

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 post-glacial 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 (Pfennig *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 (Pfennig *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, 2000; Via *et al.*, 2000; Rundle, 2002; Nosil, 2004). Such trade-offs suggest that the genetically heritable divergence is a result of

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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). However, 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 post-glacial 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 behaviour, 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, for example, 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 behaviour 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 behaviour 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 versus 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 behaviour in one and the same experiment, although this is important to identify the traits that affect feeding efficiency.

Whitefish species complexes might fulfil the four criteria that define an adaptive radiation (Schluter, 2000), that is, 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; Svårdson, 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; 2011). 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 15 000 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 (Fig. 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 (Fig. 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 (Fig. 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. On the basis of the above outlined existence of empirical evidence for the importance of both, plasticity and genetic divergence in morphology and feeding efficiency of north temperate

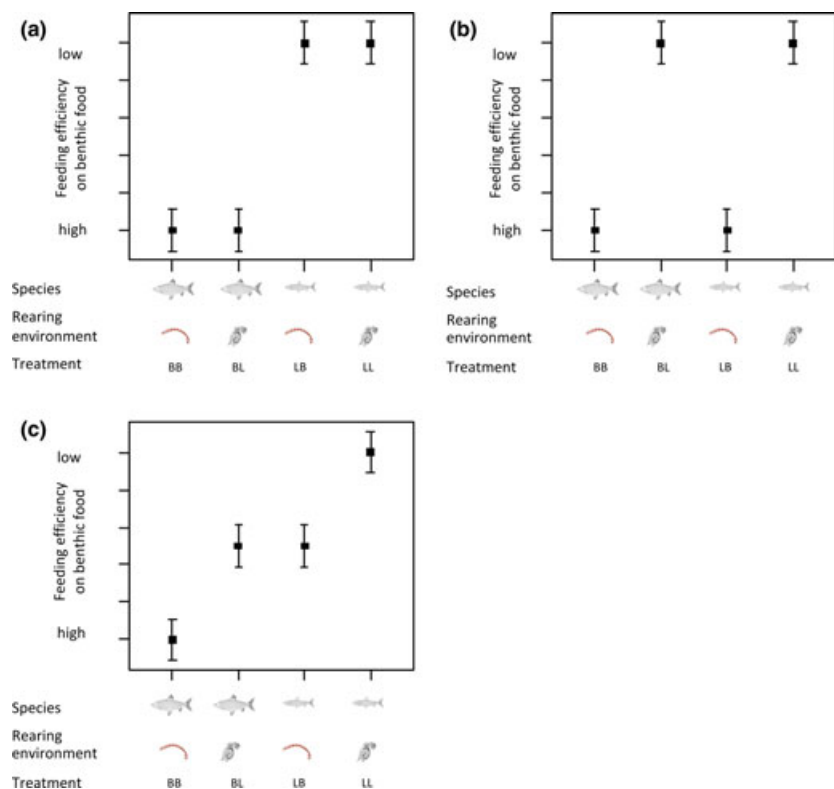
fish, we predicted that variation in feeding efficiency would have both, a genetic and an environmentally induced component.

Materials and 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*

Fig. 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).



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 are lacking, evidence for divergence in resource use 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 libitum 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 \times 71 \times 50 cm for fish from Lake Lucerne and of 120 \times 142 \times 50 cm for fish from Lake Thun. A flow through system (2.5 L min⁻¹) 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 as follows: $BB_{AQ1} = 0.03$; $BB_{AQ2} = 0.07$; $LB_{AQ1} = 0.1$. In aquaria of Lake Lucerne fish they were as follows: $BB_{AQ1} = 0.06$; $BB_{AQ2} = 0.07$; $BL_{AQ1} = 0.06$; $BL_{AQ2} = 0.2$; $LB_{AQ1} = 0.05$; $LB_{AQ2} = 0.06$; $LL_{AQ1} = 0.02$; $LL_{AQ2} = 0.09$. 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 \times 142 \times 40 cm, were divided lengthwise into two compartments using a Plexiglas wall, resulting in one compartment with a size of 33 \times 142 \times 40 cm and the other compartment with a size of 22 \times 142 \times 40 cm. Water temperature varied between 12 and 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 behavioural 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. In addition, two fish were put in the smaller back

compartment and were left there for the entire duration of the trials to stimulate natural behaviour of the single experimental fish in the front compartment (single isolated individuals did not display natural behaviour).

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. In addition, a low number of fish from Lake Lucerne (seven individuals from the LB and one 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.

Approximately 48 h 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).

Behavioural 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 could not grab it/lost it immediately after grabbing it). As 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).

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 were 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 were 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 were 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	N_{total}	N_{shape}	N_{ph}	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	

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 were 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). In addition, 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 (GLM) 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 the

Gaussian error distribution we used the identity link function, for the quasi poisson distribution we used the log link function and for the 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 *et al.*, 1992; Burnham & Anderson, 1998). We compared models using AIC, Akaike Weights (wi) 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'Abbrera, 2006). 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 as in the treatment with the smallest sample size. Subsequently, fish from the same species but different rearing environment were pooled to calculate differences between

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 with 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.

	AIC	Delta i	Likelihood	w_i	L ratio
Backward model selection Lucerne					
Time to food depletion = $(SP \times ENV) + SP + ENV + L + PC1 + PC2 + Y$	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 = $(SP \times ENV) + SP + ENV + L + PC1 + PC2 + Y$	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	–
$N_{\text{unsuccessful}}$ = $(SP \times ENV) + SP + ENV + L + PC1 + PC2 + Y$	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					
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	–

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 package 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 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 (Table S1a). 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). However, 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 (Table S1b). PC2 was lower in the benthic species, corresponding to more sub-terminal mouths in this species (see Fig. 4d). In Lake Thun, the two species did not differ in PC2 (Wilcoxon Rank Sum Test: $n = 34$, $W = 135$, $P = 0.76$, Table S1b).

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 (Fig. 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 (Table S2). Pairwise *post hoc* tests for feeding efficiency differences between treatments indicate both, a genetic basis as well as phenotypic plasticity, in feeding efficiency divergence (Table S2). All feeding efficiency variables were negatively correlated with fish length (Fig. 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, Table S3).

The observation of plasticity and species divergence in feeding efficiency as well as in fish length (Tables S1 and S2), combined with the observation of effects of fish length on feeding efficiency (Table S3), suggest that species and plasticity effects on feeding efficiency can be two-fold: 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. In addition, the effects of length on feeding efficiency can be considered 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 modelling, 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 (Fig. 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 (Fig. 4a). *Post hoc* tests revealed that all of the variables retained in the most likely model (Length, species and environment) were significantly associated with time to food depletion (Tables S2 and S3). The differences in significance levels between multivariate modelling 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 (Fig. 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 (Fig. 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 (Tables S2 and S3).

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 (Fig. 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 nonsignificant, 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 (Fig. 4d). Species and the environment were nonsignificant, but the benthic species seemed to be slightly more efficient than the limnetic species, when controlling for the effects of length, PC2 and year (Fig. 3c). Univariate *post hoc* tests revealed a similar pattern as the GLM and were only significant for length (Tables S2 and S3; and PC2: $S = 11972$, $\rho = 0.07$, $P = 0.46$).

(ii) Lake Thun

In Lake Thun, the benthic species was more efficient than the limnetic species by means of time to food

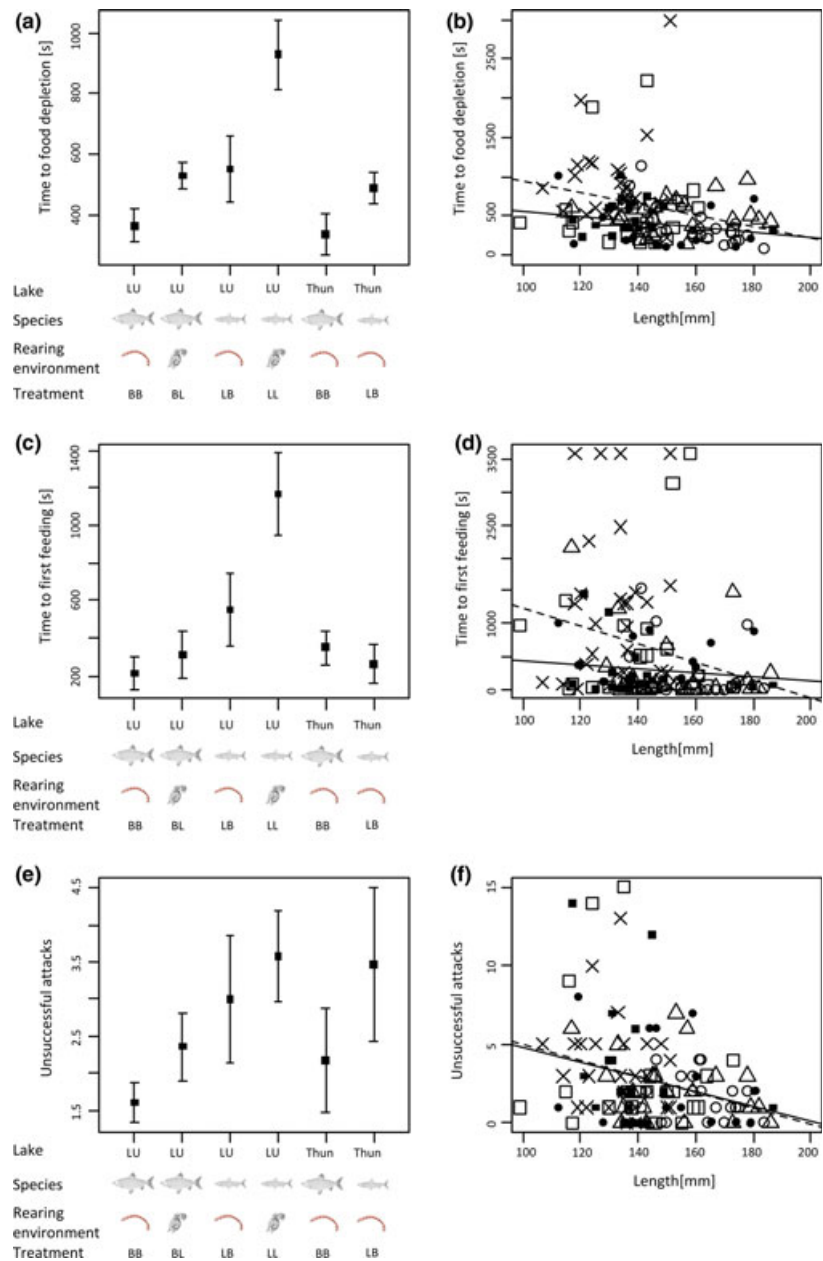


Fig. 2 Feeding efficiency versus treatments and feeding efficiency versus length. Figures on the left show the treatments (for both lakes separately) on the x-axis (see legend of Fig. 1 for more detail), and Figures on the right show total fish length on the x-axis. The y-axis shows time to food depletion [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.

depletion and the number of unsuccessful attacks (Fig. 2). Time to first feeding on the other hand was lower in limnetic than in benthic fish (Fig. 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 (Table S2). Feeding efficiency was generally higher for larger fish (Fig. 2); however, these correlations were neither significant over both species nor within species (Table S3).

Using generalized linear modelling, 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, Fig. 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 nonsignificant (Table 3, Fig. 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 (Fig. 4f). There was a trend for length; as larger fish, independent of their genetic background, their shape (PC2), and the year

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	–	–
	<i>P</i>	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
	<i>P</i>	0.26	0.03	0.08	–	0.11
$N_{\text{unsuccessful}}$ Lucerne	Coef/error	0.2/0.25	0.05/0.21	–	9.78/6.12	–
	<i>P</i>	0.42	0.81	0.01	0.11	0.076
Time to food depletion Thun	Coef/error	4.55/1.95	na	–	–	–
	<i>P</i>	0.03	na	–	–	–
Time to first attack Thun	Coef/error	–0.27/0.47	na	–	–	–
	<i>P</i>	0.56	na	–	–	–
$N_{\text{unsuccessful}}$ Thun	Coef/error	0.03/0.36	na	–0.02/0.01	–	–39.95/13.48
	<i>P</i>	0.92	na	0.07	> 0.01	0.06

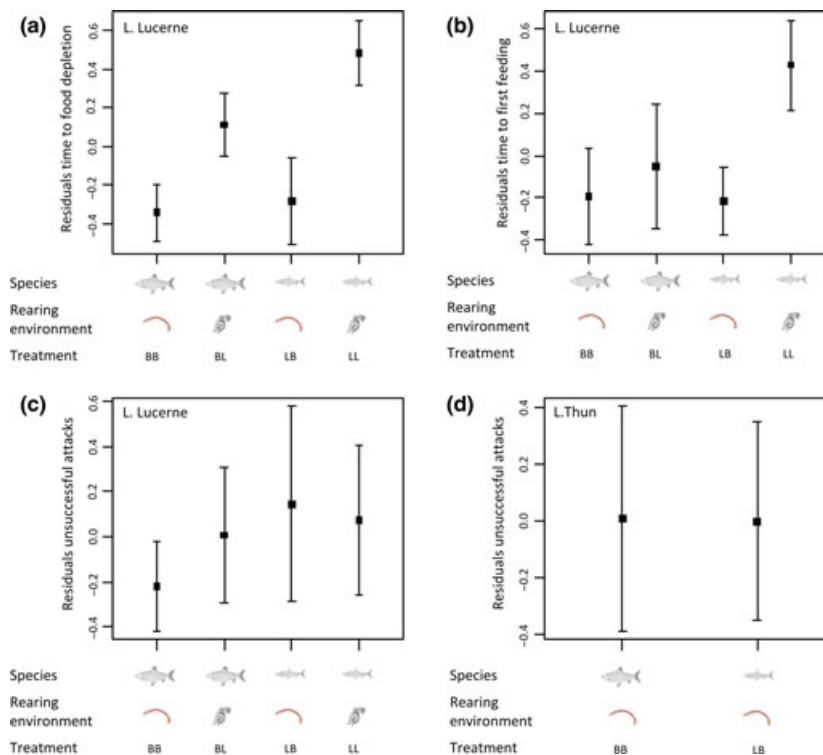


Fig. 3 Effects of species and the environment on feeding efficiency. Shown are the treatments on the x-axis (see legend of Fig. 1 for more detail) and the residuals of the most likely model excluding species (and in Lake Lucerne also the environment) from that model. This illustrates the effects of species 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, whereas 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 Fig. 2).

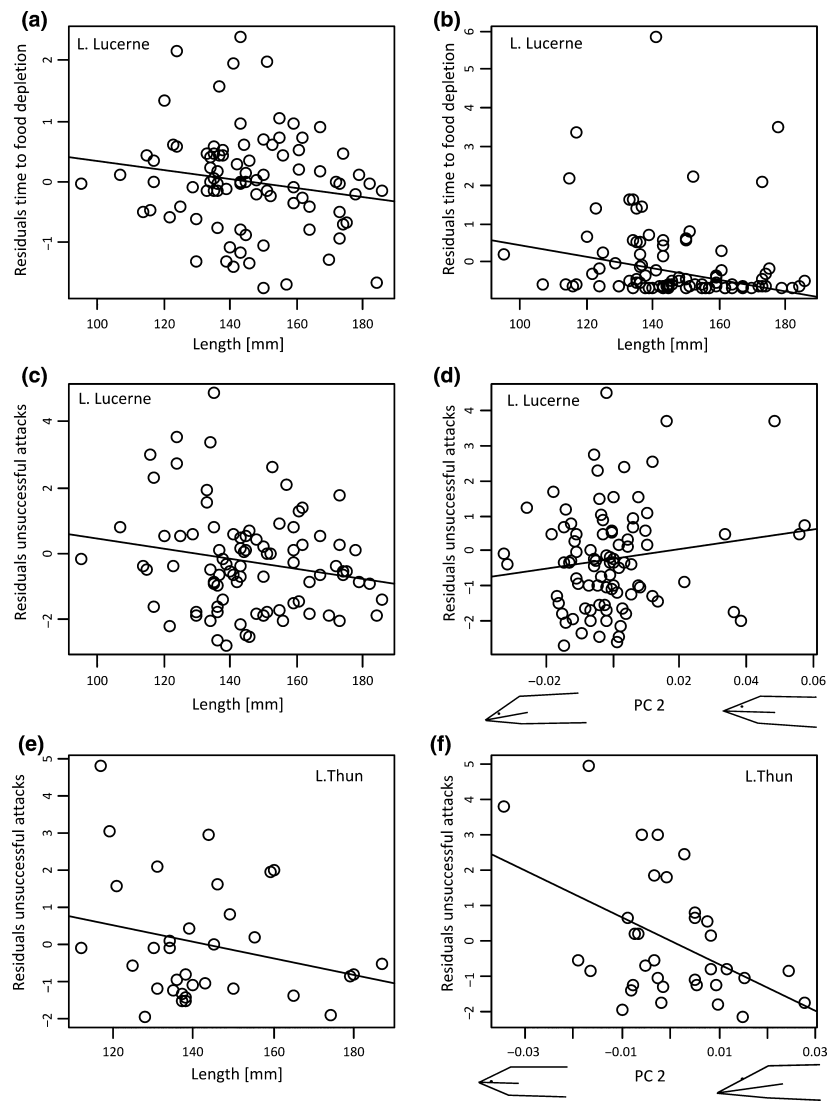


Fig. 4 Effects of fish length and shape on feeding efficiency. Shown are either length (panel a–c and e) or PC2 (panel d and f) on the x-axis and the residuals of the corresponding most likely model excluding either length or PC2. This illustrates the effect of length and PC2 corrected for the effects of their co-variables in the most likely model (residuals). Positive residuals indicate lower efficiency than predicted based on co-variables, whereas 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 three-fold 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.

when they were tested, displayed fewer unsuccessful attacks (Fig. 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 (Fig. 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 (Fig. 3d). Univariate post hoc tests were nonsignificant for an association of species and length with the number of unsuccessful attacks, while they were significant for PC2 and year (Tables S2 and S3; 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.

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 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 findings of 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 favouring 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 behaviour (Kahilainen & Lehtonen, 2002; Rogers *et al.*, 2002). As 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 & Bernatchez, 2007). Taken together, evidence for divergent selection on growth and the predominant role of size as a mate-choice signal in fish (Foote & Larkin, 1988; Sigurjonsdottir & 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 nonlabile 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 & Parsons, 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 two-fold. On one hand, they are manifested as direct behavioural 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 with 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 versus limnetic feeding, with benthic feeders having a more sub-terminal mouth (Steinmann, 1950; McCart, 1970; Caldecutt & Adams, 1998; Bernatchez *et al.*, 1999; Clabaut *et al.*, 2007; Harrod *et al.*, 2010).

The observed heritable divergence in feeding behaviour between the benthic and limnetic whitefish species

is consistent with a role for divergent natural selection favouring 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), and 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 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), although indirect evidence from comparative approaches suggests 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 behaviour), 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. Also, 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 behavioural traits (for example swimming behaviour).

Consistent with earlier work (Day & 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 behaviour 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 behavioural 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 behaviour 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. In 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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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Results of a) size and b) shape differences tests between treatments and years.

Table S2 Results of posthoc tests between treatments in a) time to first feeding, b) time to food depletion and c) number of unsuccessful attacks.

Table S3 Correlations between length and a) time to first feeding, b) time food depletion, and c) the number of unsuccessful attacks overall and within treatments.

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