species of pygmy whitefish (*Prosopium coulteri*) in three Alaskan lakes. In *Biology of Coregonid Fishes*, ed. CC Lindsey, CS Woods, pp. 81-98. Univ. Manitoba Press, Winnipeg.
- McKinnon J. S., Mori, S., Blackman, B. K., David, L., Kingsley, D. M. , Jamieson, L., Chou, J., & Schluter, D. 2004. Evidence for ecology's role in speciation. *Nature* 429: 294-298.
- Michel, M. 1996. Untersuchungen zur Nahrungsökologie von Grossfelchen im Vierwaldstättersee während des Sommerhalbjahres 1996. Master thesis EAWAG, supervised by R. Müller.
- Muschick, M., Barluenga, M., Salzburger, W. & Meyer, A. 2011. Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evol. Biol.* 11: 116.
- Mookerji, N., Heller, C., Meng, H. J., Bürgi, R., & Müller, R. 1998. Diel and seasonal patterns of food uptake and prey selection by *Coregonus sp.* in re-oligotrophicated Lake Lucerne, Switzerland. *J. Fish. Biol.* 52: 443-457.
- Nosil, P. 2004. Reproductive isolation caused by visual predation on migrants between divergent environments. *P. Roy. Soc. Lond. B Bio.* 271: 1521-1528.
- Ostbye, K., Amundsen, P. A., Bernatchez, L., Klemetsen, A., Knudsen, R., Kristoffersen, R. et al. 2006. Parallel evolution of ecomorphological traits in the European whitefish *Coregonus lavaretus* (L.) species complex during postglacial times. *Mol. Ecol.* 15: 3983-4001.

- Ostbye, K., Bernatchez, L., Naesje, T. F., Himberg, M., & Hindar, K. 2005a. Evolutionary history of European whitefish (*Coregonus lavaretus*) as inferred from mtDNA phylogeography and gill-raker numbers. *Mol. Ecol.* 14: 4371-4388.
- Ostbye, K., Naesje, T. F., Bernatchez, L., Sandlund, O. T., & Hindar, K. 2005b. Morphological divergence and origin of sympatric populations of European whitefish (*Coregonus lavaretus* (L)) in Lake Femud, Norway. *J. Evol. Biol.* 18: 683-702.
- Pfennig, D. W. & McGee, M. 2010. Resource polyphenism increases species richness: a test of the hypothesis. *Philos. T. R. Soc. B* 365: 577-591.
- Pfennig, D. W., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D. & Moczek, A. P. 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol. Evol.* 25: 459-467.
- Pigeon, D., Chouinard, A., & Bernatchez, L. 1997. Multiple modes of speciation involved in the parallel evolution of sympatric morphotypes of lake whitefish (*Coregonus clupeaformis*). *Evolution* 51: 196-205.
- Pothoven, S. A., & Nalepa, T. F. 2006. Feeding ecology of lake whitefish in Lake Huron. *J. Great Lakes Res.* 32: 489-501.
- Proulx, R. & Magnan, P. 2004. Contribution of phenotypic plasticity and heredity to the trophic polymorphism of lacustrine brook charr (*Salvelinus fontinalis* M.). *Evol. Ecol. Res.* 6: 503-522.
- R Development Core Team. 2010. R: A language and environment for Statistical Computing. Vienna, Austria. <http://www.R-project.org>.
- Robinson, B. W. 2000. Trade offs in habitat-specific foraging efficiency and the nascent adaptive divergence of sticklebacks in lakes. *Behavior* 137: 865-888.
- Robinson, B.W. & Wilson, D.S. 1994. Character release and displacement in fishes: A neglected literature. *Am. Nat.* 144: 596-627.
- Robinson, B.W. & Parsons, K. J. 2002. Changing times, spaces and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Can. J. Fish. Aquat. Sci.* 59: 1819-1833.
- Rogers, S. M. & Bernatchez, L. 2005. Integrating QTL mapping and genome scans towards characterization of candidate loci of parallel selection in the lake whitefish (*Coregonus clupeaformis*). *Mol. Ecol.* 14: 351-361.
- Rogers, S. M. & Bernatchez, L. 2007. The genetic architecture of ecological speciation and the association with signatures of selection in natural lake whitefish (*Coregonus sp.*, Salmonidae) species pairs. *Mol. Biol. Evol.* 24: 1423-1438.
- Rogers, S. M., Gagnon, V. & Bernatchez, L. 2002. Genetically based phenotype-environment association for swimming behavior in lake whitefish ecotypes (*Coregonus Clupeaformis* Mitchell). *Evolution* 56: 2322-2329.
- Rohlf, F. J. 2006. TPSDig Version 2.1. State University of New York at Stony Brook, Stony Brook, NY, USA.

- Rohlf, F. J. & Slice, D. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Syst. Zool.*, 39:40-59.
- Rundle, H. D. 2002. A test of ecologically dependent postmating isolation between sympatric sticklebacks. *Evolution* 56: 322–329.
- Sandlund, O. T., Naesje, T. F., & Jonson, B. 1992. Ontogenetic changes in habitat use by whitefish, *Coregonus lavaretus*. *Environ. Biol. Fish.* 33: 341-349.
- Schluter, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology* 76: 82–90.
- Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Schluter, D. & Nagel, L. 1995. Parallel speciation by natural selection. *Am. Nat.* 146: 292–301.
- Sigurjonsdottir H., & Gunnarsson, K. 1989. Alternative mating tactics of Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Environ. Biol. Fish.* 26: 159-176.
- Siwertsson, A., Knudsen, R., Kahilainen, K., Praebel, K., Primicerio, & R. Amundsen, P. A. 2010. Sympatric diversification as influenced by ecological opportunity and historical contingency in a young species lineage of whitefish. *Evol. Ecol. Res.* 12: 929-948.
- Skúlason, S. & Smith, T. B. 1995. Resource polymorphism in vertebrates. *Trends Ecol. Evol.* 10: 366-370.
- Smith, T. B. & Skúlason, S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu. Rev. Ecol. Syst.* 27: 111–133.
- Steinmann, P. 1950. Monographie der schweizerischen Koregonen. Beitrag zum Problem der Entstehung neuer Arten. Spezieller Teil. *Schweiz. Z. Hydrol.* 12: 340-491.
- Svårdson, G. 1979. Speciation of Scandinavian *Coregonus*. *Rep. Inst. Freshw. Res. Drott.* 57: 1-95.
- Svarvar, P. O. & Müller, R. 1982. Die Felchen des Alpnachersees. *Schweiz. Z. Hydrol.* 44: 295-314.
- Taylor, E. B. 1999. Species pairs of north temperate freshwater fishes: evolution, taxonomy, and conservation. *Rev. Fish Biol. Fisher.* 9: 299-324.
- Trudel, M., Tremblay, A., Schetagne, R., & Rasmussen, J. B. 2001. Why are dwarf fish so small? An energetic analysis of polymorphism in lake whitefish (*Coregonus clupeaformis*). *Can. J. Fish. Aquat. Sci.* 58: 394–405.
- Venables, W. N. & Ripley, B. D. 2002. *Modern Applied Statistics with S*. Fourth Edition. Springer, New York.
- Via, S., Bouck, A., & Skillman, S. 2000. Reproductive isolation between sympatric races of pea aphids. II. Selection against migrants and hybrids in the parental environments. *Evolution* 54: 1626-1637.
- Vonlanthen, P., Bittner, D., Hudson, A. G., Young, K. A., Müller, R., Lundsgaard-Hansen, B. *et al.* 2012. Eutrophication causes speciation reversal in whitefish adaptive radiations. *Nature* 482: 357-363.
- Vonlanthen, P., Roy, D., Hudson, A. G., Largiader, C. R., Bittner, D. & Seehausen, O. 2009. Divergence along a steep ecological gradient in lake whitefish (*Coregonus sp.*). *J. Evolution Biol.* 22: 498-514.

West Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20: 249-278.

West Eberhard, M. J. 2003. *Developmental plasticity and evolution*. Oxford University Press, Oxford.

Wimberger, P.H. 1994. Trophic polymorphisms, plasticity, and speciation in vertebrates. In *Theory and application in fish feeding ecology* (eds D. J. Stouder, K. L. Fresh & R. J. Feller). University of South Carolina Press, Columbia, SC.

Wund, M. A., Baker, J. A., Clancy, B., Golub, J. L. & Foster, S. A. 2008. A test of the flexible stem model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. *Am. Nat.* 172: 449-462.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D. & Fink, W.L. 2004. *Geometric Morphometrics for Biologists: A Primer*. Elsevier Academic Press, San Diego.

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. 2009. *Mixed effects models and extensions in ecology with R*. First edition. Springer, New York.

Supplementary Information Chapter 1

Supplementary Table 1: Results of a) size and b) shape differences tests between treatments and years. Given is in the first column the lake; in the second and third column the tested contrast; in the fourth column which test was used (KWA = Kruskal Wallis Anova; WRS = Wilcoxon Rank Sum Test), in the fifth and sixth column the test statistics and significance levels (ns = non significant; trend = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$). Sample sizes can be found in Table 1.

a) Size difference tests					
Lucerne	Between years	LB only	KWA	57.5	Ns
Lucerne	Between all treatments	Both years	KWA	34.35	***
Lucerne	Posthoc BB vs. BL	Both years	WRS	326	Trend
Lucerne	Posthoc BB vs. LB	Both years	WRS	446.5	***
Lucerne	Posthoc BB vs. LL	Both years	WRS	646	***
Lucerne	Posthoc BL vs. LB	Both years	WRS	338.5	Ns
Lucerne	Posthoc BL vs. LL	Both years	WRS	526.5	***
Lucerne	Posthoc LB vs. LL	Both years	WRS	489	*
Lucerne	Between all treatments	First year only	KWA	34.29	***
Lucerne	Posthoc BB vs. BL	First year only	WRS	326	*
Lucerne	Posthoc BB vs. LB	First year only	WRS	323.5	***
Lucerne	Posthoc BB vs. LL	First year only	WRS	646	***
Lucerne	Posthoc BL vs. LB	First year only	WRS	224	Ns
Lucerne	Posthoc BL vs. LL	First year only	WRS	496.5	***
Lucerne	Posthoc LB vs. LL	First year only	WRS	350	*
Thun	Between Years	BB only	WRS	24.5	Ns
Thun	Between Years	LB only	WRS	35	Ns
Thun	Between Treatments	Both years	WRS	196.5	Trend
Thun	Between Treatments	First year only	WRS	57.5	Ns
Thun	Between Treatments	Second year only	WRS	42	*
b) PC2 difference tests					
Lucerne	Between years	LB	KWA	45	Ns
Lucerne	Between all treatments	Both years	KWA	8.6	*
Lucerne	Posthoc BB vs. BL	Both years	WRS	210	Ns
Lucerne	Posthoc BB vs. LB	Both years	WRS	165	*
Lucerne	Posthoc BB vs. LL	Both years	WRS	178.5	*
Lucerne	Posthoc BL vs. LB	Both years	WRS	181	Ns
Lucerne	Posthoc BL vs. LL	Both years	WRS	207.5	Trend
Lucerne	Posthoc LB vs. LL	Both years	WRS	350	Ns
Lucerne	Between all treatments	First year only	KWA	7.71	Trend
Lucerne	Posthoc BB vs. BL	First year only	WRS	209	Ns
Lucerne	Posthoc BB vs. LB	First year only	WRS	131.5	Ns
Lucerne	Posthoc BB vs. LL	First year only	WRS	178.5	*
Lucerne	Posthoc BL vs. LB	First year only	WRS	131	Ns
Lucerne	Posthoc BL vs. LL	First year only	WRS	184.5	*
Lucerne	Posthoc LB vs. LL	First year only	WRS	224.5	Ns
Thun	Between Years	BB only	WRS	30	Ns
Thun	Between Years	LB only	WRS	23	Ns
Thun	Between Treatments	Both years	WRS	154	Ns
Thun	Between Treatments	First year only	WRS	56	Ns
Thun	Between Treatments	Second year only	WRS	26	Ns

Supplementary Table 2: Results of posthoc tests between treatments in a) time to first feeding, b) time to food depletion and c) number of unsuccessful attacks. Given is in the first column the lake; in the second column the tested contrast; in the third column the test used (KWA = Kruskal Wallis Anova; WRS = Wilcoxon Rank Sum Test); in the fourth and fifth column the test statistics and significance levels (ns = non significant; trend = $p < 0.1$; * = $p < 0.05$; ** < 0.01 ; *** < 0.001). Sample sizes can be found in Table 1.

a) Time to first feeding				
Thun	Between treatments	WRS	161	ns
Thun	Between years	WRS	133	ns
Lucerne	Between all treatments	KWA	22.06	***
Lucerne	Between environments	WRS	692	*
Lucerne	Between species	WRS	575	**
Lucerne	Posthoc BB vs. BL	WRS	217	ns
Lucerne	Posthoc BB vs. LB	WRS	205.5	ns
Lucerne	Posthoc BB vs. LL	WRS	117.5	***
Lucerne	Posthoc BL vs. LB	WRS	227	ns
Lucerne	Posthoc BL vs. LL	WRS	140	***
Lucerne	Posthoc LB vs. LL	WRS	189.5	**
Lucerne	Between years	WRS	257	ns
b) Time to food depletion				
Thun	Between treatments	WRS	72.5	*
Thun	Between years	WRS	156	ns
Lucerne	Between all treatments	KWA	29.37	***
Lucerne	Between environments	WRS	422	***
Lucerne	Between species	WRS	599	**
Lucerne	Posthoc BB vs. BL	WRS	125	**
Lucerne	Posthoc BB vs. LB	WRS	197.5	ns
Lucerne	Posthoc BB vs. LL	WRS	69	***
Lucerne	Posthoc BL vs. LB	WRS	315.5	ns
Lucerne	Posthoc BL vs. LL	WRS	121.5	***
Lucerne	Posthoc LB vs. LL	WRS	120	***
Lucerne	Between years	WRS	360	ns
c) Number of unsuccessful attacks				
Thun	Between treatments	WRS	114.5	ns
Thun	Between years	WRS	199	*
Lucerne	Between all treatments	KWA	6.6	trend
Lucerne	Between environments	WRS	696	ns
Lucerne	Between species	WRS	722	ns
Lucerne	Posthoc BB vs. BL	WRS	208.5	ns
Lucerne	Posthoc BB vs. LB	WRS	228	ns
Lucerne	Posthoc BB vs. LL	WRS	172	**
Lucerne	Posthoc BL vs. LB	WRS	263	ns
Lucerne	Posthoc BL vs. LL	WRS	219.5	ns
Lucerne	Posthoc LB vs. LL	WRS	224	ns
Lucerne	Between years	WRS	450	ns

Supplementary Table 3: Correlations between length and a) time to first feeding, b) time food depletion, and c) the number of unsuccessful attacks overall and within treatments. Given is in the first column the lake; in the second column the tested treatment(s); in the third column the spearman correlation coefficient r_{oh} ; and in the fourth column the significance levels (ns = non significant; trend = $p < 0.1$; * = $p < 0.05$; ** < 0.01 ; *** < 0.001). Sample sizes can be found in Table 1.

a) Time to first feeding			
Lucerne	ALL	-0.37	***
Lucerne	BB	-0.25	Ns
Lucerne	BL	-0.19	Ns
Lucerne	LB	-0.02	Ns
Lucerne	LL	0.007	Ns
Thun	ALL	-0.03	Ns
Thun	BB	-0.09	Ns
Thun	LB	0.04	Ns
b) Time to food depletion			
Lucerne	ALL	-0.41	***
Lucerne	BB	-0.67	***
Lucerne	BL	0.01	Ns
Lucerne	LB	-0.01	Ns
Lucerne	LL	-0.22	Ns
Thun	ALL	-0.31	Trend
Thun	BB	-0.18	Ns
Thun	LB	0.06	Ns
c) Number of unsuccessful attacks			
Lucerne	ALL	-0.28	**
Lucerne	BB	-0.23	Ns
Lucerne	BL	-0.24	Ns
Lucerne	LB	-0.09	Ns
Lucerne	LL	-0.25	Ns
Thun	ALL	-0.2	Ns
Thun	BB	-0.08	Ns
Thun	LB	-0.35	Ns

Chapter 3

Experimental evidence for trait utility of gill raker number in adaptive radiation of a north temperate fish

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Abstract

North temperate fish in postglacial lakes are textbook examples for rapid parallel adaptive radiation into multiple trophic specialists within individual lakes. Speciation repeatedly proceeded along the benthic-limnetic habitat axis, and benthic-limnetic sister species diverge in the number of gill rakers. Yet, the utility of different numbers of gill rakers for consuming benthic vs. limnetic food has only very rarely been experimentally demonstrated. We bred and raised families of a benthic-limnetic species pair of whitefish under common garden conditions, to test whether these species i) show heritable differentiation in feeding efficiency on zooplankton and ii) whether variation in feeding efficiency is predicted by variation in gill raker numbers. We used zooplankton of three different size classes to investigate prey size-dependency of divergence in feeding efficiency and to investigate the effect strength of variation in the number of gill rakers. Our results show strong interspecific differences in feeding efficiency. These differences are largest when fish were tested with the smallest zooplankton. Importantly, feeding efficiency is significantly positively correlated with the number of gill rakers when using small zooplankton, also when species identity is statistically controlled for. Our results support the hypothesis that a larger number of gill rakers is of adaptive significance for feeding on zooplankton and provide one of the first experimental demonstrations of trait utility of gill raker number when fish feed on zooplankton. These results are consistent with the suggested importance of divergent selection driven feeding adaptation during adaptive radiation of fish in postglacial lakes.

Introduction

Adaptive radiation is the evolution of phenotypic and ecological diversity within a rapidly multiplying lineage. In such a radiation, a single ancestor species diverges into an array of species that are adapted to diverse environments and evolve morphological, behavioral and physiological traits to exploit those habitats (Schluter, 2000). The process is thought to be driven either by ecological speciation where reproductive isolation arises as a consequence of divergent natural or ecologically-based sexual selection, or by non-ecological speciation followed swiftly by ecological character displacement. One important defining criterion of adaptive radiation is a persistent correlation between phenotype and environment, referred to as phenotype-environment correlation PE (Schluter, 2000). This correlation is thought to arise because of adaptive utility of the phenotype, and this trait utility is another defining criterion of adaptive radiation. Significant PEs are a first indication for such trait utility, but demonstration of trait utility in the strict sense requires experimental evidence that a trait or trait value improves performance in a particular environment or task, after controlling for potentially confounding traits. Such experimental tests are needed to resolve the mechanism underlying the PE and to rule out trivial associations between a phenotype and an environment (Wainwright, 1994; Schluter, 2000).

In adaptive radiations of north temperate fish such as Arctic charr, stickleback and whitefish, species often diverge along the benthic-limnetic habitat axes (Schluter, 1996; Smith & Skúlason, 1996; Taylor, 1999). Usually the limnetic (pelagic) morph is characterized by a compressed body, terminal mouth, large eyes, slow growth and a large number of gill rakers resulting in a high density of gill rakers (densely rakered). In contrast the benthic morph is characterized by a more robust body shape, a sub-terminal mouth, smaller eyes, fast growth and a smaller number of gill rakers resulting in a lower

density of gill rakers (sparsely rakered) (Mc Phail, 1984; Smith & Skúlason, 1996; Taylor, 1999). The repeated parallel evolution of these phenotypic differences in adaptation to similar environments in different and unrelated fish taxa strongly suggests a role for natural selection in species formation of north temperate fish (Rundle et al., 2000; Schluter, 2000). Stomach content and stable isotope analyses have shown that morphological divergence between or within species is associated with divergent resource use in nature (Malmquist et al., 1992; Schluter & McPhail, 1992; Amundsen et al., 2004; Bolnick and Paull, 2009; Harrod et al., 2010), corresponding to the phenotype-environment correlations that define adaptive radiation. The number of gill rakers is one of the traits underlying these correlations and which is thought to be of functional importance for feeding in the benthic vs. limnetic niche (Schluter, 2000). The functional prediction is that a higher density of gill rakers, i.e. the number per length of the gill arch, increases retention capability after capture of small zooplankton by increasing the filtering efficacy of the gill apparatus (Link & Hoff, 1998). Computational fluid dynamics and video endoscopy of suspension-feeding fish indeed found that gill rakers serve as cross-flow filters transporting particles from the oral cavity towards the oesophagus (Sanderson et al., 2001). There is repeated experimental evidence for divergence in feeding efficiency on limnetic prey between benthic and limnetic species of north temperate fish with differences in gill raker numbers (Schluter, 1993; Day & McPhail, 1996; Adams & Huntingford, 2002); in the direction that is consistent with the suggested functional importance of gill rakers for feeding on limnetic prey (Schluter 2000). Such studies can be considered as evidence for trait utility of the n-dimensional phenotype in regard to benthic versus limnetic feeding. And both, the repeated observation of PE between gill rakers and feeding ecology across taxa as well as the repeated documentation of feeding efficiency differences between species with differences in gill raker numbers indicate that trait utility of gill rakers for zooplankton feeding is likely. However, because the association of gill raker number with feeding performance in these studies emerged from between-species comparisons, feeding efficiency effects of gill raker number could not be separated from the effects of other traits, which also differ between species. We are aware of one study that found a correlation of gill raker spacing with feeding performance on limnetic prey within a limnetic stickleback species, suggesting a functional importance of gill rakers in feeding on zooplankton (Bentzen and McPhail 1984). In that study though, feeding performance was regressed against gill raker spacing and fish body size separately, but these two traits were highly intercorrelated ($r = 0.94$), and fish body size explained even more variation in feeding performance than gill rakers did ($r = 0.69$ versus $r = 0.65$). Because the effects of fish size and gill rakers on feeding efficiency could not be disentangled, it can, strictly speaking, not be considered as evidence for trait utility of gill rakers. But such experimental demonstration of trait utility is needed to make an even stronger argument for the adaptive significance of a trait (Schluter, 2000). Despite the ubiquity of gill raker divergence between closely related north temperate fish species, such trait utility of gill rakers for zooplankton feeding has, to our knowledge, only once been experimentally demonstrated so far (Robinson 2000). Robinson (2000) found that food intake rate (*Artemia salina* nauplii eaten/minute) was significantly positively associated with gill raker number in stickleback, also when statistically accounting for fish size and fish type (shallow- vs. open-water). Here we present more experimental evidence for trait utility of gill rakers, to our knowledge the first ever in a species of the group of salmon-like fish

including whitefish and char that are responsible for most of the adaptive radiations of north temperate fish in postglacial lakes.

In our experiments, we used a benthic-limnetic species pair of European whitefish (*Coregonus sp.*). Speciation in whitefish involves divergence along the benthic-limnetic habitat axis (Bernatchez et al., 1996; Lu & Bernatchez, 1999; Ostbye et al., 2006; Landry et al., 2007; Vonlanthen et al., 2012) and morphological differentiation between sympatric whitefish species is strongly apparent in the number of gill rakers and in adult body size (Steinmann, 1950; Bernatchez, 2004; Ostbye et al. 2005, 2006; Siwertsson et al., 2010). In this study we used *C. sp.* „Balchen” and *C. albellus*, a benthic and a limnetic whitefish species from the radiation in Lake Thun, Switzerland (Hudson et al., 2011), to experimentally test for heritable divergence in feeding efficiency on zooplankton prey. We predicted that the phenotypically limnetic species will be generally more efficient in feeding on zooplankton prey than the benthic species. To test the effects of gill rakers on zooplankton feeding efficiency we calculated the ratio between the number of zooplankton items found in the stomach of a fish after a trial and the number of attacks a fish made during a trial. Following Sanderson et al. (2001), we predict that gill rakers serve as filters to increase retention capability and that the number of gill rakers thus scales positively with the ratio between stomach content and the number of attacks not just between but also within species.

Materials and methods

Study species

Lake Thun, Switzerland, harbors five endemic whitefish species (Bittner et al., 2009; Vonlanthen et al., 2012). Speciation occurred within the lake-system following the colonization of a single species after the last glacial maxima some 15 kyr BP (Hudson et al., 2011). Two species from this adaptive radiation, a limnetic, *C. albellus*, and a benthic species, *C. sp.* „Balchen”, were used in this study. They were chosen because they phenotypically correspond to a benthic-limnetic species pair, as it has repeatedly been observed in north temperate fish (Schluter, 2000). *C. sp.* „Balchen” grows rapidly and is rather sparsely rakered with a mean gill raker number of 29 (Steinmann, 1950). The spawning grounds are in shallow water (Steinmann, 1950). *C. albellus* grows slowly and is densely rakered with a mean gill raker number of 38 (Steinmann, 1950). The spawning grounds are typically located deeper than 25m (Steinmann, 1950). Feeding efficiency experiments using benthic food and the same species suggested that *C. sp.* „Balchen” is phenotypically adapted to benthic feeding when compared to *C. albellus* (Lundsgaard-Hansen et al., 2013), whereas functional morphology predictions (Schluter, 2000; Sandersson et al., 2001) and stomach content data from other whitefish with similar phenotypes (Mookerji et al., 1998; Amundsen et al., 2004; Harrod et al., 2010) suggest that *C. albellus* is phenotypically adapted to zooplankton feeding.

Breeding and raising of experimental fish

Experimental fish were bred in winter 2006. Five ripe females and five males were caught of each species. Eggs and sperm were striped in the lab and eggs of all five females were mixed. The eggs were fertilized simultaneously with sperm from the five males, resulting in up to 25 half-sib families per species. Because we cannot rule out mortality differences between families or fertilization success differences among individuals, our results could potentially stem from only a small number

of families. However, we have no indication that this is the case. The juveniles were raised in the lab under common garden conditions. In the first year all juvenile fish were fed with zooplankton until they were large enough to be fed with red mosquito larvae (*Chironomus plumosus*). Fish were fed ad libitum once a day, except on Sundays. During a previous experimental period (from May to September 2009 and again from June to August 2010) as well as during the current experiment (from February 2012 to July 2012) fish were fed once per week with zooplankton to familiarize themselves with the limnetic food-type and to avoid food recognition effects to bias our results. The two species were distributed over four raising tanks, each with a volume of 142*122*50 cm (length x width x height). One raising tank of *C. albellus* was lost due to a technical deficiency. Therefore the limnetic species was raised just in one tank. To account for potential raising tank effects in the benthic species, we nested tank within species for all linear modeling analysis. More details about the breeding and raising of experimental fish can be found elsewhere (Lundsgaard-Hansen et al., 2013).

Experimental set-up

Four tanks, equal in size (142*55*40 cm), were used to carry out the experiments. Experiments were performed from February to July 2012 and experimental conditions were kept similar over time (water temperature always between 10 and 12 °C; illumination as described in Lundsgaard-Hansen et al., 2013). Each tank was subdivided lengthwise using a Plexiglas wall, resulting in one compartment with a volume of 142*33*40 cm and the other compartment with a volume of 142*22*40 cm. The bigger front division was used for the trials. Two fish were placed in the smaller back compartment for the entire duration of the experiments, as single isolated individuals did not display natural behavior. To prevent an observer-induced behavioral change, the front window of the tanks was covered with a reflecting mirror foil, which prevents that fish see the observer. The day before a trial, two benthic and two limnetic fish were randomly distributed over the four experimental tanks (one fish in each tank). The transfer was done by a second person so that the observer was not aware of species identity of tested fish. Fish were not fed for approximately 48 h before trials. At the day of each experiment, fresh zooplankton was caught from Lake Lucerne at a depth of 5-8 m using a plankton net (1.2 m diameter and 250 µm mesh size). Tubes with equal amounts of zooplankton suspended in 150 ml of water were prepared using sieved plankton and a measuring scoop. These were then randomly assigned to experimental tanks. On 6 occasions (6 different days) an additional tube was prepared the same way to count the number of zooplankton items offered per trial, showing that prey density in the experimental tanks was always between 25 and 30 individuals/l.

To start a trial, the suspended zooplankton was tipped into the water. The fish was filmed using a video camera until the end of the trial. After a trial the fish was immediately removed from the tank and euthanized using MS-222. Standard length (referred to as length in Table 1 and 2) was measured and the first gill arch and the stomach/gut were removed. The number of zooplankton items in the stomach and the numbers of gill rakers on the first left gill arch were then counted using a binocular.

For each individual we measured multiple feeding efficiency variables. The time until a fish started feeding is referred to as "Time to first attack". Four (three benthic and one limnetic) fish did not start feeding during 15 minutes. These fish were excluded from analyses. After the first attack, each fish was allowed to feed for five minutes. The number of attacks during the five minutes of feeding was

counted on the videotape and is referred to as „Number of attacks“. Zooplankton items in the stomach were counted and are referred to as „Stomach content“. This value was always lower than the number of attacks, for two different, but potentially interacting reasons: Fish can either fail to capture zooplankton despite an attack or they can succeed in capturing it, but fail to retain it. „Capture and/or retention capability“ (CRC) is defined as „Number of zooplankton items in the stomach“ divided by „Number of attacks“. To test our first prediction of heritable feeding efficiency divergence between species, we analyzed all variables separately. As the results were fully consistent across variables and as we use them all to test the same prediction, we did not correct for potential inter-correlation between variables. To test our second prediction of a positive relationship between gill rakers and feeding efficiency we analyzed CRC only, because the functional prediction of increased retention capability of zooplankton with increasing gill raker number specifically applies to this variable.

To test for prey size dependency of feeding efficiency, three different plankton size classes were tested. The category „Large“ contains all caught zooplankton. For the category „Medium“ we removed all plankton above a size of 500 μm using a zooplankton sieve and for the category „Small“ we removed all zooplankton larger than 355 μm . In total 55 fish were tested of which 29 belonged to *C. sp. Balchen* and 26 to *C. albellus*. 14 fish were tested using large zooplankton (8 *C. sp. Balchen* and 6 *C. albellus*), 12 fish were tested using medium zooplankton (6 each) and 29 fish (15 *C. sp. Balchen* and 14 *C. albellus*) were tested using small zooplankton.

Data analysis

All statistical analyses were conducted using R statistical software (R Development Core Team, 2010). Morphological differentiation (standard length and number of gill rakers) between species was assessed using Mann Whitney U Test. Feeding efficiency was analyzed using linear regression models doing backward model selection and always keeping the main effect (species) as well as the factor nested within it (tank). The four efficiency metrics (see above) were analyzed separately. Model selection was done by removing the variable with the highest p-value as long as the model likelihood (AIC/AICc) increased. The most likely model is the one with the lowest AIC/AICc (none of our main findings is depending on critically low AIC/AICc support, see Burnham and Anderson 1998). Model selection was done for zooplankton size categories pooled and for each zooplankton size category separately (see Table 1). AIC was calculated for the pooled zooplankton size models, while AICc (AICc is AIC corrected for sample size, applied when $n < 40$) was calculated for the models analyzing zooplankton sizes separately (Burnham and Anderson 1998). Residuals of each model were checked for normal distribution using Shapiro Wilk Test to make sure linear modeling assumptions were met. When residuals were not normally distributed, the response variable was transformed to achieve a fit to a normal error distribution (log transformation for „Time to first attack“ pooled zooplankton size categories, square root transformation for „Stomach content“ pooled zooplankton size categories).

Results

Morphological differences between species

Standard lengths was significantly lower in the limnetic species than in the benthic species (Mean length limnetic species = 125 mm, standard deviation = 8; mean length benthic species = 136mm, standard deviation = 14; Mann Whitney U Test: $n = 55$, $W = 527.5$, $P = 0.02$) and gill raker numbers were much higher in the limnetic than in the benthic species (Mean limnetic species = 36.5, standard deviation = 1.2; mean benthic species = 26.8, standard deviation = 1.9; Mann Whitney U Test: $n = 55$, $W = 781.5$, $P < 0.001$).

Species differences in feeding efficiency

When we looked at the data from all trials pooled, independent of the zooplankton size, the limnetic species was clearly more efficient than the benthic species in all efficiency metrics taken (Figure 1a-1d).

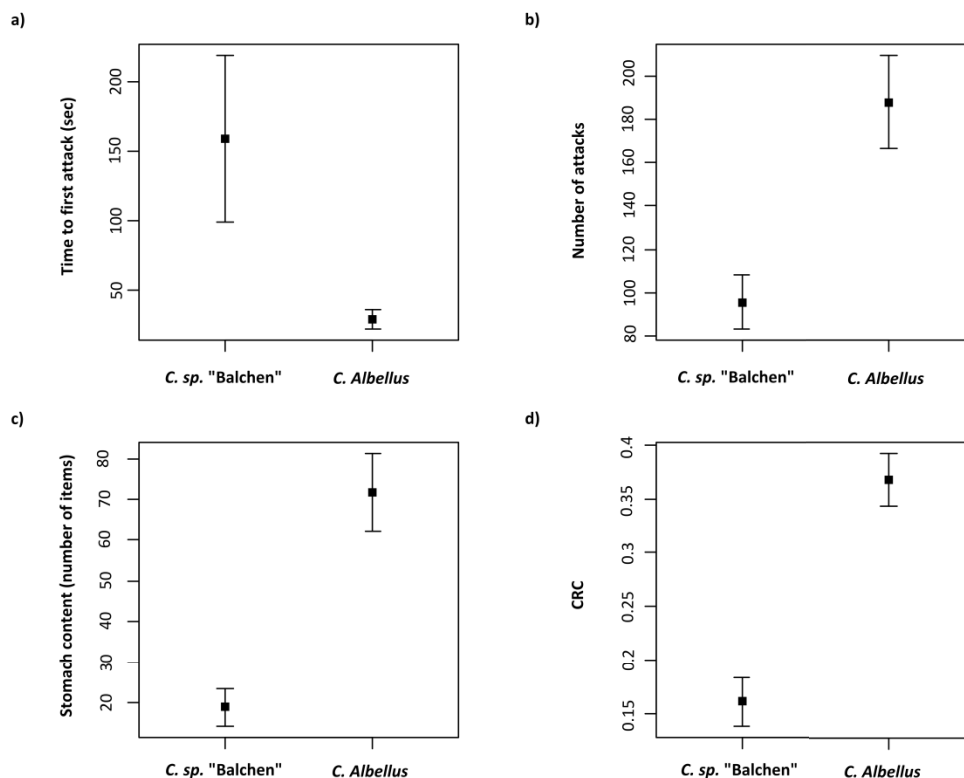


Figure 1: Feeding efficiency differentiation between species. Given is on the y-axis the mean and standard deviation per species ($N_{C. sp. Balchen} = 29$, $N_{C. albellus} = 26$) for a) time to first attack (seconds), b) the number of attacks (in five minutes of feeding), c) the number of items in the stomach after the trial, d) CRC (items in the stomach divided by the number of attacks). *C. sp. "Balchen"* is the phenotypically benthic species, *C. albellus* the phenotypically limnetic species.

When analyzing the data using linear modeling and backward model selection, the species effect was highly significant for all efficiency metrics, except for CRC (the ratio between the number of items in the stomach and the number of attacks) (Table 1). The reason that species differences are not

significant in the CRC model is the inclusion of the number of gill rakers in the most likely model, which shares a lot of information with the species factor (removal of one of these variables results in highly significant effects of the other one with p -values < 0.001). In the most likely model, gill rakers explained a larger fraction of the variation in CRC than did species (F_1 species = 0.66; F_1 Gill rakers = 2.18). This model also revealed that CRC was significantly positively associated with zooplankton size (Table 1; Figure 2a). A “species X zooplankton size”-interaction was not significant in the CRC model including all zooplankton sizes (not shown). However, when looking at the two species separately, we found significant zooplankton size effects on CRC only in the sparsely rakered species *C. sp. “Balchen”* (Model: $\text{CRC}_{\text{Benthic species}} = \text{Zooplankton Size} + \text{Tank}$, F_2 zooplankton size = 7.09, $P = 0.003$, F_1 tank = 2.1, $P = 0.15$; model: $\text{CRC}_{\text{Limnetic species}} = \text{Zooplankton Size}$, F_2 zooplankton size = 0.15, $P = 0.87$, Figure 2a). As shown by the models, rearing tank effects are non-significant, the direction of the effect of zooplankton size on CRC is the same in benthic fish coming from the two different rearing tanks (Figure 2a). But we found tank effects on time to first attack in the benthic species.

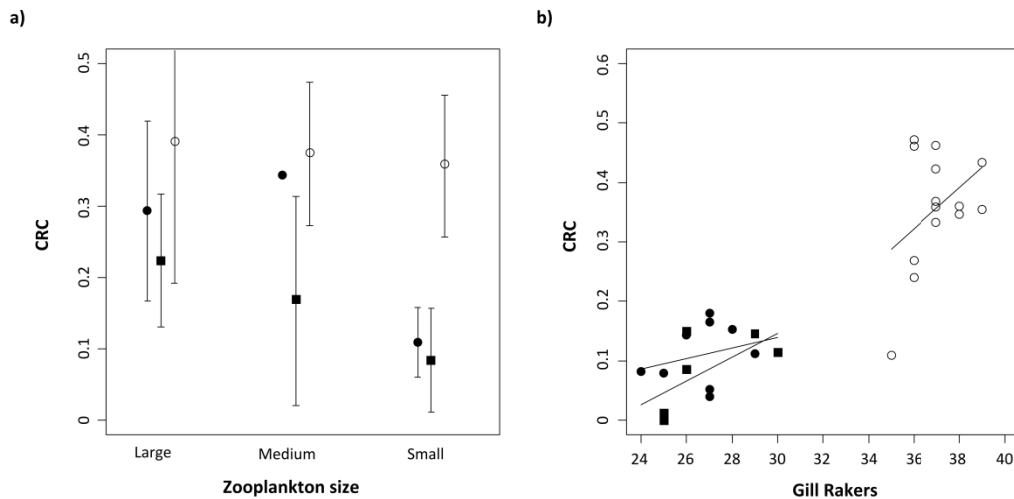


Figure 2: Capture and/or retention capability (CRC; items in the stomach divided by the number of attacks) depends on zooplankton size and the number of gill rakers. Filled symbols correspond to *C. sp. “Balchen”* (sparsely rakered) and empty symbols to *C. albellus* (densely rakered). Filled circles and squares represent the different raising tanks of the benthic species. Given is on the x-axis the CRC against a) zooplankton size and b) against the number of gill rakers when using small zooplankton size. Lines in panel b correspond to linear regression lines within species (or within tank for the benthic species) and error bars represent standard deviations. Sample sizes (from large to small zooplankton) are: *C. albellus* $n=6$, $n=6$, $n=14$; *C. sp. “Balchen”* $n=8$ (3/5), $n=6$ (1/5), $n=15$ (7/8). Numbers in brackets correspond to sample sizes per raising tank of *C. sp. “Balchen”*.

In a next step we analyzed the data separately by zooplankton size category. The limnetic species was significantly more efficient in two of the four feeding efficiency metrics for both the large and the medium zooplankton (Table 1). Neither gill raker number nor fish length affected any feeding efficiency metric when we used large or medium sized zooplankton (Table 1). With the medium sized zooplankton class we again found tank effects on the time to first feeding. When we looked at small

zooplankton only, the number of gill rakers was retained in the final CRC model, with significant positive effects on CRC, which were also retained when species was included as a factor (Table 1 and 2, Figure 2b). The relationship of gill raker number and CRC is the same in both species and is independent of the rearing tank of a fish, suggesting that tank effects are very unlikely to bias our results (Figure 2b). Further, smaller fish had a shorter latency time to first attack than larger fish, independent of species identity (Table 1).

Exact test-statistics of all linear model analysis can be found in Table 2.

Table 1: Backward linear model selection. In the first column plankton size categories included in the analysis are reported. In the second column the models are listed, with their respective AIC/AICc in the third column. The model with the lowest AIC is the most likely model. Significant variables are highlighted in bold and significant levels are indicated with *: $p < 0.05$; **: $p < 0.01$, ***: $p < 0.001$. Length corresponds to fish standard length, size corresponds to zooplankton size category.

Plankton Size	Model	AIC/AICc
All pooled	Time to first attack=Species/Tank+Gill rakers+Length+Size	197.74
All pooled	Time to first attack=Species/Tank+Gill rakers+Length	194.05
All pooled	Time to first attack=Species/ Tank* +Gill rakers	192.05
All pooled	Time to first attack= Species**/Tank*	190.42
All pooled	Number of attacks=Species/Tank+Gill rakers+Length+Size	658.68
All pooled	Number of attacks= Species**/Tank +Length+Size	656.83
All pooled	Number of attacks= Species*** /Tank+Length	653.48
All pooled	Number of attacks= Species*** /Tank	652.18
All pooled	Stomach content=Species/Tank+Gill rakers+Length+Size	278.97
All pooled	Stomach content=Species/Tank+Gill rakers+Size	277.18
All pooled	Stomach content= Species*** /Tank+Size	276.19
All pooled	Stomach content= Species*** /Tank	274.03
All pooled	CRC=Species/Tank+Gill rakers+Length+ Size*	-72.63
All pooled	CRC=Species/Tank+Gill rakers+ Size*	-74.62
Large	Time to first attack=Species/Tank+Gill rakers+Length	185.36
Large	Time to first attack=Species/ Tank +Gill rakers	179.21
Large	Time to first attack=Species/Tank	171.79
Large	Number of attacks=Species/Tank+Gill rakers+Length	169.35
Large	Number of attacks=Species/Tank+Length	162.97
Large	Number of attacks= Species* /Tank	157.92
Large	Stomach content=Species/Tank+Gill rakers+Length	152.02
Large	Stomach content=Species/Tank+Length	145.54
Large	Stomach content= Species* /Tank	140.61
Large	CRC=Species/Tank+Gill rakers+Length	9.07
Large	CRC=Species/Tank+Gill rakers	2.57
Large	CRC=Species/Tank	-2.43
Medium	Time to first attack=Species/Tank+Gill rakers+Length	60.89
Medium	Time to first attack=Species/Tank+SL	52.24
Medium	Time to first attack= Species*/Tank*	47.28
Medium	Number of attacks=Species/Tank+Gill rakers+Length	161.96
Medium	Number of attacks= Species* /Tank+Length	153.91

Medium	Number of attacks=Species/Tank	153.19
Medium	Stomach content=Species/Tank+Gill rakers+Length	143.18
Medium	Stomach content=Species/Tank+Gill rakers	135.29
Medium	Stomach content=Species/Tank	132.8
Medium	CRC=Species/Tank+Gill rakers+Length	6.25
Medium	CRC=Species/Tank+Gill rakers	-2.44
Medium	CRC= Species* /Tank	-4.26
Small	Time to first attack=Species/Tank+Gill rakers+ Length*	350.41
Small	Time to first attack=Species/Tank+ Length*	348.24
Small	Number of attacks=Species/Tank+Gill rakers+Length	362.78
Small	Number of attacks=Species/Tank+Length	359.74
Small	Number of attacks= Species* /Tank	357.28
Small	Stomach content=Species/Tank+Gill rakers+Length	312.01
Small	Stomach content= Species** /Tank+Length	308.85
Small	Stomach content= Species*** /Tank	306.55
Small	CRC=Species/Tank+Gill rakers+Length	-56.15
Small	CRC=Species/Tank+ Gill rakers*	-59.02

Table 2: Summary of the most likely models. Given are in the columns from left to right the zooplankton size included in the analysis (Size), the response variable (Variable), the error mean square of the model (Error MS) and associated degrees of freedom (df) and the explanatory variables included in the most likely model (Species, Rearing Tank (Tank), Gill Rakers, Fish Length (Length), and zooplankton size (Size)). Tank is nested within species. For each explanatory variable we reported the p-value, the F-ratio, and the degrees of freedom, separated using “;”. Empty cells indicate that a particular variable was not included in the most likely model (see Table 1), “na” indicated that zooplankton size was only included in the models with all zooplankton sizes pooled.

Size	Variable	Error MS	Df	Species	Tank	Gill Rakers	Length	Size
All	Time to first attack	1.7	52	0.002; 10.4; 1	0.01; 6.7; 1			
All	Number of attacks	7559.6	52	<0.001; 15.5; 1	0.15; 2.1; 1			
All	Stomach content	7.8	52	<0.001; 31.6; 1	0.19; 1.8; 1			
All	CRC	0.01	49	0.42; 0.7; 1	0.2; 1.7; 1	0.15; 2.2; 1		0.02; 4.2; 1
Large	Time to first attack	8977.2	11	0.55; 0.4; 1	0.09; 3.5; 1			na
Large	Number of attacks	2427.5	11	0.02; 7.4; 1	0.24; 1.5; 1			na
Large	Stomach content	704.7	11	0.02; 7.6; 1	0.29; 1.2; 1			na
Large	CRC	0.03	11	0.14; 2.6; 1	0.57; 0.3; 1			na
Medium	Time to first attack	1.3	9	0.01; 9.3; 1	0.02; 7.4; 1			na
Medium	Number of attacks	8711.3	9	0.06; 4.6; 1	0.26; 1.5; 1			na
Medium	Stomach content	1593.6	9	0.08; 3.8; 1	0.35; 0.9; 1			na
Medium	CRC	0.02	9	0.05; 5.3; 1	0.26; 1.5; 1			na
Small	Time to first attack	7216.7	25	0.18; 1.9; 1	0.77; 0.09; 1		0.03; 5.4; 1	na
Small	Number of attacks	10489.7	26	0.03; 5.6; 1	0.56; 0.3; 1			na
Small	Stomach content	1823.8	26	<0.001; 15.3; 1	0.83; 0.05; 1			na
Small	CRC	0.01	25	0.7; 0.15; 1	0.44; 0.6; 1	0.04; 4.6; 1		na

Discussion

Our experiments demonstrate genetically heritable divergence in feeding efficiency on zooplankton prey between a sympatric benthic-limnetic species pair of whitefish (*Coregonus spp.*). Feeding efficiency divergence is highly significant in all the four measured feeding efficiency metrics. And we provide one of the first experimental evidence for a role of variation in gill raker numbers in determining feeding efficiency on zooplankton prey. Below we discuss these results in more detail.

Evidence for trait utility of gill rakers

Divergence in the number of gill rakers is characteristic for many benthic-limnetic species pairs of north temperate fish (McPhail, 1984; Schluter, 1996; Smith & Skúlason, 1996; Taylor, 1999). Such widespread, phenotypically parallel divergent evolution between niches is a strong indication for the action of divergent selection on the numbers of gill rakers between the contrasting benthic vs. limnetic feeding environments (Schluter, 2000). The observation that densely rakered species usually have a higher proportion of planktonic prey in their stomach than sparsely rakered species, suggests either that densely rakered species feed more frequently or more effectively on zooplankton compared to sparsely rakered species (Amundsen et al., 2004; Harrod et al., 2010). These findings are consistent with experiments showing heritable species divergence in feeding efficiency in various taxa of north temperate fish (Schluter, 1993; Day & McPhail, 1996; Adams & Huntingford, 2002), but experimental evidence for trait utility of gill rakers for feeding in the limnetic niche is still rare (but see Robinson et al. 2000 for an example).

Our data provide several lines of evidence for such trait utility. Significant negative effects of zooplankton size on CRC (the ratio between the number of items in the stomach and the number of attacks) show that zooplankton feeding efficiency is prey-size dependent (Table 1). We find zooplankton size effects on CRC mainly in the benthic species that has the lower number of gill rakers (Figure 2a). This pattern is consistent with the common idea that a higher number of gill rakers is facilitating filter feeding especially on small zooplankton prey (Link & Hoff, 1998; Kahilainen et al., 2011). As variation in CRC can result from differences either in capture efficiency or in retention capability after capture, zooplankton size effects on CRC (Table 1) could also arise as a consequence of decreased capture efficiency with decreasing zooplankton size as opposed to decreased retention capability after successful capture. However, in the most likely model explaining CRC when using small zooplankton, gill rakers are included and have significant effects on CRC also when effects of species identity are controlled for (Table 1, Figure 2b). As differences in capture efficiency are unlikely to be driven by gill raker variation, we take this as evidence that a higher number of gill rakers increases retention capability of zooplankton. This result was confirmed when we analyzed the data with all zooplankton size classes pooled: The number of gill rakers was retained in the most likely model, it is positively correlated with CRC and explained more variation in CRC than species did ($F\text{-ratio}_{\text{species}} = 0.66$; $F\text{-ratio}_{\text{gill rakers}} = 2.18$, Table 1). The strength of the effect of gill rakers on CRC (when keeping all explanatory variables in the model, see Table 1) increased with decreasing zooplankton size ($F\text{-ratio}_{\text{gill rakers with large zooplankton}} = 0.03$; $F\text{-ratio with medium zooplankton}} = 3.24$; $F\text{-ratio with small zooplankton}} = 3.97$), again suggesting that more numerous gill rakers are facilitating zooplankton feeding especially when zooplankton is small. We cannot rule out effects on feeding efficiency of phenotypic traits linked to gill raker number (for example traits related to the

visual system or swimming skills). However, the number of gill rakers is a quantitative trait and has been shown to be controlled in other whitefish species by multiple genes from at least three different linkage groups (Rogers & Bernatchez, 2007). Physical linkage seems therefore rather unlikely to confound the interpretation of our findings. Our interpretation of trait utility of gill rakers for zooplankton feeding in whitefish is consistent with phenotype environment correlations between gill rakers and feeding ecology between species (Amundsen et al., 2004; Harrod et al., 2010), with experimental demonstration of feeding efficiency divergence between benthic and limnetic species in species pairs of north temperate freshwater fish (Bentzen and McPhail, 1984; Day & McPhail, 1996; Adams & Huntingford, 2002) and with previous experimental evidence for trait utility of gill rakers in stickleback (Robinson 2000). It is also consistent with a comparative analysis of whitefish across Scandinavian lakes, which found that mean zooplankton size is lower in lakes that have a densely rakered ecotype of whitefish besides the widespread sparsely rakered ecotype than in lakes that only have the latter (Kahilainen et al., 2007, 2011).

That fish length affected time to first attack of small zooplankton suggests larger fish are less motivated to feed on small zooplankton. Optimal foraging constraints are one possible interpretation of this result if energetic profitability of foraging on very small prey is low for larger fish (Schoener, 1971). We want to also note, that the observation of frequent and strong species effects, on other feeding efficiency variables than CRC, indicate that there are, beside gill raker number, additional traits (e. g. body shape, properties of the eye, behavioral traits and others) that differ between species and that strongly affected zooplankton feeding efficiency.

Evidence for functional trade-offs

Evidence for feeding efficiency divergence between benthic and limnetic sister species had previously been shown in other north temperate fish such as stickleback and Arctic charr (Day & McPhail, 1996; Adams & Huntingford, 2002), but here we present to our knowledge the first experimental evidence for divergence in feeding efficiency on limnetic food between benthic limnetic whitefish species. In a previous experiment we tested feeding efficiency of the same species studied here on benthic food and found the opposite pattern of efficiency divergence, with *C. sp.* “Balchen” being the more efficient forager on benthic food than *C. albellus* (Lundsgaard-Hansen et al., in press). Together with the results from this study, this shows reciprocally adaptive divergence in feeding efficiency. Such functional trade-offs between distinct environments have previously been shown in other postglacial fish (Schluter 1995; Robinson et al., 1996) and they are strong evidence for the importance of divergent natural selection in shaping environment-dependent (benthic vs. limnetic) feeding efficiencies. As functional trade-offs are necessary for divergent selection to cause evolutionary divergence, these results suggest that divergent natural selection on traits involved in foraging contribute to ecological diversification and speciation in whitefish and other fishes characterized by similar eco-morphological differentiation among species.

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References

- Adams, C. E. & Huntingford, F. A. 2002. The functional significance of inherited differences in feeding morphology in a sympatric polymorphic population of Arctic charr. *Evol. Ecol.* 16: 15-25.
- Amundsen, P. A., Knudsen, R., Klemetsen, A. & Kristoffersen, R. 2004. Resource competition and interactive segregation between sympatric whitefish morphs. *Ann. Zool. Fenn.* 41: 301-307.
- Bentzen, P. & McPhail, J. D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): specialization for alternative trophic niches in the Enos Lake species pair. *Can. J. Zoolog.* 62: 2280-2286.
- Bernatchez, L. 2004. Ecological Theory of Adaptive Radiation. An Empirical Assessment from Coregonine Fishes (Salmoniformes). In: *Evolution Illuminated* (Hendry, A. P. & Stearns, S. C., eds.). pp. 175-207. Oxford University Press, Oxford.
- Bernatchez, L., Vuorinen, J. A., Bodaly, R. A. & Dodson, J. J. 1996. Genetic evidence for reproductive isolation and multiple origins of sympatric trophic ecotypes of whitefish (*Coregonus*). *Evolution* 50: 624-635.
- Bittner, D., Excoffier, L. & Largiader, C. R. 2010. Patterns of morphological changes and hybridization between sympatric whitefish morphs (*Coregonus* spp.) in a Swiss lake: a role for eutrophication? *Mol. Ecol.* 19: 2152-2167.
- Bolnick, D., & Paull, J. F. 2009. Morphological and dietary differences between individuals are weakly but positively correlated within a population of threespine stickleback. *Evol. Ecol. Res.* 11: 1217-1233.
- Burnham, K. P. & Anderson, D. R. 1998. *Model Selection and Inference: A Practical Information-Theoretical Approach*. Springer-Verlag, New York.
- Day, T. & McPhail, J. D. 1996. The effect of behavioural and morphological plasticity on foraging efficiency in the threespine stickleback (*Gasterosteus* sp). *Oecologia* 108: 380-388.
- Grant, P. R. 1981. The feeding of Darwin's finches on *Tribulus cistoides* (L.) seeds. *Anim. Behav.*: 785-793.
- Harrod, C., Mallela, J. & Kahilainen, K. K. 2010. Phenotype-environment correlations in a putative whitefish adaptive radiation. *J. Anim. Ecol.* 79: 1057-1068.
- Hudson, A. G., Vonlanthen, P. & Seehausen, O. 2011. Rapid parallel adaptive radiations from a single hybridogenic ancestral population. *P. Roy. Soc. B-Biol. Sci.* 278: 58-66.
- Kahilainen, K. K., Malinen, T., Tuomaala, A., Alajaervi, E., Tolonen, A. & Lehtonen, H. 2007. Empirical evaluation of phenotype-environment correlation and trait utility with allopatric and sympatric whitefish, *Coregonus lavaretus* (L.), populations in subarctic lakes. *Biol.J. Linn. Soc.* 92: 561-572.
- Kahilainen, K. K., Siwertsson, A., Gjelland, K. O., Knudsen, R., Bohn, T. & Amundsen, P. A. 2011. The role of gill raker number variability in adaptive radiation of coregonid fish. *Evol. Ecol.* 25: 573-588.
- Landry, L., Vincent, W. F. & Bernatchez, L. 2007. Parallel evolution of lake whitefish dwarf ecotypes in association with limnological features of their adaptive landscape. *J. Evol. Biol.* 20: 971-984.
- Link, J. & Hoff, M. H. 1998 Relationship of Lake Herring (*Coregonus artedii*) gill raker characteristics to retention probabilities of zooplankton prey. *J. Freshwater Ecol.* 13: 55-65.

- Lu, G. Q. & Bernatchez, L. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): Support for the ecological speciation hypothesis. *Evolution* 53: 1491-1505.
- Lundsgaard-Hansen, B., Matthews, B., Vonlanthen, P., Taverna, A. & Seehausen, O. 2013. Adaptive plasticity and genetic divergence in feeding efficiency during parallel adaptive radiation of whitefish (*Coregonus* spp.). *J. Evol. Biol.* 26: 483-498.
- Malmquist, H. J., Snorrason, S. S., Skulason, S., Jonsson, B., Sandlund, O. T. & Jonasson, P. M. 1992. Diet Differentiation in Polymorphic Arctic Charr in Thingvallavatn, Iceland. *J. Anim. Ecol.* 61: 21-35.
- Mc Phail, J. D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. *Can. J. Zool.* 62: 1402-1408.
- Mookerji, N., Heller, C., Meng, H. J., Burgi, H. R. & Muller, R. 1998. Diel and seasonal patterns of food intake and prey selection by *Coregonus* sp. in re-oligotrophicated Lake Lucerne, Switzerland. *J. Fish Biol.* 52: 443-457.
- Ostbye, K., Amundsen, P. A., Bernatchez, L., Klemetsen, A., Knudsen, R., Kristoffersen, R., Naesje, T. F. & Hindar, K. 2006. Parallel evolution of ecomorphological traits in the European whitefish *Coregonus lavaretus* (L.) species complex during postglacial times. *Mol. Ecol.* 15: 3983-4001.
- Ostbye, K., Naesje, T. F., Bernatchez, L., Sandlund, O. T. & Hindar, K. 2005. Morphological divergence and origin of sympatric populations of European whitefish (*Coregonus lavaretus* (L)) in Lake Femud, Norway. *J. Evol. Biol.* 18: 683-702.
- R Development Core Team. 2010. R: A language and environment for Statistical Computing. Vienna, Austria. <http://www.R-project.org>.
- Robinson, B. W., Wilson, D. S. & Shea, G. O. 1996. Trade-offs of ecological specialization: An intraspecific comparison of pumpkinseed sunfish phenotypes.
- Robinson, B. W. 2000. Trade offs in habitat-specific foraging efficiency and the nascent adaptive divergence of sticklebacks in lakes. *Behaviour*, 137: 865-888.
- Rogers, S. M. & Bernatchez, L. 2007. The genetic architecture of ecological speciation and the association with signatures of selection in natural lake whitefish (*Coregonas* sp Salmonidae) species pairs. *Mol. Biol. Evol.* 24: 1423-1438.
- Rundle, H. D., Nagel, L., Boughman, J. W. & Schluter, D. 2000. Natural selection and parallel speciation in sticklebacks. *Science* 287: 306-308.
- Sanderson, S. L., Cheer, A. Y., Goodrich, J. S., Graziano, J. D. & Callan, W. T. 2001. Crossflow filtration in suspension-feeding fishes. *Nature* 412: 439-441.
- Schluter, D. 1993. Adaptive radiation in sticklebacks: Size, shape and habitat use efficiency. *Ecology* 74: 699-709.
- Schluter, D. 1996. Ecological speciation in postglacial fish. *Phil. Trans. R. Soc. Lond. B.* 351: 807-814.
- Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Schluter, D. & McPhail, J. D. 1992. Ecological character displacement and speciation in sticklebacks. *Am. Nat.* 140: 85-108.
- Schoener, T. W. 1971. Theory of Feeding Strategies. *Annu. Rev. Ecol. Syst.* 2: 369-404.

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- Siwertsson, A., Knudsen, R., Kahilainen, K., Praebel, K., Primicerio, R. & Amundsen, P. A. 2010. Sympatric diversification as influenced by ecological opportunity and historical contingency in a young species lineage of whitefish. *Evol. Ecol. Res.* 12: 929-948.
- Smith, T. B. & Skúlason, S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu. Rev. Ecol. Syst.* 27: 111-133.
- Steinmann, P. 1950. Monographie der schweizerischen Koregonen. Beitrag zur Entstehung neuer Arten. Spezieller Teil. *Schweiz. Z. Hydrol.* 12: 340-491.
- Taylor, E. B. 1999. Species pairs of north temperate freshwater fishes: Evolution, taxonomy, and conservation. *Rev. Fish Biol. Fisher.* 9: 299-324.
- Vonlanthen, P., Bittner, D., Hudson, A. G., Young, K. A., Muller, R., Lundsgaard-Hansen, B., Roy, D., Di Piazza, S., Largiader, C. R. & Seehausen, O. 2012. Eutrophication causes speciation reversal in whitefish adaptive radiations. *Nature* 482: 357-U1500.
- Wainwright, P. C. 1994. Functional morphology as a tool in ecological research. In *Ecological morphology*. Chicago University Press, Chicago.

Chapter 4

Ecological speciation and phenotypic plasticity in fish affect ecosystems

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Abstract

Phenotypic differences among closely related populations or species can cause contrasting effects on ecosystems; however, it is unknown whether such effects result from genetic divergence and/or phenotypic plasticity. To test this, we reared sympatric limnetic and benthic species of whitefish from a recent adaptive radiation in a common garden with two food treatments in one species. Using these fish we conducted a mesocosm experiment to test whether the contrasting ecosystem effects of the closely related species were caused by phenotypic plasticity, recent genetic divergence among species, or both. We found that strong contrasting ecosystem effects resulted more frequently from genetic divergence, but they were not stronger overall than those resulting from phenotypic plasticity. Overall, our results provide evidence that adaptive radiation can have an important role in structuring ecosystems, and, for the first time, demonstrate that phenotypic plasticity can modify the ecosystem effects of species in an adaptive radiation.

Introduction

It is well established that adaptation to contrasting ecological conditions can contribute to evolutionary diversification (Schluter, 2000; Nosil, 2012), but less is known about how phenotypic diversification, speciation, and adaptive radiation can affect ecosystem properties and functions (Harmon *et al.*, 2009; Seehausen, 2009; Schoener, 2011). Recent experimental work has revealed that closely related organisms with different phenotypes can have contrasting effects on a wide range of structural or functional aspects of ecosystems (Harmon *et al.*, 2009; Post & Palkovacs, 2009; Bassar *et al.*, 2010). For example, phenotypic differentiation in the foraging traits of allopatric alewife fish populations (*Alosa pseudoharengus*) affects the species composition, size structure, and life-history traits of their zooplankton prey (Post *et al.*, 2009; Walsh & Post, 2011). Similarly, a recent speciation event of stickleback (*Gasterosteus aculeatus*), which is associated with adaptation to divergent ecological conditions in lakes (1), affects prey community structure, as well as primary production and dissolved organic compounds in experimental aquatic mesocosms (Harmon *et al.*, 2009). Such studies suggest that the effects of evolutionary diversification can have far reaching effects on ecosystems, but because these previous experiments only used wild caught organisms they do not disentangle whether the contrasting ecosystem effects of closely related organisms are caused by genetic divergence per se or by phenotypic plasticity.

Phenotypic plasticity is a common feature of adaptive radiation (West-Eberhard, 2003). Plasticity not only affects many ecological processes, such as population dynamics, trophic cascades, and species interaction networks (reviewed in Miner *et al.*, 2005), but it could also plausibly underlie the contrasting ecosystem effects of closely related species (Harmon *et al.*, 2009) and of different populations of the same species (Post *et al.*, 2008; Bassar *et al.*, 2010; Matthews *et al.*, 2011a). It is important to distinguish between ecosystem effects caused by genetic divergence versus phenotypic plasticity, because if effects largely result from phenotypic plasticity then the buildup of reproductive isolation itself (i.e. speciation) may not be necessary to explain variation in ecosystem effects among individuals within a population (Seehausen, 2009; Hanski, 2011).

In this study, we used a sympatric benthic and limnetic species pair of whitefish, to study the ecosystem effects of phenotypic plasticity and genetic differentiation among closely related sister

species. We first raised whitefish in common garden for three years, with two food treatments in one species, and then used the reared fish as treatments in a subsequent common gardening experiment. In a common gardening experiment (*sensu* Matthews *et al.*, 2011b) the treatments are organisms with phenotypic (or genetic) differences among them, and the goal is to quantify how they differ in their effects on ecosystem properties and functions (Matthews *et al.*, 2011b). Our plasticity treatment focused on foraging traits because they are commonly implicated in ecosystem effects of predators (Palkovacs and Post, 2009) and might be particularly important in adaptive radiations where species differ in traits related to resource acquisition (Schluter, 2000).

In the adaptive radiation of Swiss whitefish, more than 40 species have arisen within less than 15'000 years (Vonlanthen *et al.*, 2012) and phylogenetic evidence suggests that this diversity has originated through at least five independent adaptive radiations situated in different lakes or lake systems (Hudson *et al.*, 2011). The species we used here (*C. sp.* "Bodenbalchen" and *C. zugensis*) belong to a monophyletic radiation endemic to Lake Lucerne (Hudson *et al.*, 2011). The benthic species *C. sp.* "Bodenbalchen" is larger at maturity and has fewer gill rakers than the limnetic species *C. zugensis* (Vonlanthen *et al.*, 2012). This divergence morphology is supported by experimental work showing strong genetic differences in feeding efficiency between *C. sp.* "Bodenbalchen" and *C. zugensis* (Lundsgaard-Hansen *et al.*, 2013). However, phenotypic differentiation between these species is not limited to foraging traits, as they also differ in spawning depth, habitat use and other traits (Vonlanthen *et al.*, 2009, Woods *et al.*, 2009; Karvonen *et al.*, 2012; Vonlanthen *et al.*, 2012).

We performed a common gardening experiment with the following three treatments: (1) the benthic species (B), *C. sp.* "Bodenbalchen", raised on benthic food (benthic benthic = BB), (2) the benthic species raised on limnetic food (benthic limnetic = BL), and (3) the limnetic species (L), *C. zugensis*, raised on limnetic food (limnetic limnetic = LL). We refer to genetic effects on ecosystems when comparing treatments with fish belonging to different species but raised on the same food (BL vs. LL), to plasticity effects when comparing treatments with the same species raised on different food (BB vs. BL), and to combined effects when comparing treatments with different species raised on food sources matching their natural habitat (BB vs. LL). We did not use a full factorial design and so the inference we make about the effects of phenotypic plasticity (BB vs. BL) pertain to the species we raised on both food types (*C. sp.* "Bodenbalchen") and the inference we make about genetic effect pertain to fish raised on limnetic food (zooplankton). In the scenario that phenotypic plasticity solely determines the ecosystem effects of whitefish (Figure 1a), we would expect mesocosms containing the same species raised on different food to generate contrasting ecosystem states, and mesocosms containing different species raised on the same food to have similar ecosystem states (see Table 1 for the suite of ecosystem metrics defining the ecosystem state). If genetic differences among species cause ecosystem effects that are independent of phenotypic plasticity, we would expect large contrasts between LL and BL and small contrasts between BL and BB (Figure 1b). If ecosystem effects result from a combination of both adaptive plasticity and genetic differences, then we would expect mesocosms with the BB and the LL treatment to have the most divergent ecosystems, while those containing the BL treatment to be intermediate (Figure 1c).

Based on our experimental design we made the following three predictions. First, we predicted that ecosystem differences among treatments would arise due to both genetic divergence and plastic differences. This was based on previous work showing effects of both genetic divergence and phenotypic plasticity on the feeding efficiency in the same whitefish species used here (Lundsgaard-Hansen *et al.*, 2013). Second, we predicted that phenotypic differences arising from plasticity would have weaker effects on ecosystems than those arising from genetic differences (Figure 1d). This is partly because our plasticity treatment was only targeted toward foraging traits, some of which are highly heritable (Bernatchez, 2004) and this may effectively limit the phenotypic differentiation caused by our plasticity treatment. Additionally, phenotypic differentiation between the different whitefish species we studied is not restricted to foraging traits, and other dimensions of phenotypic differentiation could underlie contrasting ecosystem effects (Vonlanthen *et al.*, 2009; Woods *et al.*, 2009; Karvonen *et al.*, 2012). Third, we predicted that contrasts between treatments would be larger for the direct consumptive effects of whitefish on their prey (referred to as trophic effects) rather than those resulting either from direct non-trophic effects (e.g. nutrient cycling) or from indirect trophic effects (e.g. cascading interactions) (Figure 1e). For simplicity, we collectively refer to such effects as non-trophic effects, even though some effects may be indirectly caused by trophic interactions.

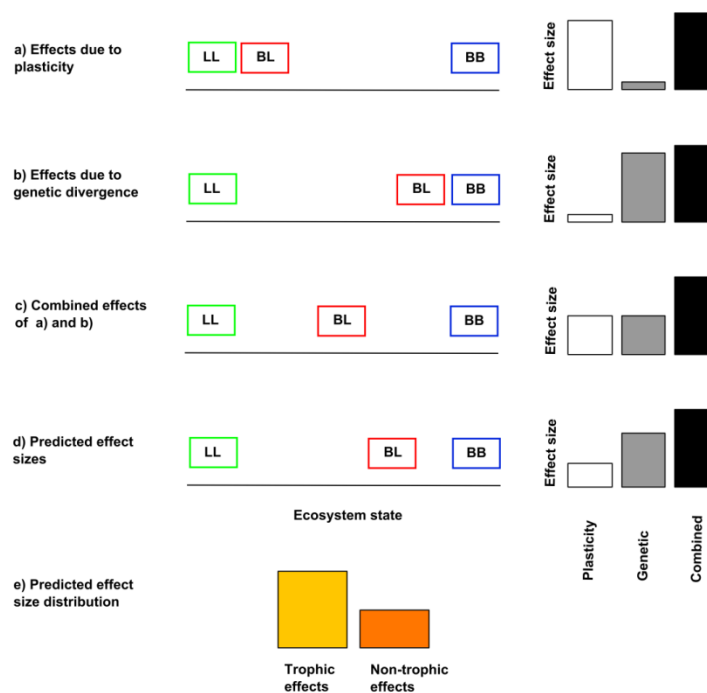


Figure 1: Conceptual Figure. Shown are expected ecosystem states (either averaged across all measured metrics or based on multivariate statistics) when ecosystem effects are a result of a) genetic species divergence, b) phenotypic plasticity, c) a combination of both. On the right hand of each panel associated effect sizes for the different contrasts (BB vs. BL = plasticity, BL vs. LL = genetics, BB vs. LL = combined) are provided. Bars are colour coded and ordered as in subsequent figures (white=plasticity, grey=genetic, black=combined). Predictions are shown in panel d): We predicted ecosystem effects to result from a combination of genetic species divergence and phenotypic plasticity, with overall strongest differences between BB and LL and stronger genetic than plasticity effects (treatment colors are as in subsequent figures, green=LL, red=BL, blue=BB). Further we predicted that effects on trophic metrics (yellow bars) are stronger than effects on non-trophic (orange bars) metrics across all contrasts (panel e).

Material & Methods

Common garden experiment

Experimental fish were bred in the winter of 2006, using five females and five males of each species. Eggs and sperm were stripped in the lab and eggs of all five females were mixed. The eggs were fertilized simultaneously with sperm from the five males, resulting in up to 25 half-sib families per species. Fish were raised for approximately three years in the lab and all juvenile fish were fed with zooplankton in the first year. After about one year the juveniles of the benthic species from Lake Lucerne were raised with two food treatments for approximately two years. Frozen mosquito larvae (*Chironomus plumosus*) were used as benthic food, and zooplankton, collected from Lake Lucerne five times a week, were used as limnetic food. This resulted in three different treatments: Genetically benthic fish raised on benthic food BB, genetically benthic fish raised on limnetic food BL, genetically limnetic fish raised on limnetic food LL.

Additional details about the rearing of fish can be found elsewhere (Lundsgaard-Hansen *et al.*, 2013).

Common gardening experiment

In September 2009, we set up 20 experimental mesocosms (1000 L) to serve as replicated ecosystems. Mesocosms were filled with gravel, sand and water from Lake Lucerne, and 2.46 g of NaNO_3 and 0.18 g NaH_2PO_4 (Phosphorous and Nitrogen) was added to each mesocosm to stimulate primary production. In order to achieve a high diversity of pelagic and benthic organisms, each tank was inoculated with sediments from three different lakes (Sempach, Roth, Lucerne) in the region of Lucerne, and additionally supplemented with zooplankton from Lake Lucerne at the beginning of the experiment.

Distribution of fish

Fish were put into the mesocosms on the 9th of October, 2009. Fish from the BB and the BL treatment were assigned to seven mesocosms each, fish from the LL treatment to six (in total 20 mesocosms). Fish biomass per mesocosm ranged from 51 g to 74 g whitefish per tank and was not significantly different among treatments (ANOVA: F-ratio=0.05, df=2, p=0.95). The number of fish varied from two to five using a total of 56 fish distributed over the 20 tanks. The inclusion of fish number as a co-variable of treatment did not change significance level of the treatment effect for any ecosystem metric we measured at the end of the experiment. Therefore we did not include fish number in further analysis. Fish behavior and health was checked daily by visual observation. Over the entire experiment two fish died (mortality rate=0.06) and one developed an eye infection. These fish were removed immediately and replaced with an individual of similar weight from the same treatment. After nine weeks the experiment was stopped and fish were removed and killed with an overdose of MS22.

Measuring ecosystem variables

We measured the following ecosystem metrics: The density (D) and size (S) of macro- and micro-zooplankton (MAZPD, MAZPS, MIZPD, MIZPS), community composition of macro zooplankton (ZPC), the abundance of snails (SNA) and *Dreissena* mussels (DRA), chlorophyll-a as a proxy for phytoplankton biomass (PPC), benthic algae cover (BAC), sedimentation rate (SED), dissolved organic

compounds (DOC) and light transmission of photosynthetic active radiation (PAR) (see Appendix S1 for measuring dates). Macrozooplankton included species that whitefish are expected to forage on directly (observed mean size range per taxa and tank: 0.4 – 2 mm), including Acarids, Bosmina, calanoid copepods, cyclopoid copepods and Daphnia; whereas microzooplankton (observed mean size range per taxa and tank: 0.1 - 0.3 mm) included copepod nauplii and Rotifera. Ecosystem metrics were divided into trophic and non-trophic metrics (see Table 1). Details on how the different ecosystem metrics were measured can be found in Supplementary Information (Appendix S2).

Statistical analysis

We used principal component analysis (PCA) and Kruskal Wallis ANOVA to test for multivariate divergence in ecosystem state among treatments (more details in Appendix S3). We used profile analysis (PA) on log transformed response variables to evaluate whether fish from different treatments differently affected temporal dynamics of ecosystems (see Table 1). Profile analysis is an alternative to repeated measure ANOVA (RMA), but the assumptions are less restrictive (Tabachnik & Fidell, 2006). PA includes three different tests: i) a flatness test, which tests whether profiles fluctuate or whether they are flat over time (similar to a time effect in RMA), ii) a levels test, which tests whether treatments differ in their average levels over time (similar to a treatment effect in RMA), iii) and a parallelism test, which tests whether different profiles are parallel over time or not (similar to a time/treatment interaction in RMA). We used linear modeling and backward model selection based on AICc (Appendix S5; Burnham & Anderson, 1998) to investigate factors influencing phytoplankton abundance across all mesocosms. We used redundancy analysis (RDA; Legendre & Gallagher, 2001) to assess differences in macro zooplankton community composition (ZPC) between treatments. We used the absolute value of Cohens d (Cohen, 1988) to calculate effect sizes for each contrast (plasticity, genetic, combined) and we tested for significance using randomizations (Bailey et al., 2009). Cohens d is the difference in means of two treatments corrected for their pooled standard deviation (Cohen, 1988). We used Kruskal Wallis ANOVA (KWA) and post-hoc Mann Whitney tests to analyze differences among ecosystem metrics for each sampling date (see Appendix S4).

All statistical analyses were performed using R (R Development Core Team, 2010).

Results

Phenotypic differences of whitefish among treatments

Fish from the three different treatments were significantly differentiated in their foraging traits such as gill rakers, body length and body shape, as well as in multiple measures of feeding efficiency on benthic food (Appendix S5; Lundsgaard-Hansen et al., 2013). The phenotypic differences between contrasts confirm a significant effect of our plasticity treatment on certain aspects of the foraging phenotypes of our whitefish (Appendix S5).

Multivariate analysis of ecosystem divergence

At the end of the experiment, multivariate analysis of ecosystem state revealed significant differences between treatments (Appendix S3 and S4), with divergence along the first principle component axis being strongest between BB and LL treatments and the BL treatment being intermediate (Figure 1d, Figure 2a, Appendix S4). Along the second axis, the three treatments were also significantly different, and divergence was strongest between BL and LL (Appendix S3, S4 and

S6). When only trophic metrics were included in this analysis, the treatments differed significantly with respect to the first axis, again with strongest differences between BB and LL (Figure 2b, Appendix S4), but not the second axis (Appendix S4 and S6). By comparison, when only non-trophic metrics were included, there were no differences between treatments on any axis (Figure 2c, Appendix S4 and S6).

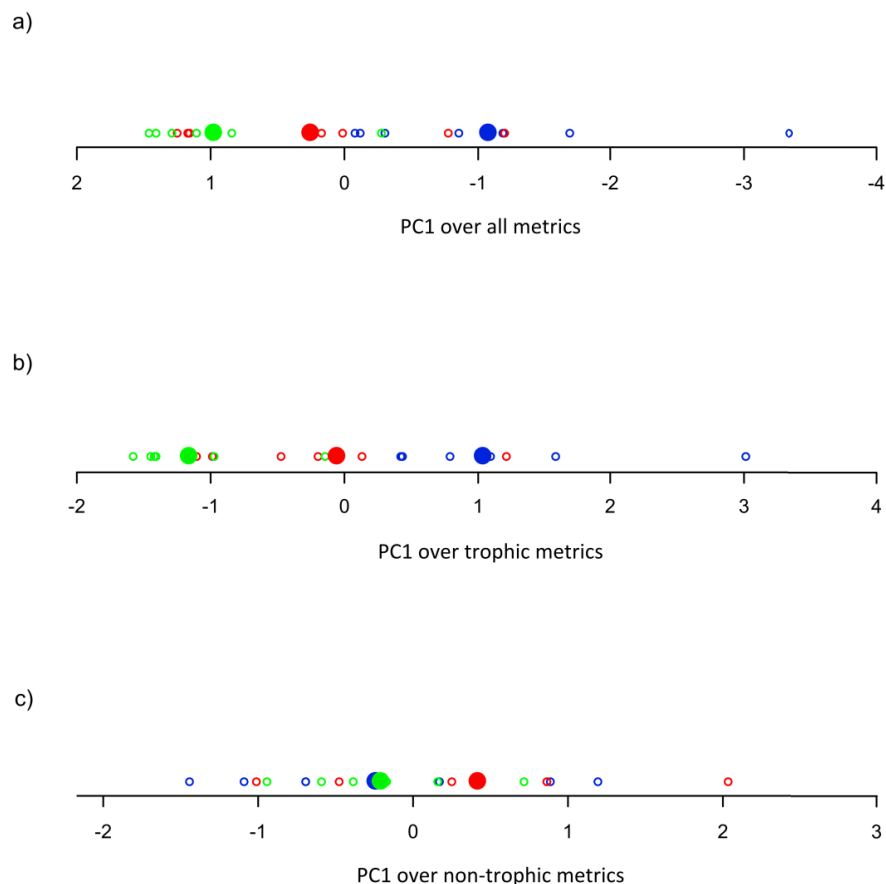


Figure 2: Multivariate ecosystem divergence at the end of the experiment. Shown are PC scores of each tank (small and empty dots; colors as in Figure 1; green=LL, red=BL, blue=BB) and treatment average scores (large and filled dots) for a) PC1 (42%) across all metrics, b) PC1 (59%) across all trophic variables, c) PC1 (65%) across all non-trophic variables. The loadings of the different metrics on the different axis are given in Appendix S3, statistical tests on PC scores are reported in Appendix S4. Variation along the second pc axis is shown in Appendix S6.

Direct trophic effects of whitefish

We found that the majority of the effects on the prey community differed significantly among whitefish treatments in their average values over the entire experiment (see the significant level tests in PA of Table 1, Figure 3). As examples, the average abundance of snails, *Dreissena* mussels, and macro zooplankton were all significantly affected by the whitefish treatments, albeit in different ways. Snail abundance was lowest in the BB treatment, intermediate in the BL treatment, and highest in the LL treatment (Figure 3a), whereas *Dreissena* abundance was lowest in the BB

treatment (Figure 3b), and macro zooplankton density was lowest in the LL treatment (Figure 3c). In addition, the different whitefish treatments had contrasting effects on the community composition of zooplankton (ZPC) at the end of the experiment (Appendix S4).

Non-trophic effects of whitefish

The temporal dynamics of phytoplankton, which is an example of the indirect effects of whitefish on ecosystems, was significantly different among the three treatments (see the significant parallelism test from PA in Table 1, Figure 3d). Such non-parallelism in the phytoplankton response across treatments probably reflect variation over the experiment in the factors regulating phytoplankton biomass. Indeed, phytoplankton concentration was negatively associated with the size of macro zooplankton in the middle of the experiment (F-value=4.6, df=1, p=0.046, Appendix S7) and was significantly negatively associated with *Dreissena* abundance at the end of the experiment (F-value=4.9, df=1, p=0.04, Appendix S7).

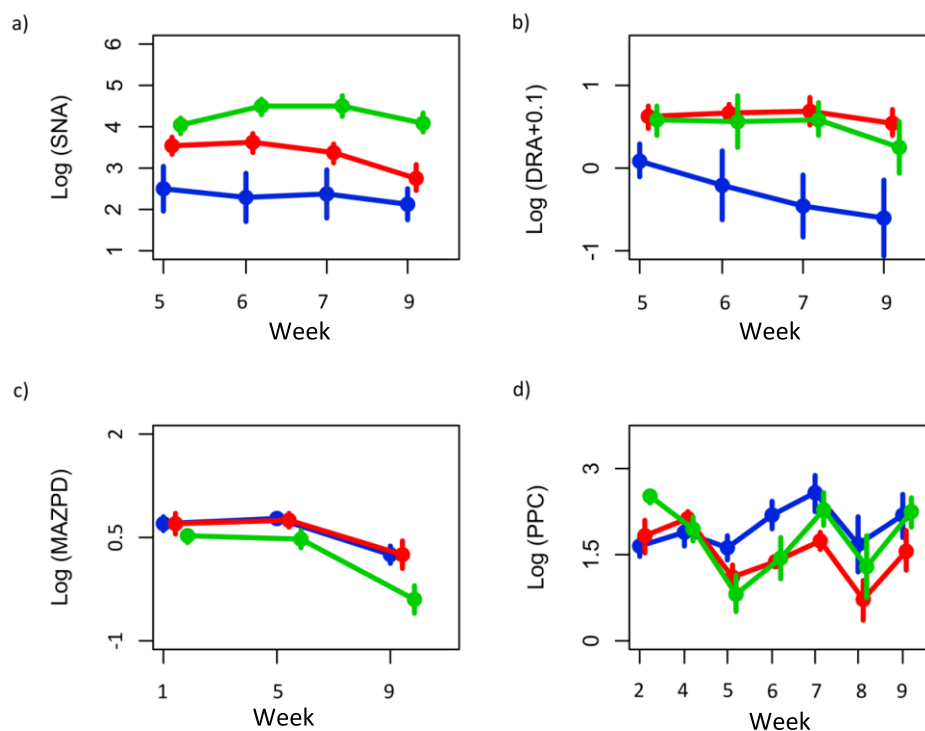


Figure 3: Analysis of temporal variation of ecosystem effects. Shown is the time (weeks) after start of the common gardening experiment on the x-axis and different ecosystem parameters on the y-axis. Acronyms of the different panels are as in Table 1 and colors are as in Figure 1 (green=LL, red=BL, blue=BB). Only metrics with either significant levels or parallelism tests in PA are shown (see Table 1): a) Snail abundance (SNA), b) Dreissena abundance (DRA), c) Macrozooplankton density (MAZPD), d) Phytoplankton concentration (PPC).

Table 1: Given are the measured ecosystem metrics, their acronym, the type of the metric, the number of times they were sampled and their significance levels of the three tests implemented in Profile Analysis (see M&M). Variables that are affected by direct trophic effects of whitefish are called trophic variables, while variables, which are not directly affected by trophic interaction of whitefish with their prey are called non-trophic variables. Metrics with less than three repeated measures and their acronyms are listed, but no profile analysis could be done for these (indicated with “-”). Redundancy analysis, which we used to estimate zooplankton community composition (ZPC) was calculated separately for the three measuring dates, values are thus not comparable across time and PA could therefore not be done on ZPC. “ns” indicates non-significant tests ($p>0.05$).

	Acronym	Type	Times sampled	Flatness Test	Levels Test	Parallelism Test
Snail abundance (N/m ²)	SNA	Trophic	4	ns	0.003	ns
<i>Dreissena</i> abundance (N/m ²)	DRA	Trophic	4	ns	0.036	ns
Macro zooplankton density (N/l)	MAZPD	Trophic	3	0.02	0.031	ns
Macro zooplankton size (mm)	MAZPS	Trophic	3	0.04	ns	ns
Macro zooplankton community	ZPC	Trophic	3	-	-	-
Micro zooplankton density (N/l)	MIZPD	Non trophic	3	ns	ns	ns
Micro zooplankton size (mm)	MIZPS	Non trophic	3	ns	ns	ns
Phytoplankton concentration (mg/l)	PPC	Non trophic	7	<0.001	ns	0.046
Dissolved organic compounds (mg/l)	DOC	Non trophic	5	0.024	ns	ns
Photosynthetic active radiation (%)	PAR	Non trophic	3	0.009	ns	ns
Benthic algae cover (%)	BAC	Non trophic	3	ns	ns	ns
Sedimentation rate (mg/d)	SED	Non trophic	1	-	-	-

Distribution of effect sizes among contrasts

At the end of the experiment, the average ecosystem effect size (absolute Cohens d) was 0.48 for the plasticity contrast (min=0.001, max=1.38), 0.83 for the genetic contrast (min=0.05, max=2.41) and 0.91 for the combined contrast (min=0.07, max=2.95; Figure 4), but these differences were non-significant (all paired t -tests non-significant). Nevertheless, across all metrics only 1 out of 12 was significant for the plasticity contrasts (based on randomization tests of Cohens d ; Bailey *et al.*, 2009), while for both the genetic and combined effects the number of significant tests was much higher (5 out of 12 each, Figure 4). The lower number of significant contrasts resulting from the plasticity treatment was also evident when looking at the density and size variation of individual zooplankton taxa. At the end of the experiment, plasticity contrasts were never significant, but 6 out of 10 cases for the genetic contrast and 5 out of 10 cases for the combined contrast were significant (Appendix S8).

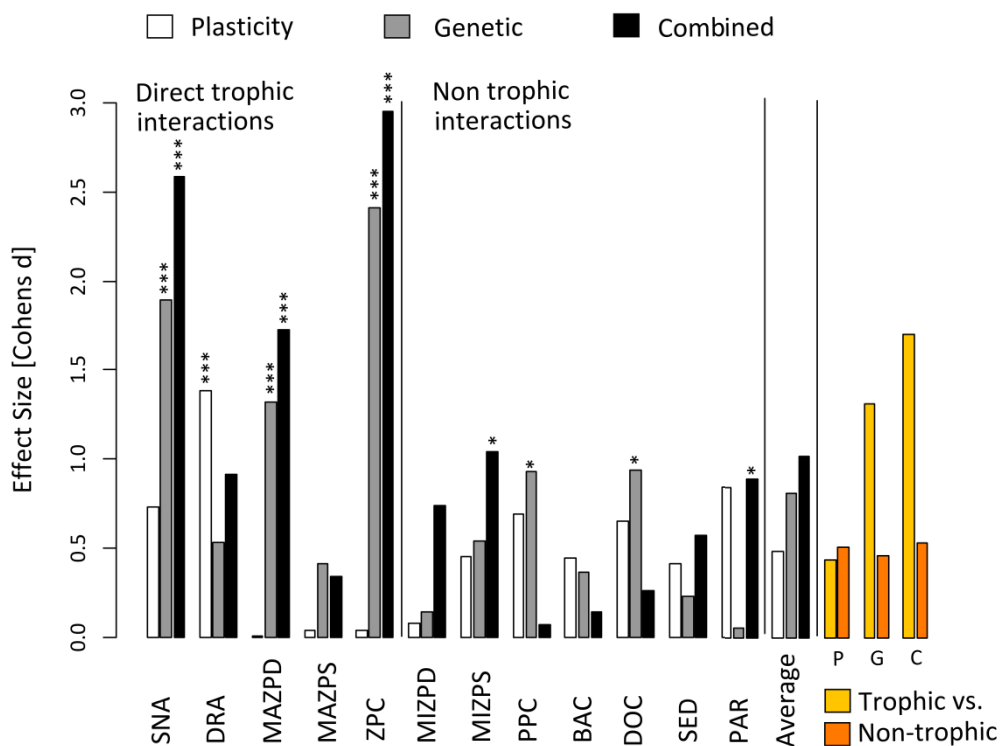


Figure 4: Distribution of effect sizes. Shown are effect sizes (Cohens d) for the different contrasts (BB vs. BL=plasticity=white, BL vs. LL=genetic=grey, LL vs. BB=combined=black) at the end of the experiment on the y-axis and the different ecosystem metrics on the x-axis. Acronyms are as in Table 1. Ecosystem parameters are divided into trophic and non-trophic effects. Significant randomization tests are indicated with $*$ = $p<0.05$, $**$ = $p<0.01$, $***$ = $p<0.001$ (Bailey *et al.*, 2009). On the right side of the graph we report for each contrast the average of all effect sizes and effect size averages of trophic (yellow bars) and non-trophic (orange bars) metrics. Trophic and non-trophic effects are ordered from plasticity (P), to genetic (G) to combined from left to right.

Discussion

The ecological mechanisms and evolutionary pathways of ecological speciation and adaptive radiation have received considerable attention (Schluter, 2000; Nosil, 2012), but much less is known about the ecosystem consequences of ecological speciation. Here, we present experimental evidence that divergent whitefish species from a recent adaptive radiation in a large pre-alpine European lake have contrasting effects on experimental aquatic ecosystems. This builds on previous work showing that evolutionary diversity of stickleback species (Harmon *et al.*, 2009), guppy (Bassar *et al.*, 2010) and alewife populations (Palkovacs & Post, 2009) can affect ecosystem properties and functions. However, because these previous studies used wild-caught fish rather than fish reared in a common garden, our study is the first to separate ecosystem effects of closely related species that are due to heritable trait divergence and due to trait differences arising from phenotypic plasticity. We found significant effects of both genetic species divergence and phenotypic plasticity. Genetic effects were more frequent than plasticity effects, although large effects were observed for both (Figure 4). Overall, this suggests that plasticity can modify the ecosystem effects of closely related species, but the phenotypic and genetic divergence among species that builds up as a consequence of speciation in adaptive radiation is the most likely explanation for contrasting ecosystem effects of species in adaptive radiation.

Whitefish divergence affects ecosystems

By the end of the experiment the multivariate analysis of ecosystem divergence revealed significant differences between treatments, and this supports some aspects of our initial predictions (Figure 1d and 1e). It supports our first prediction that ecosystem divergence results from both phenotypic plasticity and genetic divergence between whitefish species (Figure 2a&b, Appendix S4). It partially supports our second prediction, in that the contrast in ecosystem state was largest between the BB and LL treatment (Figure 2a&b, Appendix S4), but it does not support our prediction of overall stronger genetic than plasticity effects. It supports our third prediction that ecosystem effects are stronger for trophic than non-trophic interactions (Figure 2b & c, Appendix S4).

Our analysis of the temporal dynamics of ecosystem metrics over the course of the experiment showed that our whitefish treatments had strongly contrasting effects on the composition and abundance of their prey (Figure 3a-c). To some extent, the direction of these effects can be explained by known differences in phenotypes, feeding efficiency and ecology of whitefish (Appendix S5; Mookerji *et al.*, 1998; Harrod *et al.* 2010; Lundsgaard-Hansen *et al.*, 2013, Roesch *et al.*, 2013). Consistent with previous foraging trials, which used individuals reared in common garden from the same populations (Lundsgaard-Hansen *et al.*, 2013), the benthic *C. sp.* “Bodenbalchen” suppressed benthic prey more strongly than the limnetic *C. zugensis*, whereas the latter species suppressed limnetic prey more efficiently. When raised on limnetic food, the benthic species became intermediate with respect to its effects on some metrics describing the prey community (Figure 3). The strongly benthic prey *Dreissena* was similarly weakly affected by the benthic species raised on limnetic food and the limnetic species; and was suppressed only by the benthic species raised on benthic food. This finding is consistent with results from a previous study where we found plasticity in the feeding efficiency on benthic food of the same pair of whitefish species (Appendix S5; Lundsgaard-Hansen *et al.*, 2013).

In our experiment whitefish also differentially affected properties of the ecosystem other than their prey, such as the dynamics of phytoplankton through time (Figure 3d). Phytoplankton abundance in lake ecosystems is often regulated by organisms at upper trophic levels in both the pelagic and benthic food chain (Vanni & Findlay, 1990). As a result, their dynamics can be influenced by how fish feed on pelagic macrozooplankton and benthic consumers (e.g. *Dreissena*). Phytoplankton biomass was negatively correlated with the size of macrozooplankton in the middle of the experiment and with the abundance of *Dreissena* at later dates. This suggests that understanding the temporal dynamics of contrasting ecosystem effects resulting from trait differences of predators, may depend on the life history, generation time, and food web position of their prey.

In north temperate fish there are multiple species pairs with similar eco-morphological differentiation as the studied whitefish species (Schluter, 2000) and we yet know little about how repeatable their effects on ecosystems are. Future studies should test for repeatability of ecosystem effects by replicating their experiments at the population or species level. Such studies would also gain valuable additional insights if they were performed under natural or semi-natural conditions.

Distribution of effect sizes

Predicting the relative size of trophic and non-trophic effects is not trivial in reticulate food webs where consumers and predators connect multiple food chains (Teng & McCann, 2004). In aquatic ecosystems, predator-mediated cascading effects on lower trophic levels are relatively common (Borer *et al.*, 2005; Baum & Worm, 2009) and previous studies suggest that such effects might dampen as you move down the food chain (McQueen *et al.*, 1989; Micheli, 1999; Shurin *et al.*, 2002). In addition, there is some evidence that genetic and ecological effects attenuate across different levels of organization, for example from community to ecosystem properties (Bailey *et al.*, 2009; Dickie *et al.*, 2012). However, such attenuation is not always observed, and the distribution of ecosystem effect sizes may depend on which traits underlie the ecosystem effects (Bassar *et al.*, 2010; Palkovacs *et al.*, 2012). In our experiment, contrasting ecosystem effects of divergent whitefish were not idiosyncratically distributed in the ecosystem, and resulted more frequently from direct trophic interactions of whitefish with their prey. This is consistent with previous work suggesting the dampening of top-down effects in trophic cascades (Micheli, 1999; Dickie *et al.*, 2012), but it may alternatively be explained by multiple indirect effects that act in opposing directions and result in weak overall net effects (Bassar *et al.*, 2012).

We found large ecosystem effects resulting from both genetic divergence and phenotypic plasticity, but genetic effects were more common in our experiment. If this is a general finding, it suggests that the results of previous studies that used wild (caught) organisms (i. e. Harmon *et al.*, 2009; Bassar *et al.*, 2010; Palkovacs & Post, 2009) are likely attributable to phenotypic diversification resulting from genetic divergence rather than plasticity. It is also in agreement with a recent study showing that the divergence in the rates of population growth among *Daphnia* clones (reared in common garden), alters consumer-resource dynamics and ecosystem function (Walsh *et al.*, 2012). We cannot exclude that our genetic effects include some maternal effects, because we used first generation lab bred individuals. To bias our findings, maternal effects would have needed to cause differential mortality during fish raising to cause changes in trait distributions in a way that would affect ecosystems. This

seems very unlikely, as the direction of phenotypic divergence seen in our treatments is similar to species divergence observed in the wild (Appendix S5; Vonlanthen *et al.*, 2012).

Potential for eco-evolutionary feedbacks?

Here we studied species which are known to be genetically differentiated in functional traits, i.e. feeding traits and feeding behavior (Vonlanthen *et al.*, 2012; Lundsgaard-Hansen *et al.*, 2013), and found that they have strong contrasting effects on their prey and other characteristics of the ecosystem. There is independent evidence that whitefish foraging traits likely are under divergent natural selection (Rogers & Bernatchez, 2007; Vonlanthen *et al.*, 2009; Lundsgaard-Hansen *et al.*, 2013) and anthropogenic modification of trophic resources has been shown to cause contemporary evolutionary change in these fish (Bittner *et al.*, 2010). This, together with the observation of strongest ecosystem effects on ecosystem metrics related to foraging, indicates a potential for eco-evolutionary feedbacks to happen during whitefish speciation, as has been previously suggested in a comparative study of whitefish across Scandinavian lakes (Kahilainen *et al.*, 2011). Also plasticity could be an important consideration when understanding eco-evolutionary feedbacks. If plasticity in foraging traits, for example, significantly affects an ecosystem state that is preserved across generations, then the feedback between plasticity and the environment could drive phenotypic evolution through plasticity without any underlying changes in genetics (Whitman & Agrawal, 2009). Alternatively plasticity effects on ecosystems could promote the evolution of traits with a genetic basis, which could also be the genetic basis of plasticity itself (Yamamichi *et al.*, 2011).

To test for the potential importance of such eco-evolutionary dynamics in adaptive radiation and to learn whether they generally impede or facilitate diversification, future studies should measure the magnitude, dimensionality, and persistence of ecosystem effects, and quantify whether such modifications to the ecosystem alter environmental sources of selection (Yoder *et al.*, 2010). Such work would help us understand whether the contrasting ecosystem effects of species that emerge from adaptive radiations rather promote or reduce ecological opportunity for further divergence, and whether such effects play an important role in the process of diversification itself (Erwin, 2008; Losos, 2010; Yoder *et al.*, 2010).

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References

- Bailey, J. K., Schweitzer, J. A., Ubeda, F., Koricheva, J., LeRoy C. J., Madritch, M. D. *et al.* (2009). From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Philos. Trans. R. Soc. B. Biol. Sci.*, 364, 1607-1616.
- Bassar, R. D., Ferriere, R., Lopez-Sepuldre, A., Marshall, M. C., Travis, J., Pringle, C. M. *et al.* (2012). Direct and indirect ecosystem effects of evolutionary adaptation in the Trinidadian guppy (*Poecilia reticulata*). *Am. Nat.*, 180, 167-185.
- Bassar, R. D., Marshall, M. C., Lopez-Sepulcre, A., Zandon, E., Auer, S. K., Travis, J. *et al.* (2010). Local adaptation in Trinidadian guppies alters ecosystem processes. *Proc. Natl. Acad. Sci.*, 107, 3616-3621.
- Baum, J. K. & Worm, B. (2009). Cascading top-down effects of changing oceanic predator abundances. *J. Anim. Ecol.*, 78, 699-714.
- Bernatchez, L. (2004). Ecological Theory of Adaptive Radiation: An Empirical Assessment from Coregonine Fishes (Salmoniformes). In: *Evolution Illuminated* (Hendry, A. P. & Stearns, S. C., eds.). pp. 175-207. Oxford University Press, Oxford.
- Bittner, D., Excoffier, L., & Largiad, C. R. (2010). Patterns of morphological changes and hybridization between sympatric whitefish morphs between whitefish morphs (*Coregonus spp.*) in a Swiss lake: a role for eutrophication? *Mol. Ecol.*, 19, 2152-2167.
- Borer, E. T., Seabloom, E. W., Shurin, J. B., Andersson, K. E., Blanchette, C. A., Broitman, B., *et al.* (2005). What determines the strength of a trophic cascade? *Ecology*, 86, 528-537.
- Burnham, K. P. & Anderson, D. R. (1998). *Model selection and multimodel inference, a practical information-theoretic approach*. Second edition. Springer, New York.
- Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. 2nd edition. Lawrence Erlbaum Associates, New Jersey.
- Dickie, I. A., Fukami, T., Wilkie, J. P., Allen, R. B., & Buchanan, P. K. (2012). Do assembly history effects attenuate from species to ecosystem properties? A field test with wood-inhabiting fungi. *Ecol. Lett.*, 15, 133-141.
- Erwin, D. H. (2008). Macroevolution of ecosystem engineering, niche construction, and diversity. *Trends Ecol. Evol.*, 23, 304-310.
- Hanski, I. (2012). Eco-evolutionary dynamics in a changing world. *Ann. NY Acad. Sci.*, 1246, 1-17.
- Harmon, L. J., Matthews, B., Des Roches, S., Chase, J.M., Shurin, J.B. & Schluter, D. (2009). Evolutionary diversification in stickleback affects ecosystem functioning. *Nature*, 458, 1167-1170.
- Harrod, C., Mallela, J. & Kahilainen, K. (2010). Phenotype-environment correlations in a putative whitefish adaptive radiation. *J. Anim. Ecol.*, 79, 1057-1068.
- Hudson, A. G., Vonlanthen, P. & Seehausen, O. (2011). Rapid parallel adaptive radiations from a single hybridogenic ancestral population. *Proc. R. Soc. B*, 278, 58-66.
- Kahilainen, K., Siwertsson, A., Gjelland, K. Ö., Knudsen, R., Böhn, T. & Amundsen, P.-A. (2011). The role of gill raker number variability in adaptive radiation of coregonid fish. *Evol. Ecol.* 25, 573-588.

- Karvonen, A., Lundsgaard-Hansen, B., Jokkela, J. & Seehausen, O. (2012). Differentiation in parasitism among ecotypes of whitefish segregating along depth gradients. *Oikos*, 122, 122-128.
- Legendre, P. & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia*, 129, 271-280.
- Losos, J. B. (2010). Adaptive radiation, ecological opportunity and evolutionary determinism. *Am. Nat.*, 175, 623-639.
- Lundsgaard-Hansen, B., Matthews, B., Vonlanthen, P., Taverna, A. & Seehausen, O. (2013) Adaptive plasticity and genetic divergence in feeding efficiency during parallel adaptive radiation of whitefish (*Coregonus spp.*). *J. Evol. Biol.*, 26, 483-498.
- Matthews, B., Hausch, S., Winter, C., Suttle, C. A. & Shurin, J. B. 2011a. Contrasting ecosystem effects of morphologically similar copepods. *Plos ONE*, 6, e26700.
- Matthews, B., Narwani, A., Hausch, S., Nonaka, E., Peter, H., Yamamichi, M. *et al.* 2011b. Toward an integration of evolutionary biology and ecosystem science. *Ecol. Lett.*, 14, 690-701.
- McQueen, D. J., Johannes M. R. S., Post, J. R., Stewart, T. J. & Lean, D. R. S. (1989). Bottom-Up and Top-Down Impacts on Freshwater Pelagic Community Structure. *Ecol. Monogr.*, 59, 289–309.
- Micheli, F. (1999). Eutrophication, fisheries and consumer-resource dynamics in marine pelagic ecosystem. *Science*, 285, 1396-1398.
- Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K. & Relyea, R. A. (2005). Ecological consequences of phenotypic plasticity. *Trends Ecol. Evol.*, 20, 685-692.
- Mookerji, N., Heller, C., Meng, H. J., Bürgi, H. R. & Müller, R. (1998). Diel and seasonal patterns of food intake and prey selection by *Coregonus* sp. in re-oligotrophicated Lake Lucerne, Switzerland. *J. Fish. Biol.*, 52, 443–457.
- Nosil, P. (2012). *Ecological speciation*. Oxford University Press, Oxford.
- Palkovacs, E. P., Kinnison, M. T., Correa, C., Dalton, C. M. & Hendry, A. P. (2012). Fates beyond traits: ecological consequences of human-induced trait change. *Evol. Appl.*, 5, 183-191.
- Palkovacs, E. P. & Post, D. M. (2009). Experimental evidence that phenotypic divergence in predators drives community divergence in prey. *Ecology*, 90, 300-305.
- Post, D. M. & Palkovacs, E. P. (2009). Eco-evolutionary feedbacks in community and ecosystem ecology: Interactions between the ecological theatre and the evolutionary play. *Philos. Trans. R. Soc. B, Biol. Sci.*, 364, 1629-1640.
- Post, D. M., Palkovacs, E. P., Schielke, E. G. & Dodson, S. I. (2008). Intraspecific phenotypic variation in a predator affects community structure and cascading trophic interactions. *Ecology*, 89, 2019-2032.
- R Development Core Team. (2010). R: A language and environment for Statistical Computing. Vienna, Austria. Available at: <http://www.R-project.org>
- Roesch, C., Lundsgaard-Hansen, B., Vonlanthen, P., Taverna, A. & Seehausen, O. Experimental evidence for trait utility of gill raker number in adaptive radiation of a north temperate fish. In press in *J. Evol. Biol.*

- Rogers, S. M. & Bernatchez, L. (2007). The genetic architecture of ecological speciation and the association with signatures of selection in natural lake whitefish (*Coregonus sp.*, Salmonidae) species pairs. *Mol. Biol. Evol.*, 24, 1423-1438.
- Schluter, D. (2000). *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Schoener, T. W. (2011). The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science*, 331, 426-429.
- Seehausen, O. (2009). Speciation affects ecosystems. *Nature*, 458, 1122-1123.
- Shurin, J. B., Borer, E. T., Seabloom, E. W., Anderson, K., Blanchette, C. A., Broitman, B. *et al.* (2002). A cross-ecosystem comparison of the strength of trophic cascades. *Ecol. Lett.*, 5, 785-791.
- Tabachnik, B. G. & Fidell, L. S. (2006). *Using multivariate statistics*. Allyn and Bacon, Boston.
- Teng, J. & McCann. (2004). Dynamics of compartmented and reticulate food webs. *Am. Nat.*, 164: 85-100.
- Vanni, M. J. & Findlay, D. L. (1990). Trophic cascades and phytoplankton community structure. *Ecology*, 71, 921-937.
- Vonlanthen, P., Bittner, D., Hudson, A. G., Young, K. A., Müller, R., Lundsgaard-Hansen, B. *et al.* (2012). Anthropogenic eutrophication drives extinction by speciation reversal in parallel adaptive radiations. *Nature*, 482, 357-363.
- Vonlanthen, P., Roy, D., Hudson, A. G., Largiader, C. R., Bittner, D. & Seehausen, O. (2009). Divergence along a steep ecological gradient in lake whitefish (*Coregonus sp.*). *J. Evol. Biol.* 22, 498-514.
- Walsh, M. R., De Long, J. P., Hanley, T. C. & Post, D. M. (2012). A cascade of evolutionary change alters consumer-resource dynamics and ecosystem function. *Proc. Biol. Sci.*, 279, 3184-3192.
- Walsh, M. R. & Post, D. M. (2011). Interpopulation variation in a fish predator drives evolutionary divergence in prey in lakes. *Philos. Trans. R. Soc. B. Biol. Sci.*, 278, 2628-2637.
- West Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press, Oxford.
- Whitman, D. W. & Agrawal, A. A. 2009. What is phenotypic plasticity and why is it important? In "Phenotypic plasticity of insects – mechanisms and consequences", ed. by D. W. Whitman and T. N. Ananthakrishnan. Science publishers.
- Woods, P. J., Müller, R. & Seehausen, O. (2009). Intergenomic epistasis causes asynchronous hatch times in whitefish hybrids, but only when parental ecotypes differ. *J. Evol. Biol.*, 22, 2305-2319.
- Yamamichi, M., Yoshida, T. & Sasaki, A. (2011). Comparing the effects of rapid evolution and phenotypic plasticity on predator-prey dynamics. *Am. Nat.*, 178, 287-304.
- Yoder, J. B., Clancey, E., Des Roches, S., Eastman, J. M., Gentry, L., Godsoe, W. *et al.* (2010). Ecological opportunity and the origin of adaptive radiations. *J. Evol. Biol.*, 23, 1581-1596.

Supplementary Information Chapter 4

Appendix S1 – Sampling overview

Crosses indicate which measurements were taken in which week. Acronyms are SNA=snail abundance, DRA=Dreissena abundance, Zooplankton includes macro- and microzooplankton density (D) and size (S) (MAZPD, MIZPD, MAZPS, MIZPS) and macrozooplankton community composition (ZPC), PPC=phytoplankton concentration, BAC=benthic algae cover, DOC=dissolved organic compounds, SED=sedimentation, PAR=photosynthetic active radiation. Fish were put at the 9th of October and removed at the 12th of December.

Week	Date	SNA/DRA	Zooplankton	PPC	BAC	DOC	SED	PAR
1	12.10-18.10		X					
2	19.10-25.10			X	X	X		X
3	26.10-1.11.							
4	2.11-8.11			X		X		
5	9.11-15.11	X	X	X		X		
6	16.11-22.11	X		X	X	X		X
7	23.11-29.11	X		X				
8	30.11-6.12			X		X		
9	7.12-13.12	X	X	X	X		X	X

Appendix S2 - Measuring ecosystem variables

Zooplankton: Zooplankton was sampled in order to estimate the density (number/Liter) and size structure of whitefish prey (macrozooplankton) and other non-prey species (microzooplankton). A sampling tube, reaching from the tank surface to the tank bottom was used to collect 6L of water which was sieved through a 30 μ m Nitex mesh. All sampled zooplankton species belonged to one of the following taxa: Acarids, Bosmina, calanoid copepods, cyclopoid copepods, Daphnia, Ostracoda, copepod nauplii and Rotifera. Copepod nauplii and Rotifera were assigned to microzooplankton (mean size per taxa and tank always < 0.3 mm), while larger taxa were assigned to macrozooplankton (mean size per taxa and tank always >0.4 mm). This size separation is supported by stomach content analyses, which show that taxa which we assigned to microzooplankton are absent from the diet of various whitefish species, including the here studied ones (Mookerji et al., 1998; Harrod et al., 2010). Acarids, Daphnia and Ostracoda were very rare at each sampling date and within each treatment (relative abundance per treatment and date always <1%) and were thus not shown in the Appendix Figure S8, but they were included in the summed density and mean size calculations per tank (MAZPD and MAZPS, respectively). Pictures of zooplankton were taken for length measurements, and a maximum of 30 randomly chosen individuals per taxa and tank were measured.

Snails and Mussels: Starting from the middle of the experiment (week 5), the density of snails and mussels (number per m²) were counted weekly in a standardized way from the sides of tank wall. Snails belonged to the family of *Limnaidae* and *Planorbidae* but were summed for all analyses, and all mussels belonged to the species *Dreissena polymorpha*.

Sedimentation: Sedimentation traps were put in each tank in order to calculate sedimentation rate over the entire duration of the experiment. At the end of the experiment sedimentation traps were removed, sediments were dried at 60 ° C for 24 hours and the dry weight of sediments was used to calculate sedimentation rate in mg/day.

Phytoplankton and dissolved organic compounds: One liter of water was collected weekly in order to measure Chlorophyll a concentration as a proxy for phytoplankton concentration (PPC). The filtered water was used to measure the concentration of dissolved organic compounds (DOC) and nutrients (Nitrate, Phosphorus). DOC was measured on a Shimadzu TOC-V CPH and was reported in mg/L. Total dissolved phosphorus (TDP) and NO₃⁻/NO₂⁻ (DIN) were analyzed on a Lachat autoanalyzer (Zellweger Analytics, QuickChem ® 8000).

Photosynthetic active radiation: Photosynthetic active radiation (PAR) was measured at three dates at a wavelength from 400 – 700 nm to calculate light transmission through the water column using a LI-193 Underwater Spherical Quantum Sensor. The % of PAR transmitted from 10 cm below surface to the bottom of the tank was reported (high values corresponded to a high light transmission).

Benthic algae cover: Photos of the bottom were taken with a Canon S9, using an underwater cage, to estimate benthic algae cover. Photos were used to define areas on the tank bottom with high algae cover (100%), with intermediate algae cover (50%) and with no algae cover (0%) using Photoshop CS and these estimates were used to calculate the percentage of algae cover at the bottom.

Appendix S3 – PCA results summary

A summary of the sampling schedule divided up into Early (refers to weeks 1 & 2), Middle (weeks 5&6), and Late (weeks 8 & 9; see Appendix S1). See the main text (Table 1) for the distinction between trophic and non-trophic metrics that were included in the PCA. Columns 4-11 show the loadings of each metric for each principle component axis. Results of Kruskal-Wallis tests and post hoc Mann Whitney tests on PC scores can be found in Appendix S4.

Time	Metrics	Principal component	SNA	DRA	MAZPD	MAZPS	MIZPD	MIZPS	PPC	BAC	DOC	PAR
Early	All	PC1 (69.59%)	-	-	0.15	0.03	-0.03	0.01	-0.98	-0.05	-0.05	0.03
Early	All	PC2 (13.6%)	-	-	0.98	-0.04	0.12	-0.01	0.14	0.03	-0.07	-0.04
Early	trophic	PC1 (93.4%)	-	-	-1.00	0.02	-	-	-	-	-	-
Early	trophic	PC2 (6.6%)	-	-	-0.02	-1.00	-	-	-	-	-	-
Early	non trophic	PC1 (80.9)	-	-	-	-	0.04	-0.01	1.00	0.05	0.05	-0.03
Early	non trophic	PC2 (12.6%)	-	-	-	-	0.18	-0.04	0.05	-0.97	-0.15	0.04
Middle	All	PC1 (56.9%)	0.90	0.25	-0.05	-0.02	-0.21	0.00	-0.28	0.01	0.03	0.00
Middle	All	PC2 (19.01%)	-0.37	0.15	0.12	-0.04	-0.25	-0.03	-0.87	-0.05	0.07	0.01
Middle	trophic	PC1 (83.4%)	0.97	0.24	-0.07	-0.01	-	-	-	-	-	-
Middle	trophic	PC2 (11.8%)	-0.23	0.96	0.14	-0.01	-	-	-	-	-	-
Middle	non trophic	PC1 (59.7%)	-	-	-	-	-0.42	-0.02	-0.90	-0.01	0.07	0.01
Middle	non trophic	PC2 (32.9%)	-	-	-	-	0.89	0.02	-0.42	-0.16	-0.02	0.01
Late	All	PC1 (42%)	0.76	0.54	-0.08	0.00	-0.16	-0.02	-0.31	-0.02	-0.02	0.03
Late	All	PC2 (29%)	-0.58	0.49	0.20	0.00	-0.02	0.02	-0.62	-0.10	0.02	0.00
Late	trophic	PC1 (59%)	-0.88	-0.46	0.13	0.00	-	-	-	-	-	-
Late	trophic	PC2 (31%)	0.43	-0.88	-0.18	0.01	-	-	-	-	-	-
Late	non trophic	PC1 (65%)	-	-	-	-	-0.27	0.00	-0.96	-0.07	0.02	0.02
Late	non trophic	PC2 (26%)	-	-	-	-	0.93	0.08	-0.28	0.21	-0.01	-0.03

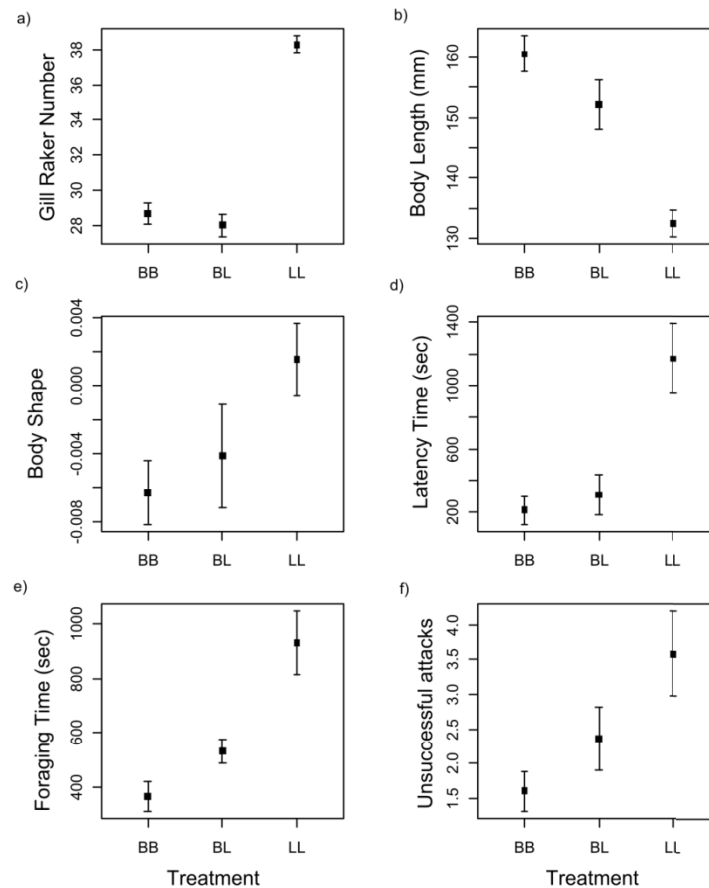
Appendix S4 – Between treatments Kruskal Wallis ANOVA and post hoc t-tests

Measured parameters are listed in the first column, the week of measurements (after the start of the experiment) in the second column, the Kruskal Wallis Chi squared (KW Chi squared) and the associated p-value in the third and the fourth columns. We report p-values from post-hoc Mann Whitney tests for each contrast (plasticity = BB vs. BL, genetic = BL vs. LL, combined = BB vs. LL). Significant tests are highlighted in bold. Acronyms are as in Appendix S1. PC1/2 trophic = principal component score 1 and 2 on trophic metrics at the end of the experiment, PC1/2 non trophic = principal component score 1 and 2 on non trophic metrics at the end of the experiment, PC1/2 overall = principal component score 1 and 2 over all metrics (see Appendix 3).

Parameter	Week	KW Chi	p-value	Plasticity p-	Genetics p-	Combined p-
SNA	5	5.87	0.05	0.26	0.18	0.02
SNA	6	8.79	0.01	0.1	0.01	0.02
SNA	7	9.15	0.01	0.21	0.01	0.01
SNA	9	9.29	0.01	0.34	0.02	0.00
DRA	5	4.68	0.10	0.05	0.82	0.12
DRA	6	3.59	0.17	0.05	0.61	0.39
DRA	7	7.84	0.02	0.01	0.88	0.04
DRA	9	4.47	0.11	0.04	0.52	0.28
MAZPD	1	1.84	0.39	0.80	0.23	0.36
MAZPD	5	4.25	0.12	1.00	0.10	0.07
MAZPD	9	2.73	0.25	0.90	0.05	0.03
MAZPS	1	4.81	0.09	0.10	0.44	0.07
MAZPS	5	4.99	0.08	0.90	0.05	0.07
MAZPS	9	0.98	0.61	1.00	0.44	0.44
ZPC	1	1.53	0.46	0.90	0.10	0.03
ZPC	5	9.60	0.01	0.26	0.01	0.00
ZPC	9	9.44	0.01	0.90	0.01	0.00
PC1 Trophic	Early	1.84	0.39	0.80	0.23	0.37
PC1 Trophic	Medium	6.55	0.04	0.13	0.10	0.03
PC1 Trophic	Late	11.32	0.00	0.07	0.03	0.00
PC2 Trophic	Early	4.82	0.09	0.10	0.45	0.07
PC2 Trophic	Medium	1.19	0.55	0.38	0.45	0.84
PC2 Trophic	Late	5.10	0.08	0.13	0.03	0.63
MIZPD	1	0.12	0.93	1.00	0.73	0.94
MIZPD	5	4.27	0.12	0.62	0.17	0.05
MIZPD	9	4.24	0.12	0.16	0.84	0.05
MIZPS	1	2.69	0.26	0.13	0.95	0.30
MIZPS	5	1.36	0.50	0.46	0.95	0.30
MIZPS	9	3.47	0.18	0.32	0.53	0.07
PPC	2	7.07	0.03	0.70	0.14	0.01
PPC	4	0.79	0.67	0.56	0.44	0.94
PPC	5	3.25	0.20	0.18	0.89	0.12
PPC	6	5.58	0.06	0.04	0.14	0.28
PPC	7	4.95	0.08	0.05	0.13	0.53
PPC	8	2.67	0.26	0.18	0.23	0.63
PPC	9	1.82	0.40	0.34	0.23	0.89
DOC	2	4.43	0.11	0.12	0.54	0.08
DOC	4	5.40	0.07	0.16	0.84	0.01
DOC	5	4.58	0.10	0.07	1.00	0.09

DOC	6	3.77	0.15	0.07	0.36	0.37
DOC	8	2.20	0.33	0.38	0.13	1.00
PAR	2	0.93	0.63	0.38	0.63	0.83
PAR	6	0.49	0.78	0.44	0.83	1.00
PAR	9	2.18	0.34	0.25	1.00	0.22
SED	1 -9	0.98	0.61	0.46	1.00	0.44
BAC	2	3.03	0.22	0.10	0.29	0.84
BAC	6	1.30	0.52	0.38	0.44	0.63
BAC	9	0.69	0.71	0.62	0.44	0.95
PC1 Non-trophic	Early	7.05	0.03	0.71	0.14	0.00
PC1 Non-trophic	Medium	5.69	0.06	0.13	0.29	0.03
PC1 Non-trophic	Late	1.93	0.38	0.32	0.23	0.84
PC2 Non-trophic	Early	3.67	0.16	0.05	0.29	0.84
PC2 Non-trophic	Medium	0.58	0.75	0.90	0.63	0.53
PC2 Non-trophic	Late	3.21	0.20	0.80	0.29	0.07
PC1 overall	Early	7.06	0.03	0.71	0.14	0.00
PC1 overall	Medium	10.07	0.01	0.05	0.10	0.00
PC1 overall	Late	9.31	0.01	0.04	0.18	0.00
PC2 overall	Early	0.46	0.79	0.62	0.63	0.94
PC2 overall	Medium	0.95	0.62	0.80	0.63	0.37
PC2 overall	Late	7.35	0.03	0.46	0.00	0.10

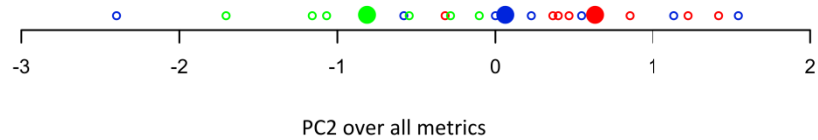
Appendix S5 – Phenotypic differences between treatments



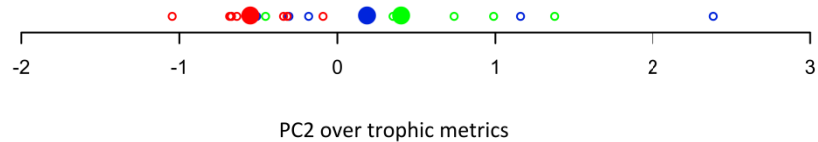
Treatment is on the x-axis. Panels a) – c) show phenotypic differences in foraging traits while panels d) – f) show differences in feeding efficiency on benthic food on the y-axis. The BL treatment is intermediate to the BB and the LL treatment in all foraging traits and behaviors, except in the number of gill rakers. Data in panel a) and b) is from the same individuals used in this experiment, data in panel c) – e) is from (18). Values in panel c) correspond to PC scores from a geometric morphometrics analysis and a low value corresponds to a more down turned head, in panels d)-e) a low value corresponds to increased feeding efficiency on benthic food (18). The three treatments differ significantly in all foraging traits and feeding efficiency measures (gill raker number: Kruskal-Wallis Chi squared = 38.8, $N = 56$, $p < 0.001$; body length: KW chi squared = 31.6, $N = 56$, $p < 0.001$; body shape: KW chi squared = 6.8, $N = 75$, $p = 0.03$; latency time: KW chi squared = 20.9, $N = 75$, $p < 0.001$; foraging time: KW chi squared, $N = 75$, $p < 0.001$; number of unsuccessful attacks: KW chi squared = 6.6, $N = 75$, $p = 0.03$). Post hoc tests for the plasticity contrast (BB vs. BL) are significant for foraging time ($N = 46$, $W = 125$, $p = 0.004$), and non-significant for others (gill raker number: $N = 32$, $W = 150$, $p = 0.36$; body length: $N = 32$, $W = 326$, $p = 0.09$; $N =$ body shape: $N = 46$; $W = 210$, $p = 0.62$; latency time: $N = 46$, $W = 217$, $p = 0.42$; number of unsuccessful attacks: $N = 46$, $W = 209$, $p = 0.31$). Post hoc tests for the genetic contrast (BL vs. LL) are significant for gill raker number ($N = 42$, $W = 396$, $p < 0.001$), body length ($N = 42$, $W = 134$, $p < 0.001$), foraging time ($N = 52$, $W = 451$, $p < 0.001$) and latency time ($N = 52$, $W = 520$, $p < 0.01$), and non-significant for body shape ($N = 52$, $W = 381$, $p = 0.08$) and the number of unsuccessful attacks ($N = 52$, $W = 352$, $p = 0.17$). Post hoc tests for the combined contrast (BB vs. LL) are significant for all foraging traits and feeding efficiency measures (gill rakers: $N = 38$, $W = 308$, $p < 0.001$; body length: $N = 38$, $W = 44$, $p < 0.001$; body shape: $N = 53$, $W = 438$, $p = 0.01$; foraging time: $N = 53$, $W = 529$, $p < 0.001$; latency time: $N = 53$, $W = 573$, $p < 0.001$; unsuccessful attacks: $N = 53$, $W = 426$, $p = 0.009$).

Appendix S6 – Multivariate ecosystem divergence II

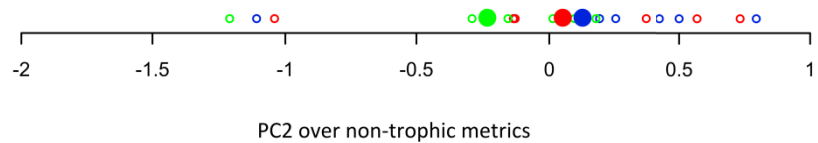
a)



b)



c)



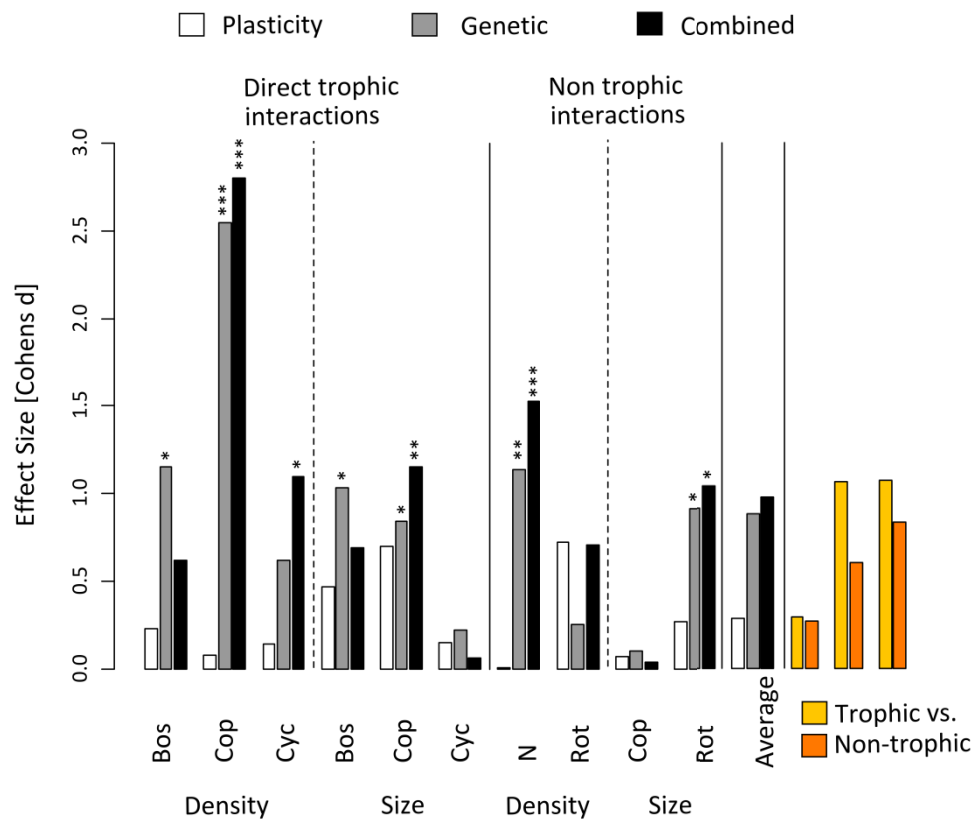
Analysis of the multivariate ecosystem divergence at the end of the experiment, where we report PC scores of each tank (small and empty dots; colors as in Figure 1; green=LL, red=BL, blue=BB) and treatment average scores (large and filled dots) for a) PC2 (29%) across all metrics, b) PC2 (31%) across all trophic variables, c) PC2 (26%) across all non-trophic variables. The loadings of the different metrics on the different axis are given in Appendix S3, statistical tests on PC scores are reported in Appendix S4.

Appendix S7 – Backward model selection phytoplankton

Backward model selection was done for three different time points in the experiment, early, middle and late, as indicated in the first column (early=weeks 1&2, middle=weeks 5&6, late=weeks 8&9). The models are given in the second column. Acronyms are the same as in Appendix S1. Model selection was done by removing variables and choosing the model with the lowest AICc. Significant variables in any model are highlighted in bold and significance levels for the most likely model are indicated with *=p<0.05, **=p<0.01, ***=p<0.001.

Time	Model	AICc
Early	PPC=MAZPD+MAZPS+ZPC+MIZPD+MIZPS+DOC+PAR+BAC	69.25
Early	PPC=MAZPD+MAZPS+ZPC+MIZPD+MIZPS+DOC+PAR	61.58
Early	PPC=MAZPD+MAZPS+ZPC+MIZPS+DOC+ PAR	56.8
Early	PPC=MAZPD+ZPC+MIZPS+DOC+PAR	53.52
Early	PPC=MAZPD+MIZPS+DOC+PAR	50.59
Early	PPC=MIZPS+DOC+PAR	47.57
Early	PPC=MIZPS+DOC	45.75
Early	PPC=DOC	44.46
Intermediate	PPC=SNA+DRA+MAZPD+MAZPS+ZPC+MIZPD+MIZPS+DOC+PAR+BAC	97.52
Intermediate	PPC=SNA+DRA+MAZPD+MAZPS+ZPC+MIZPD+DOC+PAR+BAC	84.02
Intermediate	PPC=SNA+MAZPD+MAZPS+ZPC+MIZPD+DOC+PAR+BAC	73.69
Intermediate	PPC=SNA+MAZPD+ MAZPS +ZPC+MIZPD+DOC+BAC	66.05
Intermediate	PPC=SNA+MAZPD+ MAZPS +ZPC+DOC+BAC	60.05
Intermediate	PPC=SNA+MAZPD+ MAZPS +ZPC+DOC	55.16
Intermediate	PPC=SNA+MAZPD+ MAZPS +ZPC	51.41
Intermediate	PPC=SNA+ MAZPS +ZPC	48.82
Intermediate	PPC= MAZPS +ZPC	47.09
Intermediate	PPC= MAZPS *	45.21
Late	PPC=SNA+DRA+MAZPD+MAZPS+ZPC+MIZPD+MIZPS+DOC+PAR+BAC	98.6
Late	PPC=SNA+ DRA +MAZPD+ ZPC + MIZPD +MIZPS+DOC+PAR+BAC	85.03
Late	PPC=SNA+ DRA +MAZPD+ ZPC + MIZPD +MIZPS+DOC+BAC	74.87
Late	PPC= DRA +MAZPD+ ZPC + MIZPD +MIZPS+DOC+BAC	67.06
Late	PPC= DRA +MAZPD+ ZPC + MIZPD + MIZPS +BAC	60.48
Late	PPC= DRA +MAZPD+ZPC+ MIZPD +MIZPS	58.92
Late	PPC= DRA +ZPC+MIZPD+MIZPS	57.00
Late	PPC= DRA +MIZPD+MIZPS	55.56
Late	PPC= DRA +MIZPD	55.41
Late	PPC= DRA *	53.36

Appendix S8 – Effect sizes for the different contrasts on zooplankton density and size per taxon



Shown are effect sizes (Cohens d) for the different contrasts (BB vs. BL=plasticity=white, BL vs. LL=genetic=grey, LL vs. BB=combined=black) at the end of the experiment on the y-axis and the different metrics on the x-axis. Bos=Bosmina, Cal=calanoid copepods, Cyc=cylopoid copepods, Cop=copepod nauplii and Rot= Rotifera. Acarids, Daphnia and Ostracoda were very rare (relative abundance at each date within each tank always <1%) and are thus not included here. D refers to density and S to size. Zooplankton taxa are divided into trophic and non-trophic. On the right, averaged effect size per contrast over all metrics (including densities and sizes) as well as averages for trophic and non-trophic metrics are shown. Bar colours are as in Figure 1 and 4 of the main text. Significant randomization tests are indicated with *=p<0.05, **=p<0.01, ***=p<0.001.

Chapter 5

The legacy of ecosystem effects caused by adaptive radiation

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Abstract

There is increasing evidence that closely related species have contrasting ecosystem effects, but very little is known about the temporal scale at which such effects emerge and persist. Effects that either emerge after or persist beyond the presence of the environment modifying organism in the ecosystem would have increased significance on ecosystems and on the potential for eco-evolutionary feedbacks to accompany evolutionary diversification. Here we studied lab-raised whitefish of a benthic-limnetic species pair from a postglacial adaptive radiation to test whether closely related species have contrasting ecosystem effects. We compared the strength of ecosystem effects resulting from fish presence (an ecological effect), rearing environment (plasticity effect), species differences (evolutionary effect) and an interaction between the latter two (interaction effect). In addition, we tested whether such effects decay or whether new effects emerge following the removal of fish from the ecosystem. We found strong effects on ecosystems, some of which disappeared and others that emerged only after the removal of the fish. At the end of the experiment evolutionary and ecological effects were similar in overall strength. Our results suggest that adaptive radiation can have substantial effects on the structure and function of ecosystems and that there is potential for eco-evolutionary feedbacks to accompany the process of adaptive radiation. Further, they indicate the need to not only include classical determinants of ecosystems, but to also take evolutionary processes and history into account, in order to increase our understanding of what governs structure, function and dynamics of ecosystems.

Introduction

The process of adaptive radiation typically results in groups of closely related species that are phenotypically and ecologically differentiated and coexist in close physical proximity (Schluter, 2000). Divergent selection is the primary driver of phenotypic diversification during adaptive radiation, and is typically thought to arise from contrasting environmental conditions (Schluter, 2000). However, a complementary view is that adaptive phenotypic diversification itself can modify the fitness landscape through the dynamic emergence of contrasting niches as result of competition and negative frequency dependent selection (Schluter, 1994, 1996; Schluter, 2000; Benkman, 1999; Dieckmann *et al.*, 2004). Further, phenotypic differences between closely related populations or species have been shown to directly modify a broad range of ecosystem variables (e. g. Post *et al.*, 2008; Harmon *et al.*, 2009), and this could be an overlooked agent of selection that emerges during evolutionary diversification (Losos *et al.*, 2010, Yoder *et al.*, 2011). In fact, we know little about how adaptive radiations usually unfold in response to multiple interacting agents of selection that can lead to complex fitness landscapes of high dimensionality (Losos, 2010; Yoder *et al.*, 2011; MacColl, 2011). The idea that phenotypic diversification might affect ecosystems and alter the course of a radiation is well possible during adaptive radiation, as the functional links between an organism's phenotype and its environment is thought to be particularly strong in adaptive radiations (Schluter, 2000; Pelletier *et al.*, 2009).

There are two components of timescale that are important for understanding how interactions between phenotypic diversification and ecosystem dynamics might play out in an adaptive radiation. First, on what time-scale does adaptive divergence, that causes contrasting ecosystem effects, emerge? We know that evolutionary change during adaptive radiation is often rapid (e. g. Harmon *et*

al., 2003) and there is evidence that phenotypic differences among relatively young species or populations in adaptive radiations can affect ecosystem conditions (Harmon *et al.*, 2009; Lundsgaard-Hansen *et al.*, in review). For example, phenotypic differences between 10'000 year old benthic and limnetic stickleback were shown to have ecological effects on various properties of experimental ecosystems such as zooplankton community structure, gross primary productivity and the rate of light extinction in the water column (Harmon *et al.*, 2009). Such ecosystem effects can be due either to heritable species divergence or to environmentally induced plastic differences, or both (Seehausen, 2009; Lundsgaard-Hansen *et al.*, in review). If the differences between species that cause different ecosystem effects were mostly environmentally induced (phenotypically plastic), then the ecosystem effects of contrasting phenotypes might emerge within a single generation and before genetic differences and reproductive isolation evolve.

Second, over what timescale do the contrasting ecosystem effects of closely related species persist in the environment in the face of external ecosystem drivers? This is important for determining how important evolution might be as a determinant of ecosystem structure and function and how likely ecosystem modifications are to affect selection pressures in subsequent generations (Odling-Smee *et al.*, 2003; Post & Palkovacs, 2009). In general, there is an increasing interest in how ecological and evolutionary dynamics are linked on similar time-scale (Schluter, 1994; Schluter, 2000; Dieckmann *et al.*, 2004; Hairston *et al.*, 2005; Schoener, 2011), and there are multiple frameworks to study them. The idea that ecological and evolutionary dynamics are closely linked and that evolving organisms modify their own selective landscape is at the core of adaptive dynamics theory and adaptive speciation research (Geritz *et al.*, 1998; for review see Dieckmann *et al.*, 2004). In adaptive dynamics modeling though evolutionary dynamics are assumed to happen at a slower timescale than ecological dynamics and the two processes are analytically separated (Fussmann *et al.*, 2007). The timescale of interaction between ecological and evolutionary process is also at the core of emerging work in the field of eco-evolutionary dynamics, where a considerable body of work investigates ecosystem effects of contemporary evolution and recent evolutionary history (Fussmann *et al.*, 2007; Post & Palkovacs, 2009; Schoener, 2011). Previous work suggest that the size and persistence of ecosystem effects could be important for understanding how organism mediated modification of the environment might influence other environmental sources of selection during an adaptive radiation (Odling-Smee *et al.*, 2003; Yoshida *et al.*, 2003; Palkovacs & Post, 2008, 2009; Schoener, 2011).

Here we use lab-raised fish from a benthic limnetic species pair of a young whitefish adaptive radiation (*C. zugensis* and *C. sp.* "Bodenbalchen" from Lake Lucerne, Switzerland; Hudson *et al.*, 2011) to test whether evolutionary divergence during adaptive radiation has generated contrasting ecosystem effects and to investigate the time-scale of the occurrence and persistence of such effects. We used the different species in a common gardening experiment (*sensu* Matthews *et al.* 2011a). The aim of a common gardening experiment is to use organisms with phenotypic differences between them and then measure whether their phenotypic differences have contrasting effects on an experimental ecosystem. Our experiment was divided into two phases: In a first phase the agents (different whitefish species) were present in experimental ecosystems and in the second phase they were removed (Figure 1). Our aim was to quantify ecosystem divergence at the end of phase 1 and

measure the persistency and possible emergence of new effects at the end of phase 2. We distinguish between four time patterns: If ecosystem divergence occurred in phase 1, it could either persist or disappear during phase 2. If measurable ecosystem divergence did not occur in phase 1, contrasting effects could either emerge or remain absent in phase 2. In addition to being conceptually interesting, the removal of the agent of ecosystem modification (i.e. the fish) has a clear biological relevance, because many organisms, at least in non-tropical regions, are either migratory, hibernating or display other changes in activity and behaviour over the seasons, and therefore do not continuously affect their environment in a consistent fashion.

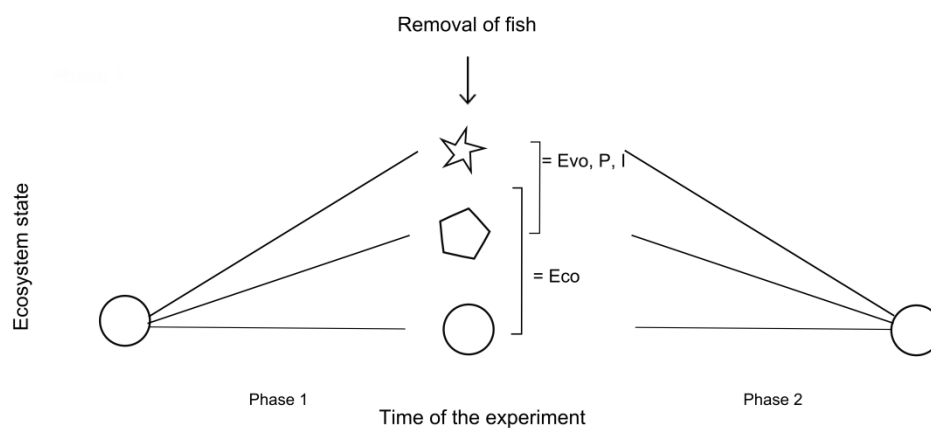


Figure 1: Schematic of the time-scale of our experimental design. In phase 1 of the experiment we used four different whitefish treatments in order to induce and quantify ecosystem divergence. In phase 2 we aimed at measuring the time-scale of persistency of the induced ecosystem divergence, after the removal of whitefish from the ecosystems. Shown is a potential scenario of how two whitefish treatments (star and polygon, respectively) diverge away from no fish tanks (circles) in their ecosystem state in phase 1 of the experiment and converge back during phase 2. Depending on which fish treatments are compared to each other (see main text), measured differences result from plasticity (P), evolutionary (Evo) or interaction effects (I). Ecological effects (Eco) are measured by comparing fish tanks to no fish tanks.

We raised families of the two whitefish species on two different food treatments each, resulting in four treatments for our common gardening experiment: The benthic species (B), *C. sp.* “Bodenbalchen”, raised on benthic (BB) or limnetic food (BL) and the limnetic species (L), *C. zugensis*, raised on benthic (LB) or limnetic food (LL). This design allowed us to partition ecosystem effects into such resulting from evolutionary species divergence and phenotypic plasticity as well as testing for interactions between the two. We refer to these contrasts as evolutionary, plasticity and interaction contrasts. Ecosystem effects resulting from evolutionary processes have previously been shown to compete in strength with classical determinants of ecological dynamics such as climatic effects, the presence/absence of a species or a species invasion (Hairston *et al.*, 2005; Ezard *et al.*, 2009; Palkovacs *et al.*, 2009; Palkovacs *et al.*, 2012). To test whether these findings also apply to effects of evolutionary divergence in adaptive radiation, we compared the strength of contrasting evolutionary ecosystem effects to that of classic ecological effects resulting from whitefish presence versus

absence by comparing tanks without whitefish (no fish = NF) to tanks with whitefish. We refer to this contrast as ecological contrast (Figure 1). Such comparisons are needed to assess how important evolutionary processes are for ecosystem dynamics relative to classical ecological determinants (Palkovacs & Hendry, 2010; Palkovacs *et al.*, 2012).

Material & Methods

Study species

We used two whitefish species, *C. zugensis* and *C. sp.* “Bodenbalchen”, from a recent adaptive radiation of Lake Lucerne, Switzerland, that began to form maximally 15'000 years ago (Hudson *et al.*, 2011). *C. zugensis* corresponds to a limnetic ecotype (Schluter, 2000) and is characterized by slow growth and small adult size, a high number of gill rakers and a streamlined body shape (Steinmann, 1950). *C. sp.* “Bodenbalchen” corresponds to a benthic ecotype (Schluter, 2000) and is characterized by faster growth and large adult size, a lower number of gill rakers and a more deep bodied shape (Steinmann, 1950). The species are strongly differentiated at neutral genetic markers (F_{ST} based on microsatellite loci ~ 0.1 , Vonlanthen *et al.*, 2012) and spawn at different water depths (*C. sp.* “Bodenbalchen”: 1-5 m, *C. zugensis*: >25 m). Recent experimental work has shown that the two species differ in their feeding efficiency on benthic vs. limnetic food, indicating that *C. zugensis* is better adapted to limnetic (zooplankton) feeding and *C. sp.* “Bodenbalchen” to benthic feeding (Lundsgaard-Hansen *et al.*, 2013; Roesch *et al.*, 2013). Differentiation in nature is not restricted to traits related to spawning and feeding, the species also differ in parasite load (Karvonen *et al.*, 2013), in metabolic traits (Blank *et al.*, 2012) and some intrinsic genetic incompatibilities were found between them (Woods *et al.*, 2009).

Common garden experiment

Of each species, five males and five females were used to breed half-sib families for this experiment. The eggs were mixed and simultaneously fertilized by the sperm of the five males, resulting in up to 25 half-sib families per species. In the first year, all juveniles were raised on zooplankton because whitefish larvae cannot efficiently be raised on benthic food. For our common garden experiment we then split juveniles of each species into two food treatments: half of the individuals were switched to a red mosquito larvae diet to simulate a benthic feeding environment, while the other half were kept on zooplankton diet to simulate a limnetic feeding environment. This resulted in four treatments for our common gardening experiment, the benthic species (*C. sp.* “Bodenbalchen”) raised on benthic (BB) and on limnetic food (LB) and the limnetic species (*C. zugensis*) raised on benthic (LB) and limnetic food (LL). More details about the raising of experimental fish are provided elsewhere (Lundsgaard-Hansen *et al.*, 2013).

Common gardening experiment

We used a total of 33 mesocosms (1000 l) to serve as replicated ecosystems. To set up the mesocosm ecosystems, we covered the bottom of each tank with a thin layer (2-3 cm) of gravel and sand collected in Lake Lucerne by a commercial gravel pit company. We overlaid this layer with a layer (3-4 cm) of sediments collected from three lakes in the region of Lucerne (Roth, Sempach and Lucerne) in order to inoculate the tanks with sediments and a diversity of aquatic organisms. We filled each tank with water from Lake Lucerne. Additionally, we supplemented the tanks with zooplankton collected

from Lake Lucerne. To stimulate primary production we once added 2.46 g of NaNO_3 and 0.18 g NaH_2PO_4 (Phosphorous and Nitrogen) to each tank. Setting-up the tanks was finished by the 29th of April 2010 and fish were introduced the 12th of May. Fish from the BB, LB and LL treatment were distributed across seven tanks each and fish from the BL treatment across 5 tanks. In total 78 fish were distributed across 26 tanks and the number of fish was varied between 2 and 5 per tank. Average fish weight per tank was 62 g and did not differ between fish treatments (ANOVA: F-ratio=0.22, df=3, p=0.88). Seven tanks were left empty (no fish = NF). Over the duration of the experiment four fish died (mortality rate=0.05). These fish were immediately replaced by an individual with similar weight and from the same treatment. After eight weeks we observed sudden high mortality rates, potentially resulting from a heat shock and/or an oxygen deficiency. In total 16 of the 78 fish died overnight and these were distributed over nine tanks, all but one belonging to the limnetic species. Dead fish were immediately removed. Surviving fish were humanely killed with an overdose of MS-22 and we stopped phase 1 of the experiment two weeks earlier than initially planned. After the removal of the fish, tanks were left as they were and all ecosystem metrics were measured again 14 weeks later, at the end of phase 2 in the first half of October 2010. We tested whether the number of dead fish in a tank had an effect on the metrics we measured at the end of the experiment by including the number of dead fish per tank in the ANOVAs as a co-variable to the main effects genetic background and rearing environments. As its effect was never significant (across 16 tests), it is very unlikely that it biased our results obtained at the end of phase 2.

Measured ecosystem metrics

We measured a broad array of biological and chemical ecosystem metrics. The following metrics were measured during phase 1 and 2 (see Table 1 for an overview): The abundance of snails (SNA), the abundance of *Dreissena* mussels (DRA), macro and micro zooplankton density (MAZPD and MIZPD), macro and micro zooplankton community composition (MAZPC and MIZPC), phytoplankton concentration (PPC), the concentration of dissolved organic compounds (DOC), benthic algae cover (BAC), transmission of photosynthetic active radiation through the water column (PAR), sedimentation rate (SED) and gross primary production (GPP). Size variation of macro and micro zooplankton (MAZPS and MIZPS) and phosphor concentration (PHO) was only measured at the end of phase 2. Additionally we screened sediments for larger benthic organisms at the end of phase 2 and this revealed the presence of loaches (LOA, *Barbatula barbatula*) in some tanks. These small benthic fish were incidentally introduced to the tanks with sediments. The fact that we have overseen them when we inoculated the mesocosms with sediments, may suggest that they have been introduced as eggs, fry or juveniles, briefly after spawning (loaches start to spawn in April; Kottelat & Freyhof, 2007). In total 10 loaches were found distributed across 8 tanks at the end of phase 2 of our experiments and their weight ranged from 1.7-2.8 g per individual. That loaches could grow from a larval stage to a weight of 1.7-2.8 g during our experiment seems well possible, as they are fast growing and have been observed to grow to a 10-fold weight increase in just 35 days in early development (Elliot *et al.*, 1996). As loaches may serve as whitefish prey or competitor for benthic food, we treated the presence/absence of loaches as a response variable in statistical analysis.

Details on how the different parameters were measured are given in Supplementary Information.

A previous experiment using the same species found that significant ecosystem contrasts of evolutionary divergence resulted most frequently from direct trophic interaction of whitefish with its prey (Lundsgaard-Hansen *et al.*, in review). Further, predictions about persistency time of contrasting ecosystem effects may be different for different functional components of the ecosystem (Matthews *et al.*, in revision). Therefore we divided measured ecosystem metrics into such resulting from direct trophic interactions of whitefish with their prey (referred to as “trophic” and including SNA, DRA, MAZPD, MAZPC, MAZPS, LOA), non-trophic metrics associated with living organic material (referred to as “non-trophic, living”, including MIZPD, MIZPC, MIZPS, PPC, BAC) and non-trophic metrics associated with chemical or physical components of the environment or with ecosystem function/processes (referred to as “non-trophic, non-living”, including DOC, PAR, GPP, PHO, SED; see Table 1). Non-trophic metrics can be affected through indirect trophic effects of whitefish (for example driven by trophic cascades; Carpenter, 1985) or through direct non-trophic effects (for example through different nutrient excretion rates), but we for simplicity collectively refer to them as non-trophic effects.

Statistical analysis

We used redundancy analysis (RDA) to measure macro and micro zooplankton community composition (Legendre & Gallagher, 2001). We used Cohens *d* to assess effect strength on the different metrics across contrast (ecological, evolutionary, interaction and plasticity) and time (end of phase 1 and end of phase 2). Cohens *d* takes the difference in the means of two groups for the variable of interest and corrects for the pooled standard deviation (Cohen, 1988). Significance levels were calculated by randomizations (Bailey *et al.*, 2009) and the effect size for the interaction was calculated as in Gruner *et al.* (2008). We took absolute values of *d* for comparison of effect strength between contrasts and phases, respectively. We used paired *t*-tests to compare effect strength between the different contrasts and phases. Metrics that were only measured at the end of phase 2 were not included in the comparison of effect strength between phase 1 and phase 2 in order to have a paired comparison.

Results

Whitefish presence, evolutionary divergence and plasticity all affect ecosystems

Ecological effects (resulting from whitefish presence) were strong on some of the ecosystem metrics at the end of phase 1 of the experiment (Table 1). Relative to NF tanks (no fish tanks), macro zooplankton density (MAZPD) and phytoplankton concentration (PPC) were elevated in fish tanks, whereas snail abundance (SNA) and *Dreissena* abundance (DRA) were diminished in fish tanks. Further, macro zooplankton community composition (MAZPC) was different between fish tanks and NF tanks, with relatively more Chydoridae and less Cyclopoids in NF tanks (Supplementary Table 1). The frequency of significant ecological effects at the end of phase 1 was 0.33.

At the end of phase 1, differences between whitefish treatments affected two out of twelve metrics. Both significant effects resulted from differences in plastic traits (Table 1). PPC was lower in tanks containing fish that were raised on limnetic food and phenotypic plasticity also significantly affected micro zooplankton community composition (MIZPC), with relatively more copepod nauplii and less *Keratella* in tanks containing fish raised on limnetic food (Supplementary Table 2). We did not find

any significant interactions between plasticity and evolution at the end of phase 1, indicating that modifications of ecosystem effects by phenotypic plasticity were independent of the species background of the fish. The frequency of significant effects of plasticity was 0.17 at the end of phase 1 of the experiment.

At the end of phase 2, ecological contrasts were significant in four of sixteen ecosystem metrics (Table 1). Relative to NF tanks, the concentration of dissolved organic compounds (DOC) was elevated and that of phosphor (PHO) diminished in fish tanks. Micro zooplankton size (MIZPS) was increased in NF tanks and fish presence significantly affected MIZPC, with increased relative abundance of *Bosmina*, Calanoids and *Ostracoda* and decreased relative abundance of Chydoridae, Cyclopoids and *Daphnidae* (Supplementary Table 1). The frequency of significant ecological effects at the end of phase 2 of the experiment was 0.25.

At the end of phase 2, differences between whitefish treatments affected six out of sixteen ecosystem metrics (Table 1). Four of these effects were a result of evolutionary divergence between species, one was a result of an interaction between evolutionary differentiation and phenotypic plasticity and one was a result of differences in phenotypically plastic traits. PPC and DOC were decreased in tanks containing the benthic species relative to tanks containing the limnetic species. Loaches (LOA) were completely absent from tanks containing whitefish of the benthic species, whereas 1 or 2 loaches were found in 5 out of 14 tanks of the limnetic species (and in 3 out of 7 NF tanks). Micro zooplankton (MIZPS) was smallest in the LL and largest in the BB treatment, with the other treatments being intermediate. Both plasticity and evolutionary effects on MIZPS were significant (Table 1). The effects on MIZPS result from a combination of size reduction within the most abundant micro zooplankton taxon (copepod nauplii) in more limnetic treatments and a community shift towards decreased relative abundance of large copepod nauplii taxa compared to smaller Rotatoria like taxa in the more limnetic treatments (Supplementary Table 1). Finally, we found a significant interaction between plasticity and evolutionary differentiation on MAZPC (Supplementary Table 1). The frequency of significant effects of evolutionary differences between whitefish species was 0.25 while that of plasticity and the interaction was 0.06 at the end of phase 2 of the experiment.

All treatment effects (including non-significant) are visualized in Supplementary Figure 1.

Table 1: Given is ecosystem effect strength (Cohens d) resulting from fish presence versus absence (Eco), for whitefish phenotypic plasticity (Pla), from evolutionary divergence (Evo) and from an interaction between plasticity and evolution (Int). In the first three columns are the analysed metrics with their categories and acronyms (Acr). In the fourth column is the phase, while “1” refers to end of phase 1 and “2” refers to end of phase 2 (see Figure 1). Metrics are ordered by phase and by category and metrics that were measured only at the end of phase 2 are listed below. Trends are in italics, significant effects are in bold and stars indicate significance levels (*= $p < 0.05$; **= $p < 0.01$, ***= $p < 0.001$).

Metric	Category	Acr	P	Eco	Pla	Evo	Int
Snail abundance (n/m ²)	Direct trophic	SNA	1	1.19*	0.26	0.36	0.17
Dreissena abundance (n/m ²)	Direct trophic	DRA	1	1.34**	0.34	0.5	0.48
Macro zooplankton density (n/l)	Direct trophic	MAZPD	1	<i>0.82</i>	0.09	0.21	0.11
Macro zooplankton community	Direct trophic	MAZPC	1	2.26***	0.21	0.33	0.15
Micro zooplankton density (n/l)	Non trophic, living	MIZPD	1	0.49	0.14	0.005	0.42
Micro zooplankton community	Non trophic, living	MIZPC	1	0.3	1.45**	0.23	0.51
Phytoplankton concentration (mg/l)	Non trophic, living	PPC	1	1*	0.97*	0.08	0.27
Benthic algae cover (%)	Non trophic, living	BAC	1	0.45	0.52	0.46	0.13
Photosynthetic active radiation (%)	Non trophic, non living	PAR	1	0.46	0.46	0.44	0.41
Dissolved organic compounds (mg/l)	Non trophic, non living	DOC	1	0.41	0.006	0.37	0.32
Gross primary production (mg O ₂ /l)	Non trophic, non living	GPP	1	0.2	0.08	0.31	0.11
Sedimentation rate (mg/d)	Non trophic, non living	SED	1	0.49	0.41	<i>0.74</i>	0.13
Snail abundance (n/m ²)	Direct trophic	SNA	2	0.63	<i>0.73</i>	0.07	0.06
Dreissena abundance (n/m ²)	Direct trophic	DRA	2	0.5	0.53	0.06	0.3
Macro zooplankton density (n/l)	Direct trophic	MAZPD	2	0.08	0.11	0.28	0.5
Macro zooplankton community	Direct trophic	MAZPC	2	0.33	0.5	0.21	0.91*
Micro zooplankton density (n/l)	Non trophic, living	MIZPD	2	0.5	0.08	0.13	0.45
Micro zooplankton community	Non trophic, living	MIZPC	2	1.88**	0.13	0.85*	0.88*
Phytoplankton concentration (mg/l)	Non trophic, living	PPC	2	0.11	0.29	<i>0.79</i>	0.1
Benthic algae cover (%)	Non trophic, living	BAC	2	0.39	0.26	<i>0.8</i>	0.02
Photosynthetic active radiation (%)	Non trophic, non living	PAR	2	0.75	0.004	0.16	0.65
Dissolved organic compounds (mg/l)	Non trophic, non living	DOC	2	1.48**	0.14	1.46**	0.27
Gross primary production (mg O ₂ /l)	Non trophic, non living	GPP	2	0.4	0.09	0.04	0.08
Sedimentation rate (mg/d)	Non trophic, non living	SED	2	0.15	0.45	0.42	0.35
Macro zooplankton size (mm)	Direct trophic	MAZPS	2	0.65	0.03	<i>0.72</i>	0.27
Abundance of loaches (n)	Direct trophic	LOA	2	0.28	0.2	0.93*	0.06
Micro zooplankton size (mm)	Non trophic, living	MIZPS	2	0.86*	0.86*	0.92*	0.02
Phosphor concentration (mg/l)	Non trophic, non living	PHO	2	1.05*	0.48	0.06	0.13

Comparing effect strength across contrasts and time

Analysis of effect strength resulting from the different contrasts indicates that evolutionary and plasticity effects are overall similarly strong at the end of phase 1 (paired t-test non-significant), with an average Cohens d of 0.34 for the evolutionary contrast and an average Cohens d of 0.41 for the plasticity contrast (Figure 2). Ecological effects of fish presence versus absence (Cohens d = 0.78) were significantly stronger than evolutionary effects (ecological vs. evolutionary: p-value = 0.03), but not stronger than effects resulting from phenotypic plasticity (ecological vs. plasticity = 0.12).

At the end of phase 2, significant evolutionary effects were more common than at the end of phase 1 and they were more common than plasticity and as common as ecological effects (4 vs. 1 vs. 4). The finding of a significant interaction indicated that plasticity can modify effects of evolutionary differentiation or vice versa (Table 1). Average evolutionary effect strength was 0.49 and that of plasticity 0.31 and these differences were not significant (paired t-test p-value = 0.18; Figure 2). Average ecological effects were 0.62 and they were significantly stronger than those resulting from plasticity (paired t-test p-value = 0.03, Figure 2), but they were not different in strength from effects resulting from evolutionary species differentiation (paired t-test p-value = 0.32, Figure 2).

Hence, over time in the course of the experiment, evolutionary effects increased in frequency and strength, while ecological and plasticity effects decreased in frequency and strength (Figure 2). The observed increase in frequency and strength of evolutionary effects indicates that effects of evolved species differences can strengthen and/or emerge even after whitefish were no longer present in the ecosystem.

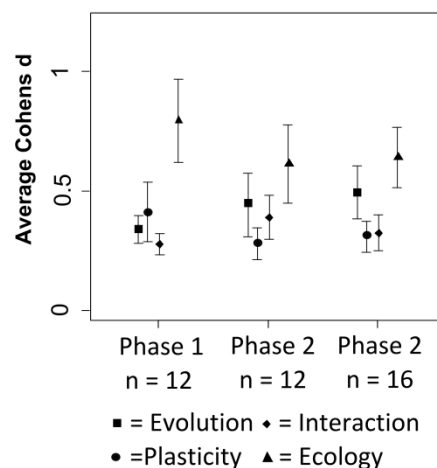


Figure 2: Effect strength of ecosystem effects observed across all measured metrics per contrast and phase of the experiment. Given is the effect strength (Cohens d) for each contrast averaged across all metrics (see Table 1) with its standard deviation on the y-axis measured at the end of phase 1 and of phase 2 (on the x-axis). Squares correspond to the evolutionary contrast, circles correspond to the plasticity contrast, diamonds correspond to the interaction contrast and triangles correspond to the ecological contrast (see main text for more detail). Fish were present in the experimental ecosystem until the end of phase 1 and they were absent during phase 2. At “Phase 2; n = 12” only metrics that were measured at phase 1 and at phase 2 of the experiment are included (see Table 1). At “Phase 2; n = 16” metrics that we only measured at the end of phase 2 are additionally included.

Discussion

We found strong and significant effects of evolved differences between closely related species on experimental mesocosm ecosystems (Table 1). This adds more evidence to recent findings that evolutionary differentiation even of very young species in adaptive radiations can induce ecosystem divergence (Harmon *et al.*, 2009; Lundsgaard-Hansen *et al.*, review). It is also consistent with previous work showing that phenotypic divergence between populations of the same species as a

result of local adaptation can induce ecosystem divergence (Post *et al.*, 2008; Palkovacs *et al.*, 2009; Bassar *et al.*, 2010). No previous study had experimentally investigated the persistency of such contrasting ecosystem effects resulting from phenotypic differentiation (either due to evolutionary divergence or phenotypic plasticity). Our data suggest that some components of ecosystem divergence disappear and others just emerge after the fish population was no longer present in the ecosystem (Figure 3). At the end of our experiment, effects of evolutionary differentiation were no less strong than ecological effects. This resulted from ecological effects decreasing and evolutionary effects increasing over the course of the experiment (Figure 2). It had previously been shown that contrasting ecosystem effects of divergent local adaptation in guppies can be stronger than ecosystem effects of invasion of guppies into habitats that previously did not harbour guppies (Palkovacs *et al.*, 2009). Our findings of similarly strong evolutionary and ecological effects add evidence to this, and strongly suggest a need to not only take classical determinants of ecological processes (such as species presence or absence or species invasions) but also evolutionary history and process into account, in order to understand the structure and function of ecosystems. Further, the finding that induction of ecosystem divergence by evolutionary species differentiation lasts beyond the period of physical presence of the organisms indicates the potential for evolution to affect selective landscapes of subsequent generations of evolving organisms.

Temporal aspects of ecosystem effects

We investigated two different components of the time-scale involved in how phenotypic differences within or between species affect ecosystems. First, we asked over what time-scales the phenotypic differences with contrasting ecosystem effects emerge. We found that phenotypic differences that are environmentally induced and emerge over the time-scale of a generation can have contrasting ecosystem effects. We also found evidence for contrasting ecosystem effects caused by phenotypic differences that emerged through evolutionary divergence over longer time-scales (maximally 15'000 years). Second, we investigated the temporal dynamics of contrasting ecosystem effects during the presence and after the removal of the environment modifying organism. None of the six significant contrasts that resulted from either the ecological, evolutionary or plasticity contrast at the end of phase 1, persisted significantly until the end of phase 2. Importantly though, some contrasting effects only emerged and became significant in phase 2 (Figure 3). Such increases of effect strength were the strongest and most frequently observed for the evolutionary contrast (Figure 2, Figure 3, Table 1). Emergent evolutionary effects were found for micro zooplankton community composition (MIZPC) and the concentration of dissolved organic compounds (DOC). Although these variables are associated with aquatic food-webs, they were neither correlated with each other at the end of phase 1 nor at the end of phase 2, making trophic cascade effects rather unlikely as explanation for these results (Carpenter, 1985). Further, evolutionary differences between whitefish species affected micro zooplankton size and the presence and absence of loaches (LOA), two metrics that we only measured at the end of phase 2. Loaches are small fish with reduced swim bladder, indicating that they are very benthic (Kottelat & Freyhof, 2007). They were completely absent from tanks containing whitefish of the benthic species. This might suggest that the benthic species fed on juvenile loaches. Because we have not measured presence/absence of loaches at the end of phase 1, we cannot exclude that the observed pattern resulted from different survival probabilities of loaches in tanks

containing benthic versus limnetic species during phase 2. And we can neither exclude that the benthic species prevented the establishment of loaches through competitive exclusion as opposed to consumption. However, independent of the exact reason for the observed effect on loaches, our findings suggest that evolutionary differentiation of whitefish species during adaptive radiation has the potential to affect fish community composition in an ecosystem.

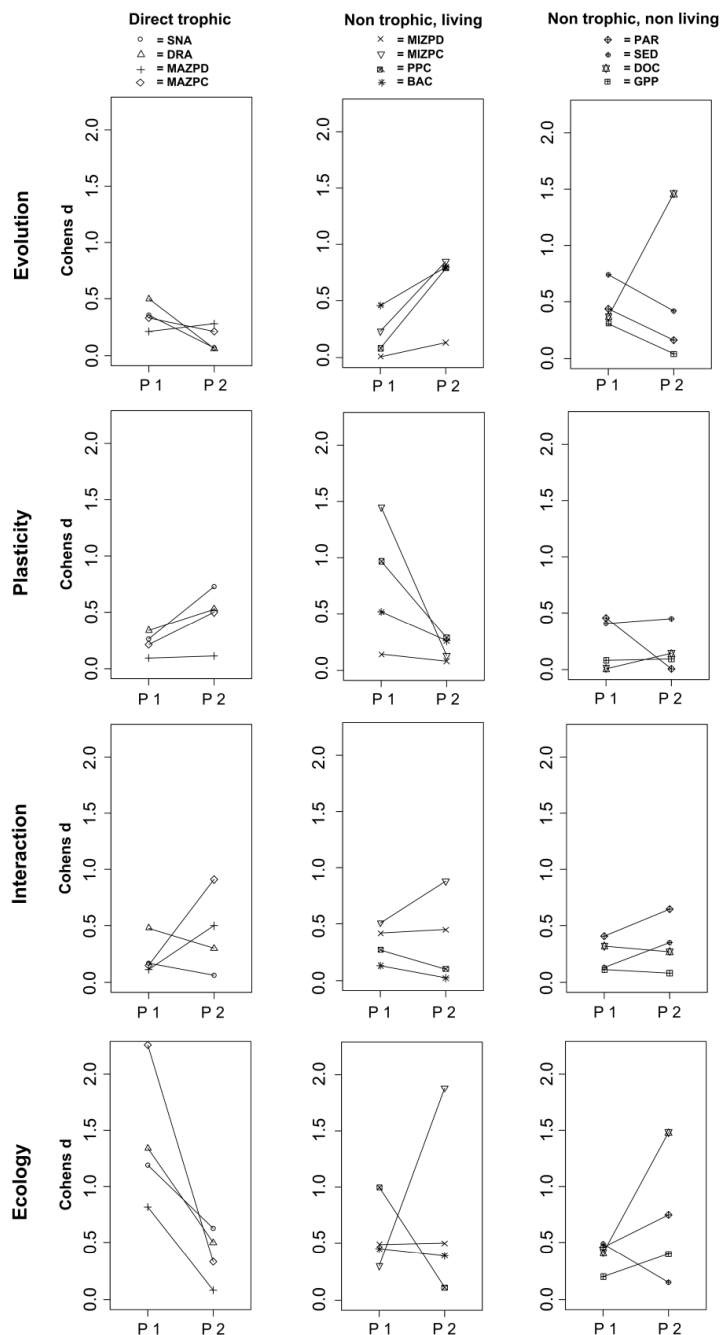


Figure 3: Effect size on single metrics per contrast and phase of the experiment. Given are effect strengths (Cohens d) for particular metrics on the y-axis and phase 1 and phase 2 on the x-axis. Evolutionary contrasts are shown at the top, followed by plasticity and interaction contrasts with ecological contrasts at the bottom. “Trophic effects” are shown at the left; “non-trophic, living” in the middle and “non-trophic, non-living” at the right. Acronyms are as in Table 1 and significance levels are reported in Table 1.

The observation that some effects of whitefish evolutionary divergence seem to be stored in the ecosystem to induce strong ecosystem divergence with some time lag adds an additional dimension to how evolution can affect ecosystems compared to previous studies, which did not measure the time-scale of persistency of contrasting effects of adaptive radiation (Harmon *et al.*, 2009; Lundsgaard-Hansen *et al.*, in review). If our observation that ecosystem effects of evolution can be stored in the ecosystem was general, this will greatly increase the importance of evolution as determinant of ecosystem structure and function and indicate the potential for eco-evolutionary feedbacks (Odling-Smee *et al.*, 2003; Post & Palkovacs, 2009). Although we did not directly test eco-evolutionary feedbacks in our experiments, we discuss below the relevance of our work for understanding eco-evolutionary feedbacks that might have accompanied whitefish adaptive radiation.

How might the observed effects be involved in eco-evolutionary feedbacks?

The whitefish species we studied here belong to a monophyletic adaptive radiation that has happened in Lake Lucerne after the last glacial maximum, with species starting to form no longer than 15'000 years ago (Hudson *et al.*, 2011). While the endemic species evolved within the lake in geographical sympatry, their genetic variation derives from two distantly related glacial lineages that formed a hybrid swarm shortly before or just after colonization of the Alpine region (Hudson *et al.*, 2011). This suggests that whitefish standing genetic variation was high from the onset of colonization which may have allowed for fast responses to selection during early diversification (Seehausen, 2004; Barrett & Schluter, 2008; Hudson *et al.*, 2011). Natural selection during whitefish speciation was multi-dimensional and included selection on spawning depth, spawning time (Vonlanthen *et al.*, 2009; Vonlanthen *et al.*, 2012), life history (Vonlanthen *et al.*, 2012), physiology (Blank *et al.*, 2012) and on foraging traits and behaviour (Vonlanthen *et al.*, 2009; Lundsgaard-Hansen *et al.*, 2013; Roesch *et al.*, 2013). Further, some traits with an important functional role for benthic vs. limnetic feeding have been shown to be genetically heritable in whitefish (Bernatchez, 2004; Lundsgaard-Hansen *et al.*, 2013; Roesch *et al.*, 2013) and fast changes in trait means, most likely through contemporary evolution, has been documented in several Swiss whitefish species (Bittner *et al.*, 2010; Vonlanthen *et al.*, 2012). All these characteristics of the whitefish species complex, in combination with our previous and current results of contrasting ecosystem effects resulting from evolutionary differentiation of whitefish species (Lundsgaard-Hansen *et al.*, in review) can facilitate eco-evolutionary feedbacks (Odling-Smee *et al.*, 2003; Yoshida *et al.*, 2003; Post *et al.*, 2008; Post & Palkovacs, 2009).

In our experiment, the two whitefish species had divergent effects on the abundance of loaches. Such effects on the abundance of a potential prey or competitor can have far-reaching effects on the nature and strength of natural selection (Schluter, 1996). The presence or absence of red squirrels has for example been shown to affect the outcome of co-evolutionary arms races between crossbills and lodgepole pines (Benkman, 1999), illustrating the potential of one species to affect eco-evolutionary feedbacks between two other species. Further, absence of species from an ecosystem could increase ecological opportunity for those present (Schluter, 2000; Losos, 2010): Ecological speciation of stickleback in Canadian lakes is constrained by which other fish species are present in a lake (Vamosi, 2003). Similarly, all known sympatric benthic limnetic species pairs of Lake whitefish in

North America are found in lakes where Cisco is absent (Pigeon *et al.*, 1997) and trout radiations in prealpine lakes of Europe are confined to lakes on the south side of the Alps where whitefish and char are absent (Seehausen, unpubl. data).

In our experiment, evolved differences among whitefish species affected several metrics associated with aquatic food webs (e.g. DOC, MIZPC) over a longer time-scale than whitefish were present in the ecosystems. Because diversification of whitefish species in prealpine lakes was at least partially driven by variation in trophic resources (Lundsgaard-Hansen *et al.*, 2013; Roesch *et al.*, in press), whitefish-induced changes in the food-web structure of lakes would likely affect the adaptive landscape for subsequent whitefish generations. Eco-evolutionary feedbacks between fish foraging traits and the zooplankton community have been documented in alewife fish and were also suggested in whitefish (Palkovacs & Post, 2008; Kahilainen *et al.*, 2011). Because foraging traits shape (through ecology) and are shaped (through evolution) by the prey community, they may particularly often be involved in eco-evolutionary feedbacks (Grant & Grant, 2006; Palkovacs & Post, 2008; Harmon *et al.*, 2009).

In order to increase our understanding of the potential for eco-evolutionary interactions in adaptive radiation, future experiments should test when along the speciation continuum divergent ecosystem effects first begin to arise (Hendry *et al.*, 2013). Further, to assess whether eco-evolutionary feedbacks may be important during adaptive radiation, they should quantify whether contrasting ecosystem states result in differences in the selective landscapes of the environment modifying organism itself. Because the same factors that constrain adaptive evolution in general can also constrain eco-evolutionary feedbacks, future studies should as a following step try to measure evolutionary responses to modified selective landscapes. Contemporary evolution (Bittner *et al.*, 2010; Vonlanthen *et al.*, 2012), large standing genetic variation (Hudson *et al.*, 2011), species specific adaptation to the trophic environment (Lundsgaard-Hansen *et al.*, 2013; Roesch *et al.*, in press) and strong and long lasting effects on the environment (in this study and in Lundsgaard-Hansen *et al.*, review) has been shown for European whitefish species. They are thus a promising study system to further investigate how eco-evolutionary feedbacks might be involved in adaptive radiation.

Plasticity and feedbacks

Studies investigating how contrasting ecosystem effects of divergent phenotypes result from plasticity and genetically heritable divergence, are rare. There is some evidence that developmentally plastic phenotypic responses to different environments can cause contrasting ecosystem effects (Miner *et al.*, 2005; Lundsgaard-Hansen *et al.*, in review; Matthews *et al.*, 2011b). Here we add further evidence for this and additionally show that plasticity can modify ecosystem effects of evolutionary divergence also over extended time scales. When keeping in mind that rapid within-population adjustment of trait distributions can be achieved through phenotypic plasticity (West-Eberhard, 2003), it is evident that plasticity cannot be neglected when studying eco-evolutionary dynamics. Plasticity effects can result in at least two different types of feedbacks: If environmental fluctuations affect the expression of a developmentally plastic trait and if this trait also affects environmental fluctuations, a reciprocal interplay between the expression of an organism's plastic phenotype and its environment can emerge (Agrawal, 2001; Whitman & Agrawal, 2009). Phenotypic

plasticity can also be involved in true eco-evolutionary feedbacks, if ecosystem effects that result from phenotypic plasticity affect subsequent evolution in a genetically heritable trait, including the genetic basis of plasticity itself (Yamamichi *et al.*, 2011). More theory, as well as more empirical case studies, will help to achieve a better understanding of how developmental plasticity affects ecosystems and the characteristics and likelihood of eco-evolutionary dynamics that emerge from such effects.

Are ecosystem effects replicated across experiments?

In a previous experiment, which investigated ecosystem effects of the same species studied here, we found that ecosystem effects of evolutionary divergence were more numerous than ecosystem effects of phenotypic plasticity at the end of the experiment (Lundsgaard-Hansen *et al.*, in review). In this study we retrieved the same pattern at the end of phase 2 of the experiment, but not at the end of phase 1 (Table 1). The parallel finding of strong and frequent effects resulting from evolutionary divergence at the end of both experiments is evidence for the importance of evolutionary processes and history in determining ecosystem structure and function. However, ecosystem effects on individual metrics were not generally consistent across experiments, neither in their strength and nor in their direction. These differences in the strength of effects on individual metrics are maybe best captured by the observation that trophic effects (those that underlie direct trophic interactions of whitefish with their prey) tended to be more strongly affected by evolutionary divergence in a previous mesocosm experiment (Lundsgaard-Hansen *et al.*, in review), while this result was neither replicated at the end of phase 1 nor at the end of phase 2 of the current experiment. Such differences in the distribution and strength of effects across ecosystem metrics may suggest that it is difficult to predict in detail how evolutionary divergence between populations or species affects ecosystems. One reason for the observed differences between experiments may be that the previous and the current experiment were conducted in different seasons of the year. The previous experiment was run from autumn to early winter, while the current was run from late summer to fall. If this was the reason for the observed differences, it would suggest that ecosystem effects of evolutionary divergence are strongly context dependent, either depending on external ecosystem drivers such as temperature or internal ecosystem dynamics and processes that differ in ecosystems between spring/summer and autumn/winter. Indeed, the result that more numerous significant ecosystem effects resulted from evolutionary divergence than from phenotypic plasticity was only replicated when measurements were taken in the same season of the year, at the end of the previous experiment and at the end of phase 2 of the current experiment, an observation which is consistent with the idea that seasonality can strongly affect ecosystem effects. However, as discussed above, effects on single ecosystem metrics were not generally consistent across experiments, also when measured at the same season of the year. It should be noted that, if measurements were taken at the same season of the year, the ecosystems differed in respect to whether whitefish were present or not. This might explain the differences seen between experiments also when comparing their ecosystem state in the same season of the year. More data will be needed to investigate how external drivers can modify ecosystem effects of evolutionary divergence.

Conclusions

Our findings of strong and frequent ecosystem effects resulting from evolutionary differentiation of very young species in an adaptive radiation strengthens the evidence that phenotypic differentiation can affect ecosystems (Post *et al.*, 2008; Harmon *et al.*, 2009, Bassar *et al.*, 2010) and that these effects can result both from phenotypic plasticity and from evolved species differences (Lundsgaard-Hansen *et al.*, in review). Our findings that, at the end of our experiment, evolutionary effects were comparable in magnitude to those of ecology, highlights the necessity to take evolutionary history and process into account when ecosystem structure, function and long term dynamics in natural systems are to be explained. Because differences in ecosystem state, which emerged as a result of evolutionary species differences, were persistent at the studied time-scale, our findings are consistent with the potential for eco-evolutionary feedbacks to emerge in and perhaps fuel adaptive radiation.

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References

- Agrawal, A. A. 2001. Phenotypic plasticity in the interactions and evolution of species. *Sciences* 294: 321-326.
- Barrett, R.D.H. & Schluter, D. 2008. Adaptation from standing genetic variation. *Trends Ecol. Evol.* 23: 38-44.
- Bassar, R.D., Marshall, M.C., Lopez-Sepulcre, A., Zandonà, E., Auer, S.K., Travis, J., Pringle, C.M., Flecker, A.S., Thomas, S.A., Fraser, D.F. & Reznick, D.N. 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. *P. Natl. Acad. Sci. U.S.A.* 107: 3616-3621.
- Bailey, J.K., Schweitzer, J.A., Ûbeda, F., Koricheva, J., LeRoy, C.J., Madritch, M.D., Rehill, B.J., Bangert, R.K., Fischer, D.G., Allan, G.J. & Whitham, T.G. 2009. From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Philos. T. Roy. Soc. B. Biol. Sci.* 364: 1607-1616.
- Blank, N., Hudson, A. G., Vonlanthen, P., Seehausen, O., Hammerschmidt, C. R. & Senn, D. 2013. Speciation leads to divergent methylmercury accumulation in sympatric whitefish. *Aquat. Sci.* 75: 261-273.
- Benkman, C.W. 1999. The selection mosaic and diversifying co-evolution between crossbill and lodgepole pine. *Am. Nat.* 153: S75-S91.
- Bernatchez, L. 2004. Ecological Theory of Adaptive Radiation: An Empirical Assessment from Coregonine Fishes (*Salmoniformes*). In: *Evolution Illuminated* (Hendry, A. P. & Stearns, S. C., eds.). pp. 175-207. Oxford University Press, Oxford.
- Bittner, D., Excoffier, L. & Largiader, C.R. 2010. Patterns of morphological changes and hybridization between sympatric whitefish morphs (*Coregonus spp.*) in a Swiss lake: a role for eutrophication? *Mol. Ecol.* 19: 2152-2167.
- Carpenter, S. R., Kitchel, J. F. & Hodgson, J. R. 1985. Cascading trophic interactions and lake productivity. *Bioscience* 35: 634-639.
- Cohen, J. 1988. Statistical power analysis for the behavioral sciences. 2nd edition. Lawrence Erlbaum Associates, New Jersey.
- Dieckmann, U., Doebeli, M., Metz, A.J. & Tautz, D. editors. 2004. Adaptive Speciation. Cambridge University Press.
- Elliott, J.M., Hurley, M.A. & Allonby, J.D. 1996. A functional model for maximum growth of immature stone loach, *Barbatula barbatula*, from three populations in north-west England. *Freshwater Biology* 36: 547-554.
- Ezard, T.H.G., Côté, S.D. & Pelletier, F. 2009. Eco-evolutionary dynamics: disentangling phenotypic, environmental and population fluctuations. *Philos. T. Roy. Soc. B.* 364: 1491-1998.
- Fussmann, G.F., Loreau, M. & Abrams, P.A. 2007. Eco-evolutionary dynamics of communities and ecosystems. *Funct. Ecol.* 21: 465-477.
- Geritz, S.A.H., Kidsi, E., Meszena, G. & Metz, J.A.J. 1998. Evolutionary singular strategies and the adaptive growth and branching of the evolutionary tree. *Evol. Ecol.* 12: 35-57.

- Grant, P.R. & Grant, B.R. 2006. Evolution of character displacement in Darwin's finches. *Science* 313: 224–226.
- Gruner, D.S., Smith, J.E., Seabloom, E.W., Sandin, S.A., Ngai, J.T., Hillebrand, H., Harpole, W.S., Elser, J.J., Cleland, E.E., Bracken, M.E.S., Borer, E.T. & Bolker, B.M. 2008. A cross-system synthesis of consumer and nutrient resource control on producer biomass. *Ecol. Lett.* 11: 740-755.
- H Hairston, N.G., Ellner, S.P., Geber, M.A., Yoshida, T. & Fox, J.A. 2005. Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.* 8: 1114-1127.
- Harmon, L.J., Matthews, B., Des Roches, S., Chase, J.M., Shurin, J.B. & Schluter, D. 2009. Evolutionary diversification in stickleback affects ecosystem functioning. *Nature* 458: 1167-1170.
- Harmon, L.J., Schulte, J.A., Larson, A. & Losos, J.B. 2003. Tempo and mode of evolutionary radiation in iguanian lizards. *Science* 301: 961-964.
- Hudson, A.G., Vonlanthen, P. & Seehausen, O. 2011. Rapid parallel adaptive radiations from a single hybridogenic ancestral population. *P. R. Soc. B* 278: 58-66.
- Hendry, A.P., Peichel, C.L., Matthews, B., Boughman, J.W. & Nosil, P. 2013. Stickleback research: the now and the next. *Evol. Ecol. Res.* In press.
- Kahilainen, K., Siwertsson, A., Gjelland, K.Ö., Knudsen, R., Böhn, T. & Amundsen, P.-A. 2011. The role of gill raker number variability in adaptive radiation of coregonid fish. *Evol. Ecol.* 25: 573-588.
- Karvonen, A., Lundsgaard-Hansen, B., Jokkela, J. & Seehausen, O. 2013. Differentiation in parasitism among ecotypes of whitefish segregating along depth gradients. *Oikos* 122: 122-128.
- Kottelat, M. & Freyhof, J. 2007. Handbook of European freshwater fishes. Kottelat, Cornol, Switzerland and Freyhof, Berlin, Germany.
- Legendre, P. & Gallagher, E.D. 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia* 129: 271-280.
- Losos, J.B. 2010. Adaptive radiation, ecological opportunity and evolutionary determinism. *Am. Nat.* 175: 623-639.
- Lundsgaard-Hansen, B., Matthews, B. & Seehausen O. Ecological speciation and phenotypic plasticity in fish affect ecosystems. In review *Proc. Natl. Acad. Sci. USA*.
- Lundsgaard-Hansen, B., Matthews, B., Vonlanthen, P., Taverna, A. & Seehausen, O. 2013. Adaptive plasticity and genetic divergence in feeding efficiency during parallel adaptive radiation of whitefish (*Coregonus* sp.). *J. Evolution. Biol.* 26: 483-498.
- Matthews, B., Narwani, A., Hausch, S., Nonaka, E., Peter, H., Yamamichi, M., Sullam, K.E., Bird, K.C., Thomas, M.K., Hanley, T.C. & Turner, C.B. 2011a. Toward an integration of evolutionary biology and ecosystem science. *Ecol. Lett.* 14: 690-701.
- Matthews, B., De Meester, L., Jones, C., Ibelings, B., Bouma, T., Nuutinen, V., van de Koppel, J. & Odling-Smee, J. Making niche construction operational in the context of eco-evolutionary dynamics. In revision.
- Matthews, B., Hausch, S., Winter, C., Suttle, C.A. & Shurin, J.B. 2011b. Contrasting ecosystem effects of morphologically similar copepods. *Plos ONE*, 6, e26700.
- MacColl, A.D.C. 2011. The ecological causes of evolution. *Trends Ecol. Evol.* 26: 514-522.

- Miner, B.G., Sultan, S.E., Morgan, S.G., Padilla, D.K. & Relyea, R.A. 2005. Ecological consequences of phenotypic plasticity. *Trends Ecol. Evol.* 20: 687-692.
- Odling-Smee, F.J., Laland, K.N. & Feldman, M.W. 2003. Niche construction. Princeton University Press.
- Palkovacs, E., Marshall, M.C., Lamphere, B.A., Lynch, B.R., Weese, D.J., Fraser, D.F. Reznick, D.N., Pringle, C.M. & Kinnison, M.T. 2009. Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Philos. T. Roy. Soc. B.* 364: 1617-1628.
- Palkovacs, E.P., Kinnison, M.T., Correa, C., Dalton, C.M. & Hendry, A.P. 2012. Fates beyond traits: ecological consequences of human-induced trait change. *Evol. Appl.* 5: 183-191.
- Palkovacs, E. P. & Post, D. M. 2008. Eco-evolutionary interactions between predators and prey: can predator-induced changes to prey communities feedback to shape predator foraging traits? *Evol. Ecol. Res.* 10: 699-720.
- Palkovacs, E.P. & Post, D.M. 2009. Experimental evidence that phenotypic divergence in predators drives community divergence in prey. *Ecology* 90: 300-305.
- Palkovacs, E.P. & Hendry, A.P. 2010. Eco-evolutionary dynamics: intertwining and evolutionary processes in contemporary time. *F1 Biology Reports*, 2: 1.
- Pelletier, F., Garant, D. & Hendry, A.P. 2009. Eco-evolutionary dynamics. *Philos. T. Roy. Soc. B* 364: 1483-1489.
- Pigeon, D., Chouinard, A., & Bernatchez, L. 1997. Multiple modes of speciation involved in the parallel evolution of sympatric morphotypes of lake whitefish (*C. clupeaformis*, Salmonidae). *Evolution*, 51: 196-205.
- Post, D.M. & Palkovacs, E.P. 2009. Eco-evolutionary feedbacks in community and ecosystem ecology: Interactions between the ecological theatre and the evolutionary play. *Philos. T. R. Soc. B, Biol. Sci.* 364: 1629-1640.
- Post, D.M., Palkovacs, E.P., Schielke, E.G. & Dodson, S.I. 2008. Intraspecific phenotypic variation in a predator affects community structure and cascading trophic interactions. *Ecology* 89: 2019-2032.
- R Development Core Team. 2010. R: A language and environment for Statistical Computing. Vienna, Austria. Available at: <http://www.R-project.org>.
- Roesch, C., Lundsgaard-Hansen, B., Vonlanthen, P., Taverna, A. & Seehausen, O. Experimental evidence for trait utility of gill raker number in adaptive radiation of a north temperate fish. In press *J. Evolution. Biol.*
- Schluter, D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. *Science* 266: 798-800.
- Schluter, D. 1996. Ecological causes of adaptive radiation. *Am. Nat.* 148: S40-S64.
- Schluter, D. 2000. The Ecology of Adaptive Radiation. Oxford University Press, Oxford.
- Schoener, T.W. 2011. The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science* 331: 426-429.
- Seehausen, O. 2004. Hybridization and adaptive radiation. *Trends Ecol. Evol.* 19: 198-207.

- Seehausen, O. 2009. Speciation affects ecosystems. *Nature* 458: 1122-1123.
- Steinmann, P. 1950. Monographie der schweizerischen Koregonen. Beitrag zum Problem der Entstehung neuer Arten. Schweiz. Zeitschr. Hydrol. 340-491.
- Vamosi, S. M. 2003. The presence of other fish species affect speciation in stickleback. *Evol. Ecol. Res.* 5: 717-730.
- Vonlanthen, P., Roy, D., Hudson, A.G., Largiader, C.R., Bittner, D. & Seehausen, O. 2009. Divergence along a steep ecological gradient in lake whitefish (*Coregonus* sp.). *J. Evolution. Biol.* 22: 498-514.
- Vonlanthen, P., Bittner, D., Hudson, A.G., Young, K.A, Müller, R., Lundsgaard-Hansen, B., Roy, D., Di Piazza, S., Largiader, C.R. & Seehausen, O. 2012. Eutrophication causes speciation in whitefish adaptive radiations. *Nature* 482: 357-363.
- West-Eberhard, M.J. 2003. Developmental plasticity and evolution. Oxford University Press, Oxford.
- Woods, P.J., Müller, R. & Seehausen, O. 2009. Intergenomic epistasis causes asynchronous hatch times in whitefish hybrids, but only when parental ecotypes differ. *J. Evolution. Biol.* 22: 2305-2319.
- Whitman, D.W. & Agrawal, A.A. 2009. What is phenotypic plasticity and why is it important? In "Phenotypic plasticity of insects – mechanisms and consequences", p. 42. Edt. by D. W. Whitman and T. N. Ananthakrishnan. Science publishers.
- Yoder, J.B., Clancey, E., Des Roches, S., Eastman, J.M., Gentry, L., Godsoe, W., Hagey, T.J., Jochimsen, D., Oswald, B.P., Roberston, J., Sarver, B.A.J., Schenk, J.J., Saer, S.F. & Harmon, L.J. 2010. Ecological opportunity and the origin of adaptive radiations. *J. Evolution. Biol.* 23: 1581-1596.
- Yoshida, T., Jones, L.E., Ellner, S.P., Fussmann, G.F. & Hairston, N.G. 2003. Rapid evolution drives ecological dynamics in a predator-prey system. *Nature* 424: 303-306.
- Yamamichi, M., Yoshida, T. & Sasaki, A. 2011. Comparing the effects of rapid evolution and phenotypic plasticity on predator-prey dynamics. *Am. Nat.* 178: 287-304.

Supplementary Information Chapter 5

Appendix S1 - Measuring ecosystem variables

Zooplankton: Zooplankton was sampled in order to estimate the abundances of macro and micro zooplankton and in order to measure size variation of zooplankton. A sampling tube, reaching from the tank surface to the tank bottom was used to collect a water probe, which was subsequently sieved through a zooplankton net. Zooplankton was then counted in the lab using a microscope. Sampled zooplankton was divided into macro and micro zooplankton as described elsewhere (Lundsgaard-Hansen *et al.*, in review). Sampled macro zooplankton individuals belonged to one of the following taxa: Acarids, *Bosmina*, calanoid and cyclopoid copepods, *Chydoridae*, *Daphnidae* and *Ostracoda*. Sampled micro zooplankton individuals belonged to one of the following taxa: Copepod nauplii and *Rotatoria*. *Rotatoria* consisted of *Keratella quadrata* and *K. cochlearis*, *Habrotorcha* and *Mega Habrotorcha*, *Alonella* and *Kellicottia*. All numbers were reported as number of individuals per liter. Pictures of zooplankton were taken for length measurements, while a maximum of 30 randomly chosen individuals per taxa and tank were measured, if more than 30 individuals of a taxon were sampled. Average sizes per taxa were weighted according to abundance of a given taxa to obtain the mean size of all zooplanktons individuals in a tank.

Snails and Mussels: Snails and mussels were counted in a standardized way in order to estimate their abundances. Numbers reported were calculated to number per m² tank wall. Observed snails belonged to the family of *Limnidae* and *Planorbidae*, but just the sum of snails was reported, independent of snail family. All counted mussels belonged to the species *Dreissena polymorpha*.

Sedimentation: Sedimentation traps were put in each tank in order to calculate sedimentation rate over the duration of the experiment. At the end of a measuring interval sedimentation traps were removed, sediments were dried at 60 ° C for 24 hours and the dry weight of sediments was used to calculate sedimentation rate in mg/day. Sedimentation rate was measured in an interval from week 5-9 at the end of phase 1 of the experiment and was measured from week 17-21 at the end of phase 2 of the experiment. We used extended measuring periods of four weeks in order to minimize biases due to measuring errors of the dry weight of sediments.

Phytoplankton and dissolved organic compounds: A one liter water probe was collected in a standardized way in order to measure Chlorophyll a concentration by standard photometry procedure as proxy for phytoplankton concentration (PPC). The filtered water was used to measure the concentration of dissolved organic compounds (DOC) and nutrients (Nitrate, Phosphor). DOC was measured doing high temperature catalytic oxidation using a Shimadzu TOC-V CPH and was reported in mg/l. Nutrients were measured doing photo spectrometry. Nitrate was always below detection limit and phosphor (PHO) was below detection limit at the end of phase 1 (<0.25 mg/l for N and <2 µg/l for P), therefore we only included PHO measured at the end of phase 2 in our analysis.

Photosynthetic active radiation: Photosynthetic active radiation (PAR) was measured at a wavelength from 400 – 700 nm to calculate light transmission through the water column using a LI-193 Underwater Spherical Quantum Sensor. The % of PAR transmitted from 10 cm below surface to the bottom of the tank was reported (high values corresponded to a high light transmission).

Benthic algae cover: Photos of the bottom were taken with a Canon S9 using an underwater cage to estimate benthic algae cover. Photos were used to define areas on the tank bottom with high algae cover (100%), with intermediate algae cover (50%) and with no algae cover (0%) using Photoshop CS and these estimates were used to calculate the percentage of algae cover at the bottom.

GPP: Gross primary production was measured using diurnal changes in oxygen levels (Wetzel and Likens, 1991; Downing, 2005). Oxygen was measured 120 times/minute using a PreSens Oxy-10 10-Channel Fiber-Optic Oxygen meter. In order to calculate diurnal changes, oxygen levels from half an hour before and after sun set and sun rise, respectively, were averaged. Respiration was calculated as the difference in oxygen between sunset and sunrise of the following day. NPP was calculated as the difference in Oxygen from sun set and sun rise (from the same day). From these GPP was calculated as $NPP+R$. Due to constraints in availability of sampling gear oxygen was measured in blocks of nine tanks. As productivity is strongly dependent on external forces that may have differed between the measuring dates of the different blocks (such as weather), we included block in the ANOVAs on GPP. For Cohens d analysis we used the residuals of the ANOVAs “GPP ~Block” to correct GPP for external influences.

References:

- Downing, L. A. 2005. Relative effects of species composition and richness on ecosystem properties in ponds. *Ecology*, 86: 701-715.
- Lundsgaard-Hansen, B., Matthews, B. & Seehausen, O. Ecological speciation and phenotypic plasticity in fish affect ecosystems. In review, *P. Natl. Acad. Sci. USA*.
- Wetzel, R. G. & Likens, G. E. 1991. *Limnological analyses*. Saunder College Publishing, New York.

Appendix S2 – Abundance macro zooplankton

Supplementary Table 1: Given is the relative abundance of macro zooplankton. Relative abundances were calculated for each tank separately and then averaged across treatment.

Phase	Treatment	Acarids	<i>Chydoridae</i>	<i>Bosmina</i>	Calanoids	Cyclopoids	<i>Ostracoda</i>	<i>Daphnidae</i>
1	No fish	0.002	0.17	0.07	0	0.70	0.03	0.03
1	Fish	0.003	0.26	0.04	0	0.62	0.03	0.04
1	BB	0.01	0.40	0.02	0	0.51	0.01	0.05
1	BL	0	0.16	0.01	0	0.77	0.02	0.03
1	LB	0.004	0.19	0.00	0	0.69	0.03	0.08
1	LL	0	0.24	0.10	0.01	0.58	0.05	0.01
2	No fish	0.01	0.20	0.01	0	0.38	0.28	0.13
2	Fish	0.02	0.09	0.06	0.01	0.32	0.43	0.07
2	BB	0.02	0.07	0.10	0	0.37	0.38	0.05
2	BL	0.01	0.12	0.15	0.04	0.30	0.28	0.10
2	LB	0.03	0.08	0	0	0.35	0.46	0.08
2	LL	0.02	0.09	0	0	0.26	0.57	0.06

Appendix S3 – Abundance micro zooplankton

Supplementary Table 2: Given are the relative abundances of micro zooplankton in panel a) and the average size per taxa (in μm , in panel b). Relative abundances and average sizes were calculated for each tank separately and then averaged across treatments.

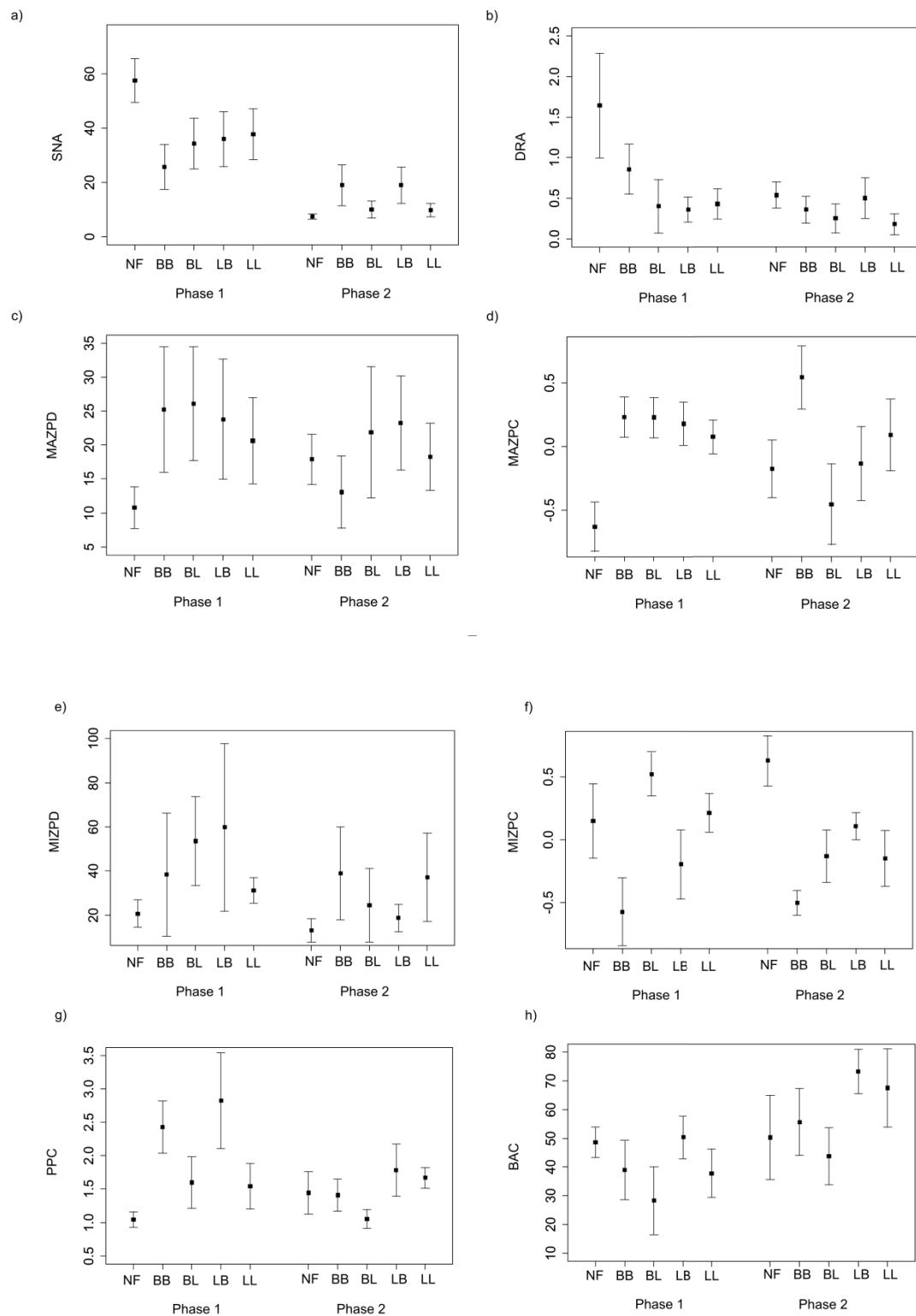
a)

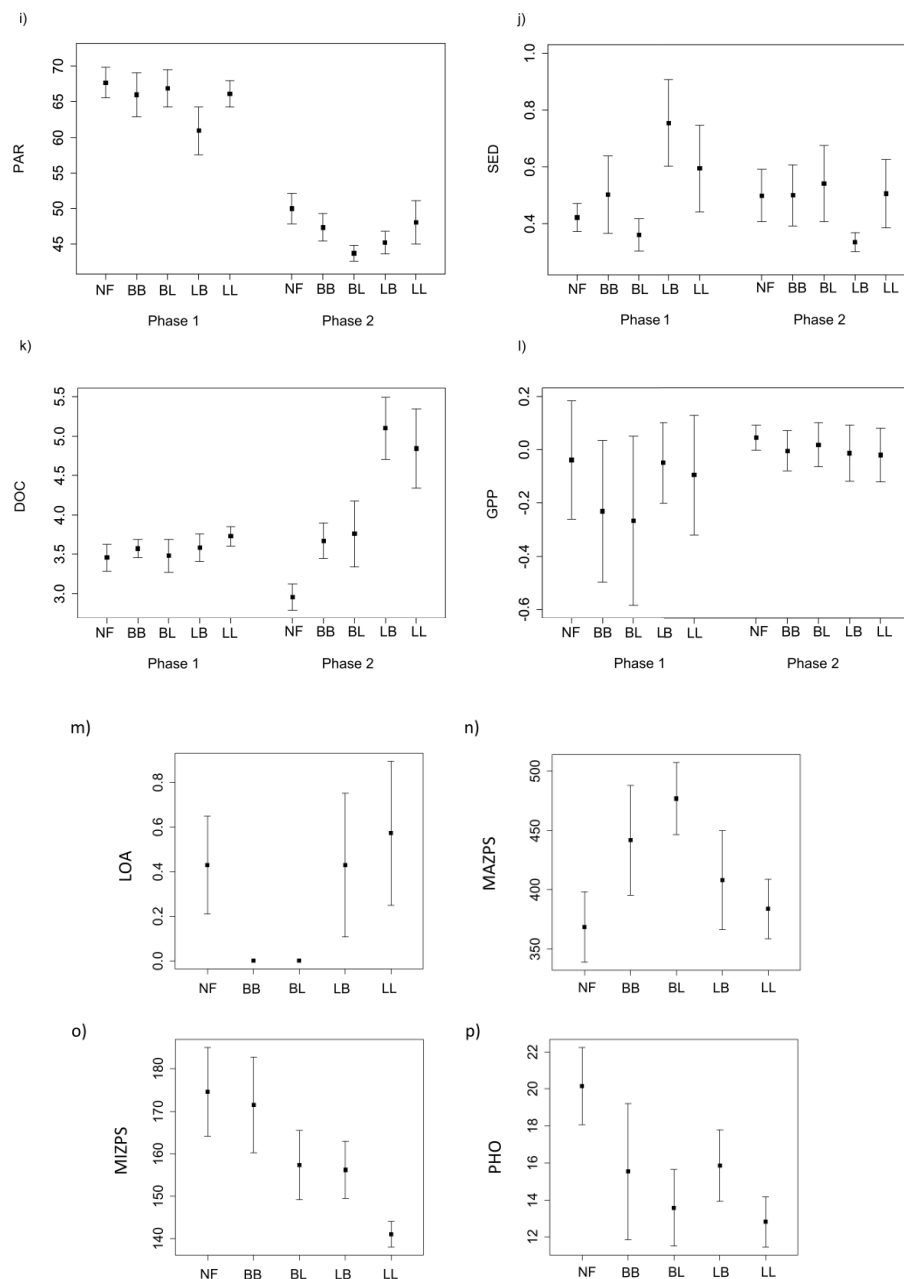
Phase	Treatment	Nauplii	<i>Habrotorcha</i>	<i>Keratella Q.</i>	<i>Alonella</i>	<i>Keratella C.</i>	<i>M. Habrotorcha</i>	<i>Kelicottia</i>
1	No fish	0.84	0	0.09	0	0	0	0.06
1	Fish	0.46	0.02	0.38	0	0	0	0.14
1	BB	0.43	0.06	0.41	0	0	0	0.10
1	BL	0.62	0	0.27	0	0	0	0.11
1	LB	0.40	0.01	0.43	0	0	0	0.16
1	LL	0.44	0	0.40	0	0	0	0.16
2	No fish	0.59	0.22	0.04	0.09	0.02	0.03	0
2	Fish	0.45	0.20	0.32	0.01	0.00	0.02	0
2	BB	0.54	0.01	0.37	0	0.001	0.08	0
2	BL	0.66	0.10	0.24	0	0	0	0
2	LB	0.41	0.34	0.23	0.03	0	0	0
2	LL	0.24	0.32	0.43	0	0.003	0	0

b)

Phase	Treatment	Nauplii	<i>Habrotorcha</i>	<i>Keratella Q.</i>	<i>Alonella</i>	<i>Keratella C.</i>	<i>M. Habrotorcha</i>	<i>Kelicottia</i>
2	No fish	189.32	132.67	108.31	164.97	79.15	167.01	-
2	Fish	177.02	142.64	131.53	154.24	129.18	210.01	-
2	BB	190.44	149.95	133.30	-	154.82	210.01	-
2	BL	168.97	139.47	130.31	-	-	-	-
2	LB	177.22	139.26	130.52	141.89	-	-	-
2	LL	167.82	147.63	131.58	166.58	103.53	-	-

Appendix S4 – Treatment effects phase 1 and phase 2





Supplementary Figure 1: Whitefish treatment effects on measured ecosystem metrics. NF refers to no fish tanks, BB is the benthic species raised on benthic food, BL is the benthic species raised on limnetic food, LB is the limnetic species raised on benthic food and LL is the limnetic species raised on limnetic food. Given is for each metric the average value per treatment with its standard deviation. The end of phase 1 is on the left side, the end of phase 2 on the right side of each panel. Metrics that were only measured at the end of phase 2 are shown in panels m-p. Acronyms and units are as reported in Table 1 of the main text. Zooplankton community state was calculated separately for the two dates, the values on the y-axis are thus not directly comparable across dates. GPP is corrected for block (see Appendix S1).

Chapter 6

Synthesis and future research

Short overall summary

Differentiation in trophic niche use is thought to be one of the most important ecological drivers of adaptive radiation in animals (Schluter, 2000). In chapters 2 and 3 of this thesis I showed that different whitefish species are heritably differentiated in their foraging traits and in their efficiency in feeding on benthic versus on limnetic food. Overall, these findings add evidence that divergence in foraging traits and trophic ecology can be an important component of adaptive radiation in lacustrine fish. Furthermore I found effects of phenotypic plasticity on feeding efficiency, which was induced by differences in the feeding environment during the raising of experimental fish. The observation that phenotypic plasticity was adaptive, meaning that a benthic rearing environment later improved feeding efficiency on benthic food, is consistent with the idea that plasticity can facilitate adaptive radiation (West-Eberhard, 2003).

In recent years evidence has accumulated that evolutionary history and evolutionary processes can strongly determine ecosystem structure and function (Schoener, 2011), but we still know little about how evolutionary divergence and adaptive radiation affect ecosystems. In chapters 4 and 5 I showed that recently evolved whitefish species have strongly contrasting effects on experimental ecosystems in mesocosms. Although contrasting ecosystem effects seemed to result more frequently from genetic divergence between species, differences in phenotypically plastic traits, induced by divergent rearing environments, also affected some ecosystem metrics. These findings suggest that genetic divergence associated with speciation in an adaptive radiation and phenotypic plasticity may both affect ecosystem structure and function.

The whitefish system as model in speciation research

Whitefish belong to the genus *Coregonus*, which is part of the family *Coregonidae* in the order of the Salmoniformes (Kottelat & Freyhof, 2007). Whitefish are distributed over large parts of the Northern hemisphere and can be found in North America, in Northern Asia, in Northern and Eastern Europe, as well as in the Alpine region of Central Europe (Bernatchez, 2004; Kottelat & Freyhof, 2007). Many lakes harbour multiple phenotypically, ecologically and genetically differentiated species in geographical sympatry, while patterns of phenotypic and ecological differences are often replicated across lakes (Steinmann, 1950; Schluter, 2000; Bernatchez, 2004; Hudson *et al.*, 2007; Siwertsson *et al.*, 2010; Vonlanthen *et al.*, 2012). Most sympatric whitefish species are found in previously glaciated areas, suggesting that they are of recent postglacial origin (Bernatchez & Wilson, 1998). Sympatric whitefish species are often differentiated along the benthic (lake bottom) and limnetic (open water) habitat axis and the number of gill rakers and adult body size are among the morphological traits that most readily diverge between benthic and limnetic whitefish species (Smith & Skúlason, 1996; Taylor, 1999). These traits seem to be associated with a benthic versus a limnetic trophic ecology, with a lower number of gill rakers and larger adult size in ecologically benthic species compared to ecologically limnetic species (Amundsen *et al.*, 2004; Harrod *et al.*, 2010; Kahilainen *et al.*, 2007, 2011). Co-existing whitefish species can also differ in the depth of their foraging habitat (Siwertsson *et al.*, 2010), in spawning depth and time (Vonlanthen *et al.*, 2009; Siwertsson *et al.*, 2010; Vonlanthen *et al.*, 2012) or in their degree of anadromy (Shubin *et al.*, 1997).

In European whitefish, the number of species per lake frequently exceeds two (Siwertsson *et al.*, 2010; Vonlanthen *et al.*, 2012) and a veritable hotspot of whitefish species diversity is found in the Alpine region of Central Europe (Steinmann, 1950; Hudson *et al.*, 2007; Hudson *et al.*, 2011; Vonlanthen *et al.*, 2012). In Swiss lakes whitefish have radiated into more than 30 different species after the last glacial maximum 15'000 – 25'000 years BP, with species diversity varying from one to at least five species per lake (Steinmann, 1950; Vonlanthen *et al.*, 2012). This Swiss whitefish diversity consists of at least five radiations that evolved in parallel, each centered on a different lake or on a system of historically connected lakes (Hudson *et al.*, 2011). Approximately one third of the historical species diversity has been lost in the last century due to human driven eutrophication of lakes (Vonlanthen *et al.*, 2012). This species loss has been most severe in heavily polluted lakes and occurred at least partially through speciation reversal (Vonlanthen *et al.*, 2012).

In this thesis I studied whitefish from two Swiss lakes, Lake Thun and Lake Lucerne. These lakes harbor independently evolved radiations and they were less strongly affected by eutrophication than many other lakes (Hudson *et al.*, 2011, Vonlanthen *et al.*, 2012). From both lakes I chose species that correspond to a benthic and a limnetic ecotype in terms of their phenotype (Schluter, 2000): The benthic *C. sp.* “Bodenbalchen” and the limnetic *C. zugensis* from Lake Lucerne and the benthic *C. sp.* “Balchen” and the limnetic *C. albellus* from Lake Thun (Vonlanthen *et al.*, 2012). Both benthic species spawn at shallower depth, have a lower number of gill rakers and larger adult size than their limnetic sister species (Vonlanthen *et al.*, 2012). Genetic differentiation between benthic and limnetic species from the same lake is around $F_{ST}=0.1$ in both lakes (Vonlanthen *et al.*, 2012). We used wild caught whitefish to breed experimental fish, which we raised in the lab under common garden conditions for at least three years (longer for the more recent experiments), with two divergent foraging environments in Lake Lucerne fish. We used deep frozen red mosquito larvae to simulate a benthic foraging environment and living zooplankton to simulate a limnetic feeding environment. In total our rearing design resulted in four treatments for Lake Lucerne whitefish (two species X two food types) and in two treatments for Lake Thun whitefish (two species X one food type). This rearing design allowed us to differentiate between genetically-based and phenotypically plastic variation in whitefish phenotypes, feeding performance, and ecosystem effects.

I experimentally tested for divergence in feeding efficiency on benthic (Chapter 2) and on limnetic food types (Chapter 3) between whitefish of different species and from different rearing treatments; I tested which traits are of functional importance for exploiting different food types (Chapter 2 and Chapter 3); and I investigated what role phenotypic plasticity and natural selection may have played in their divergence (Chapter 2). In a next step I experimentally investigated whether phenotypic differences between closely related whitefish species have contrasting effects on experimental ecosystems and whether these contrasting effects result from genetic differences between species and/or whether they are caused by phenotypically plastic trait differences induced by divergent rearing environments (Chapter 4 and Chapter 5).

Ecological drivers of adaptive radiation in north temperate fish

Evidence for adaptive radiation

The observation that genetically and phenotypically differentiated whitefish species are found in geographical sympatry within many lakes of the northern hemisphere, together with the replicated occurrence of phenotypically similar ecotypes among lakes, indicate that whitefish species likely originated by adaptive radiation. Schluter (2000) described four criteria that characterize an adaptive radiation: i) Common ancestry, ii) phenotype-environment correlation, iii) trait utility and iv) fast speciation (see chapter 1 for more details). All of these criteria except trait utility have been demonstrated to be satisfied for the whole genus *Coregonus* (reviewed elsewhere, Bernatchez, 2004) as well as for species of the European whitefish species complex: Common ancestry has been shown for individual lake flocks from the Alpine region as well as for the entire Alpine radiation (Douglas *et al.*, 1999; Hudson *et al.*, 2011). Demonstrations of monophyly of individual lake flocks is likewise evidence for fast speciation, because the Alpine lakes are of recent post-glacial origin (Douglas *et al.*, 1999; Hudson *et al.*, 2011). Phenotype-environment correlations have been repeatedly shown for Scandinavian whitefish species, where benthic versus limnetic resource use is correlated with the number of gill rakers and with adult body size (Amundsen *et al.*, 2004; Harrod *et al.*, 2010; Kahilainen *et al.*, 2011). Such documentations that the same phenotypic traits are repeatedly associated with resource use in nature suggest an important role for trophic adaptation in whitefish adaptive radiation and it points to a functional role of these traits in resource acquisition. However, experimental evidence for feeding efficiency differences between benthic and limnetic whitefish species and experimental evidence for trait utility had so far been lacking.

In chapters 2 and 3 of this thesis I provided evidence for heritable species differences in foraging traits and feeding efficiency on benthic and on limnetic food types. Also when raised in a common garden, the benthic species *C. sp.* “Bodenbalchen” and *C. sp.* “Balchen” had a lower number of gill rakers and faster growth (larger size) than their limnetic sister species *C. zugensis* and *C. albellus*. Both benthic species were more efficient in feeding on benthic mosquito larvae than their limnetic sister species, while the limnetic species *C. albellus* was more efficient in feeding on zooplankton than the benthic species *C. sp.* “Balchen” (feeding efficiency experiments using zooplankton were only done with fish from Lake Thun). These findings add evidence that adaptation to trophic niches was involved in whitefish diversification. Further, they are evidence for trait utility of the n-dimensional phenotype, including behavior, to exploit either benthic or limnetic resources. These species differences in feeding efficiency might result from divergence in the traits that underlie PEs in nature (Schluter, 2000). In whitefish these are essentially the number of gill rakers and adult body size. Despite the ubiquity of divergence in gill raker number between benthic and limnetic species of fish of various taxa, experimental evidence for their functional significance is still very rare. The functional morphology prediction is that a higher number of gill rakers (an increased density of gill rakers) increases retention capability (filter efficiency) for zooplankton prey (Link & Hoff, 1998; Sanderson *et al.*, 2001). To my knowledge, there is only one unambiguous experimental demonstration for a role of gill rakers in facilitating zooplankton feeding, which found that food (*Artemia salina*) intake rate is positively associated with gill raker number within and across benthic and limnetic species of lacustrine stickleback (Robinson, 2000). For salmonid-like fish, which are

responsible for a considerable fraction of the species diversity in postglacial lakes of the northern hemisphere (Smith & Skúlason, 1996; Taylor 1999), evidence for trait utility of gill rakers has so far not at all been available. In chapter 3 of this thesis I provide the first evidence for trait utility of gill rakers in a salmonid-like fish: An increased number of gill rakers was positively associated with retention capability (measured as the ratio between the number of attacks and the number of zooplankton items found in the stomach after the experiment) for small zooplankton prey within and across whitefish species. Overall, retention capability for zooplankton decreased with decreasing zooplankton size, while the effect strength of gill raker number on retention capability increased with decreasing zooplankton size, a result fully consistent with the idea that gill rakers serve as filters to retain small prey (Sanderson *et al.*, 2001). In the benthic feeding efficiency trials, increased body size was positively associated with multiple feeding efficiency measures (again within and across species). Increased fish size though did not increase feeding efficiency on zooplankton prey, indicating that larger size is not generally increasing feeding efficiency. Thus I interpret the positive association of fish size with benthic feeding efficiency as evidence for trait utility of large size for feeding in the benthic niche.

The experimental demonstrations of trait utility of gill rakers and adult size adds evidence that whitefish have arisen by adaptive radiation. The finding of a functional significance of these traits is consistent with the observation that gill rakers and adult size are among those traits that are most often differentiated between sympatric benthic and limnetic whitefish species (Siwertsson *et al.*, 2010; Vonlanthen *et al.*, 2012), and our results probably equally apply to other fish taxa with similar eco-morphological differentiation (Schluter, 2000).

Replicated heritable feeding efficiency divergence is consistent with the importance of natural selection

According to the ecological theory of adaptive radiation, diversification and speciation in adaptive radiation is driven by divergent ecologically based natural or sexual selection (Schluter, 2000). The results from chapter 2 of my thesis revealed that functional traits such as gill rakers and growth are heritably differentiated between sympatric species in parallel across two lakes with independently evolved radiations. Further, the observed phenotypic differences affected benthic feeding efficiency on benthic food in a consistent fashion across radiations. Such parallel ecotypic differentiation in independently evolved adaptive radiations is evidence for divergent natural selection between contrasting foraging environments, because drift is unlikely to produce similar phenotypic outcomes across independently evolved radiations (Rundle *et al.*, 2000; Schluter, 2000). This interpretation is supported by the repeated occurrence of similar phenotypes across many lakes (Bernatchez, 2004; Siwertsson *et al.*, 2010; Vonlanthen *et al.*, 2012) and by evidence for the action of natural selection on foraging traits in independently evolved whitefish radiations (Rogers & Bernatchez, 2007; Vonlanthen *et al.*, 2009).

Further I showed that divergence in feeding efficiency is reciprocally adaptive, with higher feeding efficiency of phenotypically benthic species on benthic food and higher feeding efficiency of the phenotypically limnetic species on limnetic food. The presence of such functional trade-offs between foraging niches is consistent with the importance of divergent natural selection between contrasting

foraging environments. Trade-offs occur when adaptation to one environment or task results in a cost for performance in another environment or task, and they are needed for phenotypic differences to evolve (Schluter, 1995). Strong functional fitness trade-offs are also found in other taxa of north temperate fish (Schluter, 1995) and they may help to explain why speciation along the benthic and limnetic habitat axis is so common in these fish (Smith & Skúlason, 1996; Taylor, 1999).

I interpret the above discussed findings as evidence that divergent natural selection on foraging traits was most likely involved early in the speciation process of Alpine whitefish. The alternative of ecological character displacement upon secondary contact after initialization of speciation in different glacial refugia seems unlikely to be responsible for the observed phenotypic divergence between species, as speciation is most likely recent and happened in geographical sympatry within lakes or historically connected lake systems (Hudson *et al.*, 2011). However, it remains uncertain whether trophic adaptation was a driving force initializing the process of speciation or whether it has rather occurred as a by-product after speciation has been initialized, for example by divergent adaptations to different spawning depths (Vonlanthen *et al.*, 2009; Lundsgaard-Hansen *et al.*, in prep.; Ingram *et al.*, 2012; Vonlanthen *et al.*, 2012).

Phenotypic plasticity also affects feeding efficiency

Quite a few species of young radiations, including fish such as stickleback, char and cichlids, are characterized by considerable amounts of phenotypic plasticity in traits that diverge over the course of the radiation (i. e. Wimberger, 1994; Skúlason *et al.*, 1999; Bouton *et al.*, 2002; Robinson & Parson, 2002; West-Eberhard, 2003; Wund *et al.*, 2008). Despite these observations, the ecological theory of adaptive radiation does not directly deal with phenotypic plasticity (Schluter, 2000). The flexible stem model of adaptive radiation on the other hand, suggests that phenotypic plasticity in the ancestor of a radiation can affect the course of evolution during the radiation (West-Eberhard, 2003). Under this model adaptation to new environments is initially achieved through phenotypic plasticity, while phenotypes are subsequently genetically assimilated what allows them to respond to natural selection through evolution (West-Eberhard, 2003). Direct evidence for the flexible stem model of adaptive radiation is available for very different taxa such as frogs and fish (Gomez-Mestre & Buchholz, 2006; Wund *et al.*, 2008). The finding of parallel heritable differentiation in foraging traits and feeding efficiency across different radiations, as well as the observation that phenotypic plasticity in feeding efficiency was adaptive, are consistent with the flexible stem model of adaptive radiation. However, as we could not quantify levels of plasticity in the ancestor of the Alpine whitefish radiation, we could not directly test the flexible stem model of adaptive radiation.

In our experiment mainly behavioural traits were responding to different environments through phenotypic plasticity. Such behavioural plasticity might be particularly powerful in allowing for colonization of new niches. Mayr (1963) even hypothesized that “a shift into a new niche or adaptive zone is almost without exception initiated by a change in behaviour”. When plasticity facilitates the colonization of new niches, non-plastic traits with a heritable basis can be exposed to new selection pressures and their adaptive fit to the environment can be increased through evolution in response to natural selection. This mechanism of how plasticity can affect evolutionary divergence has been referred to as genetic accommodation (Price *et al.*, 2003; West-Eberhard, 2003). We cannot directly

assess whether behavioural plasticity facilitated the colonization of new niches by whitefish, but our results are consistent with this hypothesis.

The results from chapter 2 and 3 confirm that adaptation through natural selection and adaptation through phenotypic plasticity should not be considered two mutually exclusive alternatives. The finding of both genetic differences and phenotypic plasticity in functional traits and feeding efficiency, suggest that plasticity and natural selection on heritable traits may have acted in concert during the build-up of diversity in whitefish adaptive radiation.

Why do Alpine whitefish tend to speciate so frequently?

The Alpine whitefish radiation has many features that are known to have the potential to facilitate diversification and speciation: i) It is of hybridogenic origin, indicating that genetic variation was probably high from the onset of colonization of Swiss lakes (Hudson *et al.*, 2011). Compared to evolutionary adaptation through the accumulation of new mutations, adaptation from standing genetic variation can be fast (Seehausen, 2004; Barrett & Schluter, 2008). ii) The post-glacial origin of whitefish suggests that ecological opportunity was high after colonization of potentially “empty lakes” (Losos *et al.*, 2010). More whitefish species originated in deeper lakes, suggesting that the availability of ecological opportunity was indeed an important driver for whitefish speciation (Vonlanthen *et al.*, 2012). iii) In this thesis we have shown that whitefish have the ability to display strong adaptive plasticity in behaviour. Maybe it is this combination of high standing genetic variation, ecological opportunity and the ability to display strong behavioural plasticity, that allowed whitefish to diverge along multiple ecological niche axes such as foraging habitat, spawning depth and spawning time. The dimensionality of ecological niche divergence may be an important factor affecting the likelihood of speciation (Nosil & Harmon, 2009; Nosil *et al.*, 2009). Maybe it is the interaction of the above reviewed features of the Alpine whitefish radiation that makes it one of the most rapid and most diverse vertebrate radiations known worldwide.

Ecosystem consequences of whitefish adaptive radiation

The currently ongoing synthesis between evolutionary biology and ecosystem science focuses mainly on the question of how evolution affects ecosystems (Schoener, 2011). Within this synthesis a considerable body of work investigates how phenotypic differentiation between populations or species affects ecosystem structure and function. By now a handful of empirical examples show that phenotypic differences between populations of the same species or between closely related species can have contrasting ecosystem effects (i. e. Post *et al.*, 2008; Harmon *et al.*, 2009; Bassar *et al.*, 2010). A limitation of these previous studies is that they could not distinguish whether contrasting ecosystem effects were caused by genetic differences between populations or species, or whether they result from environmentally induced phenotypically plastic differences, because they studied wild or wild-caught individuals.

We performed two separate common gardening experiments (*sensu* Matthews *et al.*, 2011) using whitefish from Lake Lucerne, which were previously raised in a common garden in two different trophic environments. The goal of a common gardening experiment is to quantify how organisms with phenotypic (or genetic) differences affect properties and function of initially identical experimental ecosystem (Matthews *et al.*, 2011). The experiments presented in chapter 4 and 5 of

this thesis are the first that can differentiate whether contrasting ecosystem effects are caused by genetic differences or whether they can be explained by environmentally induced phenotypically plastic differences. This is important, because if environmentally induced differences between individuals, populations and species alone cause contrasting ecosystem effects, evolutionary divergence is not needed to explain variation in ecosystem effects (Seehausen, 2009). From here on I will for simplicity refer to “effects caused by environmentally induced phenotypically plastic differences” as effects of phenotypic plasticity and to “effects resulting from genetic differences” to effects of evolutionary divergence.

Do contrasting ecosystem effects of phenotypic differentiation result from evolutionary divergence or phenotypic plasticity?

In chapters 4 and 5 I found that ecosystem effects of evolutionary divergence seemed to be more common than those of phenotypic plasticity. In our first experiment, which was conducted from fall to beginning of winter 2010, evolutionary species divergence mainly affected metrics associated with direct trophic interactions of whitefish with their prey, such as prey abundance and prey community composition. In the second experiment, conducted from spring to fall 2011 (with fish being present from May to June), evolutionary divergence rather affected lower trophic levels such as micro zooplankton composition, as well as chemical properties such as DOC. The replicated result of numerous strong ecosystem effects resulting from evolutionary divergence in both experiments is consistent with previous studies, and together with these, strongly suggests that evolutionary adaptation, speciation and adaptive radiation cannot be neglected when studying structure, function and dynamics of ecosystems (i. e. Post *et al.*, 2008; Harmon *et al.*, 2009; Bassar *et al.*, 2010). Although less frequently than evolutionary effects, we also found some strongly contrasting ecosystem effects resulting from phenotypic plasticity. In the first experiment it was exclusively the abundance of zebra mussels (*Dreissena polymorpha*), a strongly benthic organism, that was significantly affected by phenotypic plasticity. In the second experiment phenotypic plasticity again affected important components of the aquatic food web such as micro and macro zooplankton community composition. The finding of significant ecosystem effects caused by phenotypic plasticity are consistent with previous evidence showing that ecological consequences of plasticity can be manifold (Miner *et al.*, 2005), and include modifications of direct and indirect species interactions (Agrawal, 2001; Peacor & Werner, 2001).

Similar experiments as ours, with many different organisms, are needed to achieve a better understanding of the generality of the results obtained in this thesis. If our finding that effects of evolutionary divergence tend to be more numerous than effects of phenotypic plasticity are applicable to other systems, previous findings of contrasting ecosystem effects resulting from wild born individuals of different species or populations (i. e. Post *et al.*, 2008; Harmon *et al.*, 2009; Bassar *et al.*, 2010) can probably largely be attributed to evolutionary divergence and only to a lesser extent to phenotypic plasticity. Nevertheless, we documented strongly contrasting ecosystem effects of phenotypic plasticity on some ecosystem metrics, a finding that clearly indicates the need to differentiate between ecosystem effects caused by phenotypic plasticity and those caused by evolutionary divergence in future studies.

Are ecosystem effects of adaptive radiation long lasting and strong enough to really matter for ecosystems?

We divided the experiment presented in chapter 5 into two phases, in order to measure how long lasting ecosystem effects of whitefish phenotypic differences are. In a first phase whitefish of one or another treatment were present in the mesocosm ecosystems to eventually induce ecosystem divergence. In a second phase whitefish were removed from the mesocosm ecosystems to investigate the temporal dynamics of ecosystem effects beyond the time of presence of whitefish. This experiment is the first to experimentally address the question whether ecosystem contrasts induced by phenotypic divergence between populations (resulting from evolutionary divergence or phenotypic plasticity) persist beyond the presence time of the environment-modifying-organisms in the ecosystem. We found that contrasting ecosystem effects of whitefish evolutionary divergence can be long lasting and some only emerged after whitefish had been removed from the ecosystem. If the observation that contrasting ecosystem effects of evolutionary divergence can be stored in an ecosystem and subsequently be strengthened over time was general, it would greatly increase the power of evolution to affect ecosystems.

In order to assess how relatively important effects of evolution on ecosystems are, it is important to compare the strength of ecosystem effects caused by evolutionary processes or evolutionary history to the strength of effects caused by classic non-evolutionary determinants of ecosystem structure and function (Hairston *et al.*, 2005; Schoener, 2011). In the experiment presented in chapter 5 we thus included tanks without whitefish to assess effects of whitefish presence or absence, what we consider an ecological contrast. At the very end of the experiment, ecosystem effects of evolved species differences were very similar in magnitude to effects resulting from whitefish presence versus absence. Further, significant effects of evolutionary divergence were as common as significant effects resulting from fish presence and absence at the very end of the experiment. This is striking, as the whitefish versus no whitefish contrast seems to be a very strong ecological contrast in comparison to the contrast of phenotypically and genetically differentiated but very recently evolved whitefish species. Previous studies found that effects of contemporary evolution on population dynamics compete in strength with classic ecological determinants such as predation, resource availability or climatic effects (Hairston *et al.*, 2005; Ezard *et al.*, 2009). Similarly, a recent study found that the strength of contrasting ecosystem effects of local adaptation of guppy populations is statistically indistinguishable from the strength of effects caused by guppy invasion (Palkovacs *et al.*, 2009). Such findings clearly indicate the need to incorporate evolution in ecological studies in order to increase our understanding of the structure, function and dynamics of natural systems.

Non-parallel aspects of ecosystem effects of adaptive radiation

Comparison of the strength and direction of effects on individual ecosystem metrics across the two common-gardening experiments revealed that individual metrics were rarely similarly affected in the two experiments. These differences between the experiments are maybe best captured by the observation that in the first but not in the second experiment, effects of evolutionary divergence on trophic metrics (those that underlie direct trophic interaction with whitefish) were stronger than those on other metrics. Such fundamental differences in the distribution of strong ecosystem effects between the experiments indicate that predicting in detail how phenotypic differentiation causes

contrasting ecosystem effects may be challenging. A potential explanation for this striking difference between our two experiments might be the different seasonal aspects. While the first experiment was conducted during fall from October to December, the second experiment was conducted from spring to fall, with fish being present only from May to June. If this explanation was true, it would suggest that ecosystem effects of fish are strongly context dependent, for example depending on external forces (such as weather) and internal ecosystem dynamics (such as processes that differ in emerging ecosystems in spring and in closing ecosystems in autumn). Our data seem to partially support this interpretation: At the end of the first experiment we found contrasting effects of evolutionary divergence to be more numerous than contrasting effects due to phenotypic plasticity. We found the same pattern at the end of phase 2 of the second experiment (when fish were no more present in the mesocosms), but not at the end of phase 1 of the second experiment (when fish were present). So the result of more frequent significant effects of evolutionary divergence compared to effects of phenotypic plasticity was really only replicated when ecosystem metrics were measured roughly in the same season of the year (end of experiment 1 (beginning of December) versus end of phase 2 of experiment 2 (mid of October)). But still, effects on individual metrics were not generally consistent in direction and strength across experiments even when measured in the same season. These differences in the effects on individual metrics may have to do with fish being present at that time of the experiment in the first experiment, but no more being present at that time of the experiment in the second experiment. More data is needed to draw robust conclusions about how seasonality of external factors modifies ecosystem effects of evolutionary divergence.

Potential for eco-evolutionary feedbacks and niche construction?

Ecosystem effects of evolution can result in eco-evolutionary feedbacks, when they affect fitness landscapes for the population of the environment-modifying organisms themselves. Additionally or alternatively, they can result in niche construction, if they affect evolution of other species in the community (Odling-Smee *et al.*, 2003; Post & Palkovacs 2009). Eco-evolutionary feedbacks and niche construction (collectively referred to as evolutionary consequences from here onwards) may be particularly common in adaptive radiation, where the fit between an organism's phenotype and the environment is usually strong (Schluter, 2000; Pelletier *et al.*, 2009). A basic requirement for evolutionary consequences to arise is a persistence time of environmental modification that allows for an evolutionary response to happen (Odling-Smee *et al.*, 2003). This persistency can either be achieved through continuous environmental modification by the modifying population or, if organisms are only intermittently present or active in the ecosystem, by an extended persistence time of environmental modification (Odling-Smee *et al.*, 2003; Palkovacs & Post, 2008). Intermittent environmental modification may be common in non-tropical species, as among those many are either migratory, hibernating or display other changes in behaviour or activity through the seasons. Another factor causing intermittent environmental modification in the wild may be strong age structure in short lived organisms, with adults only being present in parts of the year not because of migration but due to mortality. Therefore it is important to look at the persistency of ecosystem effects of evolutionary divergence in order to assess how likely evolutionary consequences might be.

In this thesis I showed that whitefish species are heritably differentiated in foraging morphology and behaviour (chapter 2 and 3); that their evolutionary divergence affected the prey community of

whitefish as well as other important components of aquatic food webs (chapter 4 and 5); and that strong effects of evolutionary divergence can be present beyond the time of presence of whitefish in the ecosystem (chapter 5). From previous studies we know that the Alpine whitefish radiation is of hybridogenic origin and that genetic variation thus was probably high from the onset of colonization of Alpine lakes (Hudson *et al.*, 2011), that contemporary evolution is happening (Bittner *et al.*, 2010; Vonlanthen *et al.*, 2012) and that whitefish can be considered keystone species in many Swiss lakes (Vonlanthen *et al.*, 2012, unpublished data Projet Lac). All these features of the Alpine radiation make whitefish species good candidates for eco-evolutionary feedbacks to happen during their evolutionary diversification (Odling-Smee *et al.*, 2003; Yoshida *et al.*, 2003; Post & Palkovacs, 2009). This claim is supported by theoretical considerations, which show that modification of the fitness landscape through resource exploitation by the evolving populations can be important for speciation and adaptive radiation (Dieckmann & Doebeli, 1999; Ito & Dieckmann, 2007). However, strong empirical evidence that speciation can be driven by dynamically evolving fitness landscapes rather than from extrinsically rugged adaptive landscapes is still rare (Dieckmann *et al.*, 2004; but see Meyer & Kassen, 2007 for an example).

In chapter 5 I showed that phenotypic plasticity can affect ecosystems over extended time-scales too. These findings indicate that ecosystem modification through phenotypic plasticity may lead to evolutionary consequences too and that plasticity should not be neglected when trying to understand eco-evolutionary dynamics. This interpretation is consistent with earlier studies: Niche construction through plasticity in phenological and dispersal traits is probably quite ubiquitous in plants (Donohue, 2004) and theoretical considerations suggested that eco-evolutionary feedbacks during predator-prey cycling can result in evolutionary change of the genetic basis of plasticity (Yamamichi *et al.*, 2011).

Human-driven environmental modification and eco-evolutionary dynamics

Climate change and other human-driven environmental modifications are strong selective events that can cause fast ecological and evolutionary change (Palumbi, 2001). There are not (yet) many studies about ecological consequences of human induced evolution and how they might cause eco-evolutionary feedbacks, but the few that are available indicate that effects of human induced evolution on ecosystems can be strong (Lavergne *et al.*, 2010; Palkovacs *et al.*, 2011). Nevertheless, modelling approaches trying to predict consequences of human driven environmental modification such as climate change and eutrophication do usually ignore eco-evolutionary dynamics. This may question their validity, as highlighted by a recent study, which showed that including eco-evolutionary dynamics in forecasting models of climate change can render unexpected biodiversity responses, most notably because the interaction between evolution and ecological species interactions continued to cause extinctions long after climate stabilization (Norberg *et al.*, 2012). An example of how human driven environmental change can cause trait change and biodiversity loss can also be found in the Alpine whitefish radiation: Recent eutrophication of Alpine lakes has caused substantial diversity loss which was mainly driven by speciation reversal and this diversity loss was most severe in heavily polluted lakes (Vonlanthen *et al.*, 2012). Our results of heritable differentiation in functional traits (Chapter 2 and 3) combined with the finding of strongly contrasting ecosystem effects of different whitefish species from only weakly affected lakes (chapter 4 and 5)

imply that the human induced collapse of genetically distinct species likely modified the effects whitefish have had on the ecosystem, and by doing so might have reinforced the ecosystem perturbations that had induced speciation reversal (Seehausen, 2009). A better understanding of how environmental modifications by humans modify eco-evolutionary interactions will be of fundamental importance to predict ecological, evolutionary and biodiversity dynamics in the future; and this is needed in order to minimize negative impacts on biodiversity (Seehausen *et al.*, 2007; Lavergne *et al.*, 2010).

Open questions and future research directions

The findings of this thesis generated a large number of relevant follow on research questions. I here want to outline just a few potential directions of future research.

Whitefish evolutionary ecology

We have evidence that different whitefish species differ in their use of foraging habitat (Amundsen *et al.*, 2004; Harrod *et al.*, 2010; Ingram *et al.*, 2012) and their efficiency to exploit different trophic resources (chapter 2 and 3). But empirical data also shows that phenotypically clearly differentiated whitefish species can broadly overlap in trophic resource use during most of the year (Mookerji *et al.*, 1998; Michel, unpub. data). The question of how phenotypic divergence in traits related to trophic ecology between closely related species can result in partitioning of resources over the seasons has been resolved in cichlid fish a while ago (McKaye & Marsh, 1983; Bouton *et al.*, 1997; Bouton *et al.*, 1999). Studying how whitefish feeding performance depends on environmental settings, for example variation in prey density (the establishment of functional response curves) or light conditions (in nature light will vary with foraging depth and water clarity), combined with an investigation of resource availability and use in nature, could help to understand in more detail how dietary specialization of whitefish species originated and how they partition resources.

Evidence that a larger number of gill rakers increases feeding efficiency in the limnetic habitat exists (Robinson, 2000; chapter 3 of this thesis), but direct experimental evidence that a lower number of gill rakers is advantageous for benthic feeding is lacking. In north temperate fish, the number of gill rakers is repeatedly decreased in benthic species compared to their limnetic sister species (Smith & Skúlason, 1996), an observation which strongly suggests that a larger number of gill rakers is costly for feeding in the benthic habitat. Most benthic feeding fish suck their prey from the sediment and by doing so also ingest sediment particles. Subsequently they separate the prey from the sediments to spit the latter out through the mouth or to release it through the gill rakers. One idea why many gill rakers might be detrimental for benthic feeding is that a higher number of gill rakers may be disadvantageous for releasing ingested sediment particles through the gills. Under this hypothesis processing time for benthic food items should be associated with gill raker number. An alternative, not mutually exclusive, hypothesis is the occurrence of a hydrodynamic trade-off between suction and filter feeding, with an increased number of gill rakers reducing suction force (van den Berg *et al.*, 1992). Under this hypothesis prey capture efficiency of benthic foragers should be associated with the number of gill rakers. Because I kept experimental fish alive in the experiment presented in chapter 2, I could not quantify their variation in gill raker number and thus I could not test for the

above outlined hypothesis. We are currently working on such experiments and these will help to further increase our understanding of the evolutionary ecology of feeding specialization in fish.

Whitefish speciation

In a yet unpublished study we found that genetic distance between individuals of three Lake Lucerne whitefish species is best predicted by their variation in individual growth rate (Lundsgaard-Hansen *et al.*, in prep.). Further, we found significant genetic differentiation between species, despite mixing of individuals of different species on their spawning grounds at spawning time. Taken together, these findings can be interpreted as indirect evidence for behavioural reproductive isolation, maybe due to mate choice based on size or a correlated trait. In chapter 2 and 3 of this thesis I have shown that there is heritable differentiation in body size between whitefish species and that body size is associated with feeding efficiency on benthic food. Evidence that adult size is under natural selection between benthic and limnetic whitefish species is available from other whitefish radiations (Rogers & Bernatchez, 2007; Vonlanthen *et al.*, 2009). In combination, these findings suggest that natural selection on size might be involved in the evolution of reproductive isolation between whitefish species, as it has been suggested for other north temperate fish such as other salmonids or stickleback (Foote & Larking, 1988; Sigurjonsdottir & Gunnarsson, 1989; McKinnon *et al.*, 2004). To date, mate choice trials using whitefish species are not available, probably partially because of their rather long generation time, large adult size and/or the difficulties to collect adults in the wild and to subsequently keep them alive and make them spawn in captivity. However, such trials are urgently needed to improve our understanding of how whitefish species arise and get lost. Further, from such a study one could learn about basic questions of adaptive radiation research such as which mechanisms of reproductive isolation are involved in ecological speciation during adaptive radiation and which traits might serve as mate-choice signals.

Genetics of eco-evolutionary dynamics

An exciting direction of future research consists of generating F2-hybrids between benthic and limnetic specialist species. F2-hybrids can span a wide range of phenotypes and genotypes, while specific traits of interest may segregate independently of other traits. Using F2-hybrids in feeding efficiency trials could shed more light on the functional significance of specific traits, because linkage disequilibrium between genes coding for different traits is not expected in F2-hybrids unless genes are tightly physically linked. If combined with a QTL approach such a study could help to reveal the genetic basis of foraging traits and feeding efficiency divergence. Such knowledge would enable a broader understanding of how genetic variation is translated into variation in resource use (Irschick *et al.*, 2013) and a better understanding of the genetics of speciation (Wu & Ting, 2004; Schluter & Conte, 2009).

Using the F2-hybrids in mesocosm trials would allow one to identify ecosystem effect traits. Further, ecosystem effects of F2-hybrids could be compared to ecosystem effects of both parental specialists together in order to test for ecosystem consequences of speciation and speciation-reversal (Seehausen, 2009).

Along similar lines, one could quantify ecosystem effects of adaptive radiation by comparing mesocosms containing a generalist whitefish species to mesocosms containing multiple specialist

species. Genetically variable F2 fry could then be introduced to mesocosm ecosystems previously modified by either a generalist or multiple specialist species. Analysis of phenotypic variation across tanks would allow one to identify phenotypic traits under “adaptive radiation driven selection”, divergent selection that is caused by ecosystem effects of specialization and diversification during adaptive radiation. Combined with a “genome wide outlier analysis”, which compares whether allele frequencies at multiple loci across the genome are significantly different between F2-fry from tanks previously modified by generalists and F2-fry from tanks previously modified by specialists, one could also measure the signature of “adaptive radiation driven selection” across the genome. Such a study would shed some light on how the process of adaptive radiation itself affects fitness landscapes and this is important to understand how different sources of selection might interact during adaptive radiation (Pelletier *et al.*, 2009; Losos, 2010; Yoder *et al.*, 2011).

Closing remark

Adaptive radiation has been suggested to be responsible for a considerable fraction of Earth’s biodiversity (Simpson, 1953). I hope that the work I have performed during my thesis contributes to a better understanding of the eco-evolutionary dynamics and ecosystem relevance of adaptive radiation. Such an understanding can almost certainly be useful to protect and restore natural biodiversity, because effective conservation and restoration of biodiversity needs insights into ecological and evolutionary processes that generate and maintain it. As we are today losing biodiversity at alarming rates, maybe higher than ever before in Earth’s history (Barnosky *et al.*, 2012), more effective biodiversity protection is urgently needed if we want to conserve the most valuable of all legacies for next generation, the legacy of nature in all its forms and beauty.

References

- Agrawal, A.A. 2001. Phenotypic plasticity in the interaction and evolution of species. *Science* 294: 321-326.
- Amundsen, P.A., Knudsen, R., Klemetsen, A. & Kristoffersen, R. 2004. Resource competition and interactive segregation between sympatric whitefish morphs. *Ann. Zool. Fenn.* 41: 301-307.
- Barnosky, A.D., Matzke, N., Tomiya, S., Wogan, G.O.U., Swartz, B., Quental, T.B., Marshall, C., McGuire, J.L., Lindsey, E.L., Maguire, K.C., Mersey, B. & Ferrer, E.A. 2011. Has the Earth's sixth mass extinction already arrived? *Nature* 471: 51-57.
- Barret, R.D. & Schluter, D. 2008. Adaptation from standing genetic variation. *Trends Ecol. Evol.* 23: 38-44.
- Bassar, R.D., Marshall, M.C., Lopez-Sepulcre, A., Zandon, E., Auer, S.K., Travis, J., Pringle, C.M., Flecker, A.S., Thomas, S.A., Fraser, D.F. & Reznick, D.N. 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. *P. Natl. Acad. Sci. USA* 107: 3616-3621.
- Bernatchez, L. 2004. Ecological Theory of Adaptive Radiation. An Empirical Assessment from Coregonine Fishes (*Salmoniformes*). In: Evolution Illuminated (Hendry, A. P. & Stearns, S. C., eds.). pp. 175-207. Oxford University Press, Oxford.
- Bernatchez, L. & Wilson, C.C. 1998. Comparative phylogeography of Nearctic and Palearctic. *Mol. Ecol.* 7: 431-452.
- Bittner, D., Excoffier, L. & Largiadèr, C.R. 2010. Patterns of morphological changes and hybridization between sympatric whitefish forms (*Coregonus spp.*) in a Swiss lake: a role for eutrophication? *Mol. Ecol.* 19: 2152-2167.
- Bouton, N., Seehausen, O. & van Alphen, J.J.M. 1997. Resource partitioning among rock-dwelling haplochromines (Pisces: *Cichlidae*) from Lake Victoria. *Ecol. Freshw. Fish* 6: 225-240.
- Bouton, N., Witte, F. & Van Alphen, J.J.M. 2002. Experimental evidence for adaptive phenotypic plasticity in a rock-dwelling cichlid fish from Lake Victoria. *Biol. J. Linn. Soc.* 77: 185-192.
- Bouton, N., Witte, F., van Alphen, J.J.M., Schenk, A. & Seehausen, O. 1999. Local adaptation in populations of rock-dwelling haplochromines (Pisces: *Cichlidae*) from southern Lake Victoria. *P. R. Soc. B.* 266: 355-360.
- Dieckmann, U. & Doebeli, M. 1999. On the origin of species by sympatric speciation. *Nature* 400: 354-357.
- Dieckmann, U., Doebeli, M., Metz, A.J. & Tautz, D. editors. 2004. Adaptive Speciation. Cambridge University Press, Cambridge.
- Donohue, K. 2004. Niche construction through phenological plasticity: life history dynamics and ecological consequences. *New Phytol.* 166: 83-92.
- Douglas, M.R., Brunner, P.C. & Bernatchez, L. 1999. Do assemblages of *Coregonus* (*Teleostei: Salmoniformes*) in the Central Alpine region of Europe represent species flocks? *Mol. Ecol.* 8: 589-603.
- Ezard, T.H.G., Côté, S.D. & Pelletier, F. 2009. Eco-evolutionary dynamics : disentangling phenotypic, environmental and population fluctuations. *Philos. T. Roy. Soc. B.* 364: 1491-1498.

- Hairston Jr., N.G., Ellner, S.P., Geber, M.A., Yoshida, T. & Fox, J.A. 2005. Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.* 8: 1114-1127.
- Foote, C.J. & Larkin, P.A. 1988. The role of mate choice in the assortative mating of anadromous and non-anadromous sockeye salmon, *Oncorhynchus nerka*. *Behavior* 106: 43-62.
- Gomez-Mestre, I. & Buchholz, D.R. 2006. Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *P. Natl. Acad. Sci. U.S.A.* 103: 19021-19026.
- Harmon, L.J., Matthews, B., Des Roches, S., Chase, J.M., Shurin, J.B. & Schluter, D. 2009. Evolutionary diversification in stickleback affects ecosystem functioning. *Nature* 458: 1167–1170.
- Harrod, C., Mallela, J. & Kahilainen, K. 2010. Phenotype-environment correlations in a putative whitefish adaptive radiation. *J. Anim. Ecol.* 79: 1057-1068.
- Hudson, A.G., Vonlanthen, P., Müller, R. & Seehausen, O. 2007. Review: The geography of speciation and adaptive radiation of coregonines. *Arch. Hydrob. Spec. Iss. Adv. Limnol.* 60: 111-146.
- Hudson, A.G., Vonlanthen, P. & Seehausen, O. 2011. Rapid parallel adaptive radiations from a single hybridogenetic ancestral population. *P. R. Soc. B.* 278: 58-66.
- Ingram, T., Hudson, A.G., Vonlanthen, P. & Seehausen, O. 2012. Does water depth or diet divergence predict progress towards ecological speciation in whitefish radiations? *Evol. Ecol. Res.* 14: 487-502.
- Ito, H.C. & Dieckmann, U. 2007. A new mechanism for recurrent adaptive radiations. *Am. Nat.* 170: E96-E111.
- Irschick, D.J., Albertson, R.C., Brennan, P., Podos, J., Johnson, N.A., Patek, S. & Dumont, E. 2013. Eco-devo beyond morphology: from genes to resource use. *Trends. Ecol. Evol.* 28: 267-273.
- Kahilainen, K.K., Malinen, T., Tuomaala, A., Alajärvi, E., Tolonen, A. & Lehtonen, A. 2007. Empirical evaluation of phenotype-environment correlation and trait utility with allopatric and sympatric whitefish (*Coregonus lavaretus* (L.)) in subarctic lakes. *Biol. J. Linn. Soc.* 92: 561-572.
- Kahilainen, K.K., Siwertsson, A., Gjelland, K.O., Knudsen, R., Bohn, T. & Amundsen, P.-A. 2011. The role of gill raker variability in adaptive radiation of coregonid fish. *Evol. Ecol.* 25: 573-588.
- Kottelat, M. & Freyhof, J. 2007. Handbook of European freshwater fishes. Kottelat, Cornol, and Freyhof, Berlin.
- Lavergne, S., Moquet, N., Thuiller, W. & Ronce, O. 2010. Biodiversity and climate change: Integrating evolutionary and ecological responses of species and communities. *Annu. Rev. Ecol. Evol. S.* 41: 321-350.
- Link, J. & Hoff, M.H. 1998. Relationship of Lake Herring (*Coregonus artedii*) gill raker characteristics to retention probabilities of zooplankton prey. *J. Freshw. Ecol.* 13: 55-65.
- Lundsgaard-Hansen B., Vonlanthen, P., Hudson, A.G., Lucek, K. & Seehausen, O. Ecological speciation on a water depth gradient in Alpine whitefish. In preparation.
- Losos, J.B. 2010. Adaptive radiation, ecological opportunity and evolutionary determinism. *Am. Nat.* 175: 623-639.

- Matthews, B., Narwani, A., Hausch, S., Nonaka, E., Peter, H., Yamamichi, M., Sullam, K.E., Bird, K.C., Thomas, M.K., Hanley, T.C. & Turner, B.C. 2011. Toward an integration of evolutionary biology and ecosystem science. *Ecol. Lett.* 14: 690-701.
- Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge.
- Mc Kaye, K.R. & Marsh, A. 1983. Food switching by two specialized algae-scraping cichlid fishes in Lake Malawi, Africa. *Oecologia* 56: 245-248.
- Mc Kinnon, J.S., Mori, S., Blackman, B.K., David, L., Kingsley, D.M., Jamieson, L., Chou, J. & Schluter, D. 2004. Evidence for ecology's role in speciation. *Nature* 429: 294-298.
- Meyer, J.R. & Kassen, R. 2007. The effects of competition and predation on diversification in a model adaptive radiation. *Nature* 446: 432-435.
- Michel, M. 1996. Untersuchungen zur Nahrungsökologie von Grossfelchen im Vierwaldstättersee während des Sommerhalbjahres 1996. Master thesis EAWAG.
- Miner, B.G., Sultan, S.E., Morgan, S.G., Padilla, D.K. & Relyea, R.A. 2005. Ecological consequences of phenotypic plasticity. *Trends Ecol. Evol.* 20: 687-692.
- Mookerji, N., Heller, C., Meng, H.J., Bürgi, R. & Müller, R. 1998. Diel and seasonal patterns of food uptake and prey selection by *Coregonus* sp. in re-oligotrophicated Lake Lucerne, Switzerland. *J. Fish. Biol.* 52: 443-457.
- Norberg, J., Urban, M.C., Vellend, M., Klausmeier, C.A. & Leouille, N. 2012. Eco-evolutionary responses of biodiversity to climate change. *Nature Climate Change* 2:747-751.
- Nosil, P., Harmon, L. & Seehausen, O. 2009. Ecological explanations for (incomplete) speciation. *Trends Ecol. Evol.* 24: 145-156.
- Nosil, P. & Harmon, L. 2009. Niche dimensionality and ecological speciation. In "Speciation and patterns of diversity" (R. Butlin, J. Bridle, D. Schluter, eds), Cambridge University Press, Cambridge.
- Odling-Smee, F.J., Laland, K.N. & Feldmann, M.W. 2003. Niche construction – the neglected process in evolution. Princeton University Press, Princeton.
- Palkovacs, E.P., Marshall, M.C., Lamphere, B.A., Lynch, B.R., Weese, D.J., Fraser, D.F., Reznick D.N., Pringle, C.M. & Kinnison, M.T. 2009. Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Philos. T. Roy. Soc. B.* 364: 1617–1629.
- Palkovacs, E.P. & Post, D.M. 2008. Eco-evolutionary interactions between predators and prey: can predator-induced changes to prey communities feed back to shape predator foraging traits? *Evol. Ecol. Res.* 10: 699-720.
- Palkovacs, E.P., Kinnison, M.T., Correa, C., Dalton, C.M. & Hendry, A.P. 2011. Fates beyond traits: ecological consequences of human induced trait change. *Evol. Appl.* 5: 183-191.
- Palumbi, S.R. 2001. Humans as the world's greatest evolutionary force. *Science* 293: 1786-1790.
- Peacor, S.D. & Werner, E.E. 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. *P. Natl. Acad. Sci. USA* 98: 3904-3908.
- Pelletier, F., Garant, D. & Hendry, A.P. 2009. Eco-evlutionary dynamics. *Philos. T. Roy. Soc. B.* 364: 1483-1489.

- Post, D.M. & Palkovacs, E.P. 2009. Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. *Philos. T. Roy. Soc. B.* 364: 1629–1640.
- Post, D.M., Palkovacs, E.P., Schielke, E.G. & Dalton, S.I. 2008. Intraspecific phenotypic variation in a predator affects community structure and cascading trophic interactions. *Ecology* 89: 2019–2032.
- Price, T.D., Qvarnström, A. & Irwin, D.E. 2003. The role of phenotypic plasticity in driving genetic evolution. *P. R. Soc. B.* 270: 1433–1440.
- Projet Lac: Assessing biodiversity of fish in Swiss lakes. Ongoing project of Vonlanthen, P., Périat, G. Brodersen, J. & Seehausen, O.
- Robinson, B.W. 2000. Trade offs in habitat-specific foraging efficiency and the nascent adaptive divergence of sticklebacks in lakes. *Behaviour* 137: 865–888.
- Robinson, B.W. & Parsons, K.J. 2002. Changing times, spaces and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Can. J. Fish. Aquat. Sci.* 59: 1819–1833.
- Rogers, S.M. & Bernatchez, L. 2007. The genetic architecture of ecological speciation and the association with signatures of selection in natural lake whitefish (*Coregonus sp.*, *Salmonidae*) species pairs. *Mol. Biol. Evol.* 24: 1423–1438.
- Rundle, H.D., Nagel, L., Boughmann, J.W. & Schluter, D. 2000. Natural selection and parallel speciation in sympatric sticklebacks. *Science* 287: 306–308.
- Sanderson, S.L., Cheer, A.Y., Goodrich, J.S., Graziano, J.D. & Callan, W.T. 2001. Crossflow filtration in suspension-feeding fishes. *Nature* 412: 439–441.
- Schluter, D. 1995. Adaptive radiation in sticklebacks: Trade-offs in feeding performance and growth. *Ecology* 76: 82–90.
- Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Schluter, D. & Conte, G.L. 2009. Genetics and ecological speciation. *P. Natl. Acad. Sci. USA* 106: 9955–9962.
- Schoener, T.W. 2011. The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science* 331: 426–429.
- Seehausen, O. 2004. Hybridization and adaptive radiation. *Trends Ecol. Evol.* 19: 198–207.
- Seehausen, O. 2009. Speciation affects ecosystems. *Nature* 458: 1122–1123.
- Seehausen, O., Takimoto, G., Roy, D. & Jokela, J. 2007. Speciation reversal and biodiversity dynamics in changing environments. *Mol. Ecol.* 17: 30–44.
- Shubin, Y.P., Chelpanova, T.I., Efimsteva, E.A. & Shubin, P.N. 1997. Genetic differentiation between resident and semianadromous Siberian whitefish, *Coregonus lavaretus pidschian*. *Jpn. J. Ichtyol.* 37: 634–638.
- Sigurjonsdottir, H. & Gunnarson, K. 1989. Alternative mating tactics of Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. *Environ. Biol. Fish.* 26: 159–176.

- Siwertsson, A., Knudsen, R., Kahilainen, K., Praebel, K., Primicerio, R. & Amundsen, P.A. 2010. Sympatric diversification as influenced by ecological opportunity and historical contingency in a young species lineage of whitefish. *Evol. Ecol. Res.* 12: 929-948.
- Skulason, S., Snorrason, S.S. & Jonsson, B. 1999. Sympatric morphs, populations and speciation in freshwater fish with emphasis on Arctic charr. In: Magurran, A. E., May, R., eds. *Evolution of biological diversity*, pp. 70-92. Oxford University Press.
- Smith, T.B. & Skulason, B. 1996. Evolutionary significance of resource polymorphism in fishes, amphibians and birds. *Annu. Rev. Ecol. S.* 27: 111-133.
- Steinmann, P. 1950. Monographie der schweizerischen Koregonen. Beitrag zum Problem der Entstehung neuer Arten. Spezieller Teil. *Schweiz. Z. Hydrol.* 12: 340-491.
- Taylor, E.B. 1999. Species pairs of north temperate freshwater fishes: evolution, taxonomy, and conservation. *Rev. Fish Biol. Fisher.* 9: 299-324
- Van den Berg, C., Sibbing, F.A., Osse, J.W.M. & Hoogenboezem, W. 1992. Structure, development and function of the branchial sieve of bream (*Abramis brama*), white bream (*Blicca bjoerka*) and roach (*Rutilus rutilus*). *Environ. Biol. Fish.* 33: 105-124.
- Vonlanthen, P., Bittner, D., Hudson, A.G., Young, K.A., Müller, R., Lundsgaard-Hansen, B., Roy, D., Di Piazza, S., Largiadèr, C.R. & Seehausen, O. 2012. Eutrophication causes speciation reversal in whitefish adaptive radiations. *Nature* 482: 357-363.
- Vonlanthen, P., Roy, D., Hudson, A.G., Largiadèr, C.R., Bittner, D. & Seehausen, O. 2009. Divergence along a steep ecological gradient in lake whitefish (*Coregonus sp.*). *J. Evolution. Biol.* 22: 498-514.
- West-Eberhard, M.J. 2003. *Developmental plasticity and evolution*. Oxford University Press, Oxford.
- Wimberger, P.H. 1994. Trophic polymorphisms, plasticity, and speciation in vertebrates. In *Theory and application in fish feeding ecology* (eds D. J. Stouder, K. L. Fresh & R. J. Feller). University of South Carolina Press, Columbia, SC.
- Wu, C.I. & Ting, C.T. 2004. Genes and speciation. *Nat. Rev. Genet.* 5: 114-122.
- Wund, M.A., Baker, J.A., Clancy, B., Golub, J.L. & Foster, S.A. 2008. A test of the flexible stem model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. *Am. Nat.* 172: 449-462.
- Yamamichi, M., Yoshida, T. & Sasaki, A. 2011. Comparing the effects of rapid evolution and phenotypic plasticity on predator-prey dynamics. *Am. Nat.* 178: 287-304.
- Yoder, J.B., Clancey, E., Des Roches, S., Eastman, J.M., Gentry, L., Godsoe, W., Hagey, T.J., Jochimsen, D., Oswald, B.P., Roberston, J., Sarver, B.A.J., Schenk, J.J., Saer, S.F. & Harmon, L.J. 2010. Ecological opportunity and the origin of adaptive radiations. *J. Evolution. Biol.* 23: 1581-1596.
- Yoshida, T., Jones, L.E., Ellner, S.P., Fussmann, G.F. & Hairston, N.G. 2003. Rapid evolution drives ecological dynamics in a predator-prey system. *Nature* 424: 303-306.

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Erklärung

gemäss Art. 28 Abs. 2 RSL 05

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Publications

Karvonen, A., Lundsgaard-Hansen, B., Jokkela, J., & Seehausen, O. 2012. Divergence in parasitism in whitefish species segregating along depth gradients. *Oikos*, 122: 122-128.

Lundsgaard-Hansen, B., Matthews, B., Vonlanthen, P., Taverna, A., & Seehausen, O. 2013. Adaptive plasticity and genetic divergence in feeding efficiency during parallel adaptive radiation in whitefish (*Coregonus spp.*). *Journal of Evolutionary Biology*, 26: 483-498.

Lundsgaard-Hansen, B., Roesch, C., Vonlanthen, P., Taverna, A., & Seehausen, O. 2013. Experimental evidence for trait utility of gill raker number in a north temperate fish. *Journal of Evolutionary Biology*, 26: 1578-1587.

Magalhaes, I.S., Lundsgaard-Hansen, B., Mwaiko, S., & Seehausen, O. 2012. Evolutionary diversification along depth gradients in replicate pairs of Lake Victoria cichlids. *Ecology & Evolution Research*, 14: 381-401.

Vonlanthen, P., Bittner, D., Hudson, A. G., Young, K. A., Müller, R., Lundsgaard-Hansen, B., Roy, D., Di Piazza, S., Largiadèr, C. R., & Seehausen, O. 2012. Anthropogenic eutrophication drives extinction by speciation reversal in parallel adaptive radiation of whitefish. *Nature*, 482: 357-362.

Reports

Vonlanthen, P., Hudson, A. G., Lundsgaard-Hansen, B., Roy, D., & Seehausen, O. Untersuchungen zur Verwandtschaft der Felchen aus dem Lago di Como, di Lugano und Maggiore. 2008. Zu Handen Kanton Tessin.

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