Food, Fear and Defense –

Behavioral and morphological adaptations of juvenile perch under the risk of predation

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Martina Heynen

aus Köln

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Berichterstatter: (Gutachter)	PD. Dr. habil. Jost Borcherding Prof. Dr. Hartmut Arndt

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CONTENTS

INTRODUCTION

Predation is one of the major structuring forces in animal communities (e.g. Sih et al. 1985; Eklöv & Hamrin 1989; Kelly et al. 2003; Langerhans et al. 2004; Sharma & Borgstrom 2008; Soykan & Sabo 2009). Because most predators hunt selectively (e.g. Christensen & Persson 1993; Bueno & Motta-Junior 2008; Kishida & Nishimura 2005; Bell & Sih 2007), they influence not only species assemblage but also the distribution and abundance of phenotypes within species (e.g. Kelly et al. 2003; Losos et al. 2006; Bell & Sih 2007). Losos et al. (2006) demonstrate how rapid predator-driven selection may influence prey populations. The introduction of the terrestrial predatory lizard *Leiocephalus carinatus* to small Bahamian islands led to an increase in limb length in the populations of the resident lizard, *Anolis* s*argei*, within only 6 months. Individuals with longer legs were probably better able to evade predation (Losos et al. 2006). However, with a behavioral response to the predator, *Anolis sargei* became increasingly arboreal, which reversed the direction of selection within another 6 months, because shorter limbs are better suited to movment on twigs (Loses et al. 2006). This illustrates the complexity of predator-driven selection in the field, operating on behavioral and morphological traits.

Morphological characteristics of animals are indeed important factors determining the outcome of predator-prey interactions. Physiological and morphological abilities of the predator affect whether it is fast and/or large enough to pursue, capture and ingest a certain prey (e.g. Christensen 1996). On the other hand, morphological characteristics of the prey that facilitate the avoidance of predation will increase the prey´s probability of survival and reproduction (Lind & Cresswell 2005). Correspondingly, different morphological defense strategies, like armor (Vamosi 2002), spines (Kolar & Wahl 1998; Dahl & Peckarsky 2002), camouflage (Tollrian 1995; Ryer et al. 2008) or increased body depth (Nilsson et al. 1995; Kishida & Nishimura 2005) are found throughout many species. For example, the long caudal filaments of mayfly larvae (*Drunella colordensis*) were found to reduce predation rate by fish (Dahl & Peckarsky 2002), and neck teeth on the dorsal surface of the head increases survival of *Daphnia pulex* in the presence of Chaoborus midge larvae (*Chaoborus crystallinus*) (Tollrian 1995). Chaoborus larvae themselves are almost transparent, which reduces their vulnerability to visually oriented predators (Giguère & Northcote 1987). In the freshwater snail *Physa acuta*, thicker and larger shells were found to be an effective morphological defense against predation from crayfish (*Procambarus acutus*) (Auld & Relyea 2011).

In recent years, the fitness consequences of behavioral traits have also received considerable attention, discussed as key in evolutionary diversification of populations (Wyles et al. 1983; Wcislo 1989; Losos et al. 2004), as well as in the light of the evolution of personalities (Dall et al. 2004; Lind & Cresswell 2005; Wolf et al. 2007). Between and within populations, individuals were found to vary in a number of behavioral traits (Hayes & Jenkins 1997; Koolhaas et al. 1999; Sih et al. 2004; Dingemanse et al. 2007). Among vigilance (e.g. Dugatkin 1992; Godin & Davis 1995; Treves 2000) and exploratory behavior (e.g. Yoder et al. 2004), relative differences in boldness between individuals were found to influence an individual’s likelihood to fall victim to predation (e.g. Smith & Blumstein 2008). In a meta-analysis, Smith & Blumstein (2008) found that bold individuals had a higher reproductive success, but also suffered from higher predatory mortality. Correspondingly shyer, less active individual sticklebacks (*Gasterosteus aculeatus*) were found to be less vulnerable to predation (Moodie et al. 1973). Similarly, damselfly larvae (*Enallagma geminatum*) exhibiting less foraging activity had lower mortality rates than more active individuals (Strobbe et al. 2011). Furthermore, bolder, more active individual rock agama (*Agama planiceps*), which had a lower flight initiation distance, were found to suffer more often from tail losses than their shyer conspecifics (Carter et al. 2010). However, traits that reduce predation risk i.e. defense strategies often bear costs confronting the prey with time and/or resource allocation trade-offs (Steiner & Pfeiffer 2007). Behavioral defenses, like watching out for potential threats, inactivity and hiding are largely incompatible with foraging (e.g. Treves 2000; Lind & Cresswell 2005), hence individuals allocating their time towards predator avoidance simultaneously lose foraging opportunities. With respect to morphological adaptations, increased body depth, though effective against gape limited predators (Nilsson et al. 1995), was also found to reduce competitive abilities (Pettersson & Brönmark 1997) and increase swimming costs in crucian carp (Pettersson & Brönmark 1999).

Indeed, behavioral and morphological defenses are often plastic, where expression of a defense trait is induced by sensation of predation risk (e.g. Brönmark & Miner 1992; Eklöv & Jonsson 2007; Robinson et al. 2008). This allows an individual to optimize the energy expenditure to reduce predation risk, because defenses are expressed only when necessary. The potential plasticity of morphological and behavioral traits was documented for many taxa (Agrawal 2001; Price et al. 2003; Crispo 2008). For example tadpoles (*Rana pirica*) were found to increase in body depth and to decrease in activity in the presence of gape-limited predatory salamander larvae (*Hynobius retardatus*) (Kishida & Nishimura 2005; Kishida et al. 2009). Similarly, pumpkinseed sunfish (*Lepomis gibbosus*) were found to increase spine length, body depth, and their behavioral reaction to a predator when previously exposed to the odor of walleyes (*Sander vitreus*) (Robinson et al. 2008), and the presences of green crabs (*Carcinus maenas*) induced adaptive changes in burring behavior and siphon morphology in the soft-shell clam *Mya arenaria* (Whitlow 2010).

Between-population and between-individual differences in plasticity *per se* (Nussey et al. 2007; Briffa et al. 2008; Dingemanse et al. 2010) suggest that phenotypic plasticity might be a trait under selection pressure (Via et al. 1995; Carere et al. 2005; Dingemanse et al. 2010). The mechanisms controlling the expressed range of variation might have an independent basis (Stearns 1989; Via et al. 1995) and theoretical results indicate that among adaptiveness and associated costs of a given phenotype, environmental variability determines whether a trait becomes fixed or remains plastic (Sultan & Spencer 2002). When phenotypic diversity (independent of its origin) represents an adaptive response to a constant selection pressure, traits may evolve and/or become genetically fixed (Scheiner 1993; Sultan & Spencer 2002; Price et al. 2003). The presence or absence of a predator as well as differences in predator assemblages have been shown to lead to trait differences between populations (Pettersson et al. 2001; Langerhans et al. 2004; Kishida & Nishimura 2005; Kishida et al. 2007). In a comparison of five lakes in northern Sweden Magnhagen & Heibo (2004) demonstrated that body depth and dorsal fin ray length in young-of-the-year European perch (*Perca fluviatilis*) were positively correlated with the relative predation risk posed by pike. Investigating nutritional effects in juvenile perch using fish from the two lakes that showed the biggest differences in size-specific predation risk, Borcherding & Magnhagen (2008) observed morphological head and body depth differences between the two populations that persisted even after changes in food availability. However, the authors were not able to state conclusively whether these observations were based on phenotypic plasticity or had a genetic basis.

European perch is an ideal model organism to study multiple plastic defense strategies in prey organisms, because several studies indicate multiple trait plasticity (Eklöv & Jonsson 2007; Olsson et al. 2007; Magnhagen & Borcherding 2008), behavioral (Magnhagen & Bunnefeld 2009) and morphological (Borcherding & Magnhagen 2008) reaction norms and trait mediating indirect effects (Eklöv & Svanbäck 2006) in the response of juvenile perch to predation risk. With respect to the study of Borcherding and Magnhagen (2008), studies in the first chapter concentrated on observed morphological differences between perch of two lakes hypothesizing that their adaptations might have a genetic basis, created by the differences in size-specific predation risk (Magnhagen & Heibo 2004; Magnhagen 2006; Borcherding & Magnhagen 2008).

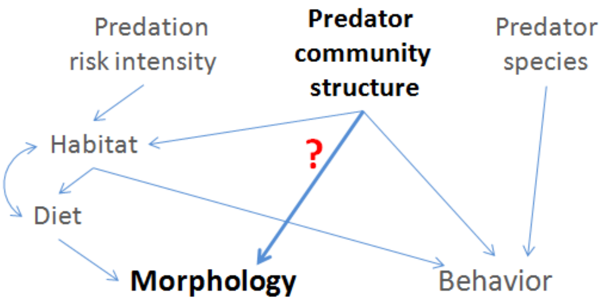

Figure 1: Conceptual illustration of the factors shaping predation risk, influencing direct and/or indirectly morphological and behavioral traits of juvenile perch. Bold type indicates the aspect investigated in the first chapter.

Thus juvenile perch from Lake Fisksjön and Lake Ängersjön were reared in a predator-free common garden setup to study the morphological variations among the pond perch and to compare the results with analyses of young-of-the-year fish that were collected from the wild. Assuming stable population structures and therewith constant differences between both lakes, it could be expected to find the same morphological differences between the wild perch as in previous studies. If these morphological differences are heritable, then they should be maintained under common garden rearing. Such results would indicate that differences in size-specific predation pressure generated by the same predator species create heritable morphological differences.

Predators can also have a variety of indirect effects on prey that could potentially lead to evolutionary responses (Lima 1998). Eklöv & Svanbäck (2006) demonstrated that a change in predation risk in varying habitats caused shifts in behavior of the prey, leading to differences in habitat and resource use, i.e. induced resource polymorphism.

Although behavior has been considered to be even more plastic than morphology (e.g. Price et al. 2003) and has been shown to vary with predation risk (e.g. Christensen & Persson 1993), habitat complexity (e.g. Snickars et al. 2004) or nutrition level (e.g. Borcherding & Magnhagen 2008), relatively few studies have focused on the behavioral consequences of resource polymorphism.

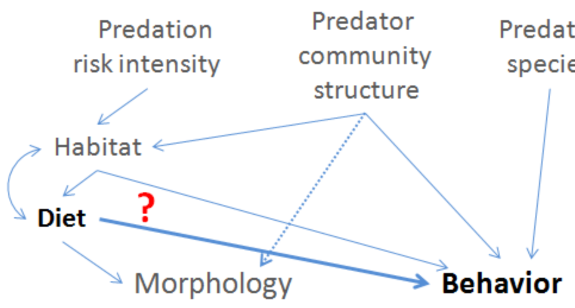

Figure 2: Conceptual illustration of the factors shaping predation risk, influencing direct and/or indirectly morphological and behavioral traits of juvenile perch. Bold type indicates the aspect investigated in the second chapter.

In the second chapter I reared young-of-the-year perch on different prey to investigate whether the utilization of divergent resources changes the reaction to a novel surrounding and the behavior under the threat of predation.

Due to gape size limitations of many piscivores, vulnerability of prey changes with size (Lundvall et al. 1999). The size range during which growing juveniles have the highest predation risk, termed vulnerable size window, is shaped by the structure of the associated predator community (Claessen et al. 2002; Borcherding et al. 2010). Correspondingly, intensity of defense expression over ontogeny should also be influenced by the size structure of the predator community, i.e. be proportional to the actual imposed predation risk. Indeed, boldness of perch from two nearby Swedish lakes differed between lakes and age classes and their behavioral patterns were not consistent but were connected to the actual experience of cannibalistic predation (Magnhagen & Borcherding 2008). The disadvantage of such empirical field studies is, however that numerous factors like different habitat structures (Brown & Warburton 1997; Svanbäck & Eklöv 2002), different hunger levels (Heermann et al. 2007; Borcherding & Magnhagen 2008) or different water transparencies (Skov et al. 2007) may influence the results.

Thus, basic functionality of a certain ecological adaptation should be analyzed additionally with a defined experimental approach, in which only the independent factors of interest are varied and all other possible factors are kept constant.

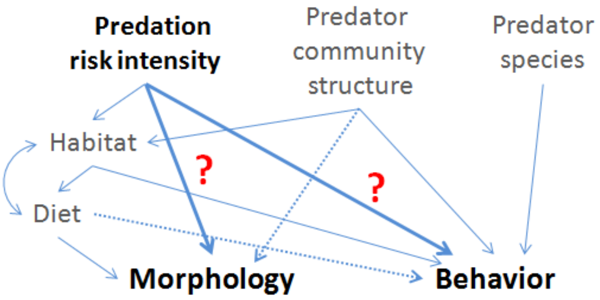

Figure 3: Conceptual illustration of the factors shaping predation risk, influencing direct and/or indirectly morphological and behavioral traits of juvenile perch. Bold type indicates the aspect investigated in the third chapter.

Based on the results outlined by Magnhagen & Borcherding (2008), the third chapter used also 0+ and 1+ perch. Groups of perch were held in mesocosms with and without predatory perch. As these predators were able to feed on the prey perch, the analyses could only use the remaining juvenile perch of the mesocosm experiments. It was hypothesized that these surviving perch would differ in their behavior and their morphology according to the perceived predation risk. With increasing risk of predation it was expected (1) that perch should become shyer in the tradeoff between food and shelter (Magnhagen 2006), and (2) should develop deeper body morphology (Eklöv & Jonsson 2007). In the natural environment a prey typically faces multiple predators (Sih et al. 1998), which might differ in size (Scharf et al. 2000), density (Magnhagen & Heibo 2004), habitat use (Krupa & Sih 1998), diel activity (e.g. Turesson & Bronmark 2004) and/or hunting strategy (e.g. Turesson & Bronmark 2004; Kishida & Nishimura 2005), imposing different predator specific selection forces on the shared prey. However, although previous studies found juvenile perch to respond morphologically (Eklöv & Jonsson 2007) and behaviorally to predation risk (Magnhagen 2006), the relative selection advantages of morphological and behavioral traits with respect to predation have not yet been quantified, or even compared for different predators.

Therefore, behavioral and morphological traits in young-of-the-year perch were measured in the final study, and compared in response to the two most common predators, adult perch and pike.

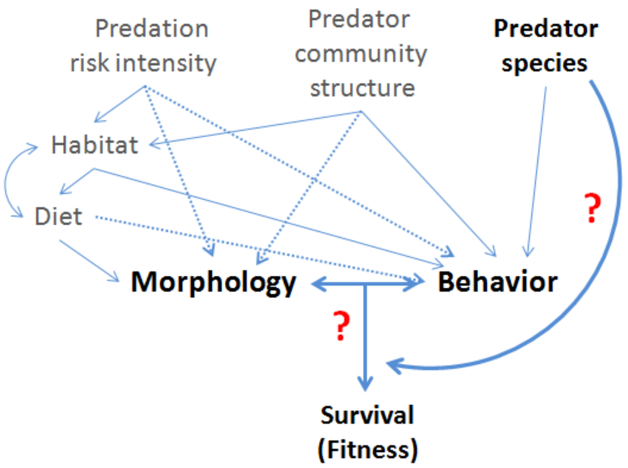

Figure 4: Conceptual illustration of the factors shaping predation risk, influencing direct and/or indirectly morphological and behavioral traits of juvenile perch. Bold type indicates the aspect investigated in the fourth chapter.

Boldness towards a predator and morphological features describing the body shape were measured before groups of juvenile perch faced either a piscivorous pike or perch. To analyze the selective value of the different phenotypic traits, we compared the initial morphological and behavioral characteristics of the juvenile fish that survived with the characteristics of the juvenile fish that were preyed upon. Using mixed effects models and model averaging to analyze our data, we quantified and compared the selectivity of pike and perch predators upon the different morphological and behavioral phenotypes within the population of juvenile perch.

Studies on model organisms like perch provide a promising direction to facilitate the understanding of the complex operating multiple defense trait dynamics in predator-prey interactions. The composition of the studies includes experiments in natural and semi-natural, ponds and mesocosms and in laboratory aquaria environments, balancing the need to simplify and to identify causal effects without losing sight of the “big picture”. Shedding light on the complexity of predator-prey interaction by investigating heritability and direct and indirect effects on plastic response of multiple traits, this study tried to analyze, behavioral and morphological defense traits for juvenile perch and their possible interactions and compensation, in response to different predators.

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CHAPTER I

Does morphological variation between young-of-the-year perch from two Swedish lakes depend on genetic differences?

Martina Heynen[1], Gustav Hellström[2], Carin Magnhagen[2] and Jost Borcherding[1]

[1]Zoological Institute of the University of Cologne, Department of General Ecology & Limnology, Ecological Field Station Grietherbusch, D-50923 Cologne, Germany
[2]Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden

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Abstract

Different local environmental conditions have often been found to generate phenotypic diversity. In the present study we examined morphological differences between young-of-the-year perch from two lake populations with differences in size-specific predation risk. A common garden setup was used to examine the genetic and environmental components of the morphological variation. We found differences in head and jaw length and slight differences in body depth between the wild young-of-the-year perch from Lake Ängersjön and Lake Fisksjön. The differences found between the wild fish from the two lakes were not maintained under common garden rearing. The observed morphological divergence between the wild young-of-the-year perch from Lake Ängersjön and Lake Fisksjön seems to stem mainly from a plastic response to different environmental conditions in the two lakes. It is clear that the morphological traits are not influenced by direct reaction to the size-specific risk of cannibalism, but probably stem from a combination of different environment characteristics, including resource and habitat use, and the density of other piscivores, such as pike.

Introduction

Different local environmental conditions have often been found to generate phenotypic diversity, which may result from either genetic differentiation or phenotypic plasticity (Langerhans et al. 2004; Andersson et al. 2006; Sharpe et al. 2008). When phenotypic diversity (independent of its origin) represents an adaptive response to a constant selection pressure, new traits may evolve and become genetically assimilated (Sultan & Spencer 2002; Price et al. 2003). One important structuring force in freshwater communities is predation (Eklöv & Hamrin 1989; Langerhans et al. 2004; Sharma & Borgström 2008). Most predators hunt selectively (Moodie et al. 1973; Christensen & Persson 1993; Kishida & Nishimura 2005; Bell & Sih 2007), influencing not only species assemblage but also the distribution and abundance of phenotypes within species (Kishida & Nishimura 2005; Bell & Sih 2007). In many taxa, the morphological characters of the prey are important factors determining the outcome of predator-prey interactions (fish: Nilsson et al. 1995; Lundvall et al. 1999; Domenici et al. 2008, amphibian larvae: Kishida & Nishimura 2005, cladocera: Swaffar & O`Brien 1996; Kolar & Wahl 1998). Furthermore, the presence or absence of a predator as well as differences in predator assemblages have been shown to lead to morphological differences between populations (Pettersson et al. 2001; Langerhans et al. 2004; Kishida & Nishimura 2005; Kishida et al. 2007). For example, Crucian carp (*Carassius carassius*) developed a deeper body (Brönmark & Pettersson 1994) in the presence of pike (*Esox lucius*), which is an advantage when confronted with gape-limited piscivores (Nilsson et al. 1995; Domenici et al. 2008). Similarly, Langerhans et al. (2004) found morphological differences between populations of western mosquitofish (*Gambusia affinis*) that experience different levels of predation pressure, where the fish from populations with higher predation pressure exhibited morphological features that facilitate increased acceleration, which in turn increased escape speed and therewith survival (Domenici 2003; Langerhans et al. 2004). However, predators can also have a variety of indirect effects on prey that could potentially lead to evolutionary responses (Lima 1998). Eklöv & Svanbäck (2006) demonstrated that a change in predation risk in varying habitats caused shifts in behaviour of the prey that, in turn, affected the prey’s morphology. Therefore, it is important to disentangle the genetic and environmental contributions and their components when investigating a highly plastic character like morphology. This permits a better understanding of the ongoing mechanisms and operating dynamics that promote the observed diversification (Crispo 2008; Sharpe et al. 2008).

In a comparison of five lakes in northern Sweden, Magnhagen & Heibo (2004) demonstrated that body depth and dorsal fin ray length in young-of-the-year (YOY) perch (*Perca fluviatilis*) were positively correlated with the relative predation risk posed by pike. Investigating nutritional effects in juvenile perch using fish from the two lakes that showed the biggest differences in size-specific predation risk, Borcherding & Magnhagen (2008) observed morphological head and body depth differences between the two populations that persisted even after changes in food availability. These findings raised the question as to whether the observed morphological differences might have a genetic basis, created by the differences in size-specific predation risk (Magnhagen & Heibo 2004; Magnhagen 2006; Borcherding & Magnhagen 2008).

We reared juvenile perch from Lake Fisksjön and Lake Ängersjön in a predator-free common garden setup to study the morphological variations among the pond perch and to compare the results with analyses of YOY fish that were collected from the wild. Assuming stable population structures and therewith constant differences between both lakes, we expected to find the same morphological differences between the wild perch as in previous studies. If these morphological differences are heritable, then they should be maintained under common-garden rearing. Such results would indicate that differences in size-specific predation pressure generated by the same predator species create heritable morphological differences. Additionally we are taking into account the diet of the fish in the different environments, which has earlier been shown to strongly influence morphology (e.g. Hjelm et al. 2001). Thus, we could evaluate to what extent genetic and plastic predator related effects and dietary effects actually influence the morphological patterns observed in the field.

Material and method

The study populations

We used YOY perch originating from Lake Ängersjön and Lake Fisksjön. Both lakes are located near Umeå (63° 47´N; 20°17´E), Sweden. The perch populations in the two lakes were investigated earlier with regards to life history, population structure, behaviour, predation regimes and morphology (Magnhagen & Heibo 2001; Magnhagen & Heibo 2004; Heibo & Magnhagen 2005; Magnhagen 2006; Borcherding & Magnhagen 2008; Magnhagen & Borcherding 2008). The fish species composition is similar in the two lakes and dominated by perch, pike and roach (*Rutilus rutilus*; Magnhagen & Heibo 2001). However, there are some differences in the fish communities that have been consistent over years (Magnhagen & Heibo 2001, Magnhagen & Borcherding 2008, also confirmed by test fishing in 2008, Hellström, G., Heynen, M., Borcherding, J. & Magnhagen, C. in prep.). Lake Fisksjön has a high density of similarly sized, small perch while the perch population in Lake Ängersjön has a more variable size distribution with generally larger perch (Magnhagen & Heibo 2001). In Fisksjön, the risk of cannibalism is, already at a length of 80 mm, only 50% of the risk at the most vulnerable size (Magnhagen & Borcherding 2008). In contrast, in Ängersjön, the decrease in risk to 50% was reached first at 120 mm (Magnhagen & Borcherding 2008). Additionally, pike (Magnhagen & Heibo 2001) and perch (Borcherding, J. & Magnhagen, C. unpubl. data) have larger gapes, compared to the predators of equal length in Lake Fisksjön. This generates a higher direct size-specific predation risk for perch of the analysed size in Lake Fisksjön (Magnhagen & Borcherding 2008), but a longer size-specific predation window for the juvenile fish in Lake Ängersjön (Magnhagen & Borcherding 2008). The two lakes are similar in size and morphology (Lake Ängersjön: 1.45 km² surface area, 0.9 m mean depth, 3.5 m maximum depth, Lake Fisksjön: 0.75 km², 1.9 m, 3.1 m), in productivity (Lake Ängersjön: 21 µg/l total P; Lake Fisksjön: 18 µg/l total P; Magnhagen & Heibo 2004) and in the amount of littoral vegetation (Gustav Hellström, pers. obs.).

Sampling and preparations

To study the morphological variations among the pond perch from the two lakes and to compare them to wild perch, we reared juvenile perch from Lake Fisksjön and Lake Ängersjön in a common garden setup over two consecutive years. During the first two weeks of May, eyed eggs from Lake Ängersjön and Lake Fisksjön were collected at several different locations in the littoral zone of each lake and carefully transported to a pond on the university property outside of the town. The pond (32 x 12 m, depth 1.5m) was divided into two equally sized halves by a fish-proof plastic barrier. On one side we stocked perch eggs from Lake Ängersjön and on the other side those from Lake Fisksjön. Approximately 2500 eggs, in equal numbers from 10 different females per lake were used each year. The vegetation in the pond was dominated by *Carex* spp. and *Potamogeton* spp. (approx 50% cover).

YOY perch from Lake Ängersjön, Lake Fisksjön and from the pond were collected during the first week of September by beach seining (Table 1). In 2008, additional YOY perch were caught for stomach content analyses. Perch were caught using a beach seine between mid-August and mid-September in Lake Fisksjön, Lake Ängersjön and the pond. The stomachs were dissected and weighed full and empty. Stomach contents were identified to the lowest taxonomic level possible, five individuals per prey type were measured and the size and abundance of each prey were estimated. The biomass of the different prey taxa were pooled into two different functional diet categories: pelagic (zooplankton) and benthic (macrozoobenthos and benthic cladocerans) prey. After an arcsin(sqrt) transformation the differences in the consumed proportion of benthic prey between the fish from the pond and the two lakes were analysed with Mann-Whitney U-Tests.

All fish from the study were killed with an overdose of MS222, measured to the nearest 1mm, weighed to the nearest 0.1g and deep frozen for later analyses (Table 1). Furthermore Fulton’s condition factor ($K=10^5 M*TL^{-3}$, where M=weight in g and TL=total length in mm; Bagenal & Tesch 1978) was calculated.

Morphometric analyses

For morphometric analyses, perch were grouped according to origin (Lake Ängersjön wild, Lake Ängersjön pond, Lake Fisksjön wild and Lake Fisksjön pond). Fish were defrosted and placed on a carved piece of Styrofoam to prevent deformation. After fixing the fins with needles, perch were photographed together with a ruler using a digital camera. 18 homologous landmarks (12 defined points to describe the outer shape, two for the pectoral fin, two the mouth and one for the eye and the operculum spine respectively) were digitized on the left side of each specimen using tpsDigit and tpsUtility software from Rohlf (available at: http://life.bio.sunysb.edu/morph/; Stony Brook, NY, USA). All following analyses were performed with Integrated Morphometrics Package (IMP) developed by Sheets (which is available at: http://www2.canisius.edu/~sheets/morphsoft.html; Buffalo, NY, USA). All specimens were transformed to the same baseline orientation and length, using IMP software CoordGen6. Nonshape-variations were removed, using the Procrustes superimposition option of the IMP software. This involved rescaling and rotating the raw configurations to minimize the sum of squared errors (i.e., the offset between corresponding landmarks) between forms (see Webster et al. 2001 and references therein). Shape differences between groups were analyzed with a Canonical Variates Analysis (CVA). Using the software CVAGen6, partial warp scores were computed and a MANOVA (Wilk´s lambda value $p<0.05$) followed by a CVA was conducted. CVAGen6 was also used to visualize the CVAplot and the morphometric distinction along the significant CVA axes as vectors on landmarks.

Table 1: Catch date, origin, hatching place, n per group, condition factor (mean ± SD), weight [g] (mean ± SD) and length [mm] (mean ± SD) for all perch used in the different analyses of the presented study.

Analysis	**Catch date**	**Origin**	**Hatched**	**n**	**Condition factor**	**Weight [g]**	**Length [mm]**
Morphology	04.09.2007	Ängersjön	pond	21	0.90 ± 0.07	2.31 ± 0.38	63.5 ± 3.6
Morphology	12.09.2007	Ängersjön	wild	20	0.88 ± 0.07	1.61 ± 0.27	56.6 ± 3.2
Morphology	04.09.2007	Fisksjön	pond	22	0.92 ± 0.07	2.63 ± 0.62	65.4 ± 5.3
Morphology	12.09.2007	Fisksjön	wild	23	0.88 ± 0.06	1.94 ± 0.38	60.3 ± 3.6
Morphology	10.09.2008	Ängersjön	pond	20	0.96 ± 0.08	1.89 ± 0.73	57.3 ± 6.9
Morphology	03.09.2008	Ängersjön	wild	21	0.94 ± 0.05	1.72 ± 0.35	56.6 ± 3.9
Morphology	10.09.2008	Fisksjön	pond	20	1.06 ± 0.09	1.81 ± 0.69	54.9 ± 5.9
Morphology	03.09.2008	Fisksjön	wild	20	0.95 ± 0.07	1.94 ± 0.38	58.8 ± 3.3
Stomach content	10.09.2008	Ängersjön	pond	23	0.99 ± 0.08	2.22 ± 1.7	57.7 ± 12.2
Stomach content	08.09.2008	Ängersjön	wild	20	0.89 ± 0.06	1.39 ± 0.3	53.6 ± 3.7
Stomach content	10.09.2008	Fisksjön	pond	27	1.05 ± 0.09	1.74 ± 0.69	54.3 ± 6.3
Stomach content	05.08.2008	Fisksjön	wild	16	0.92 ± 0.11	0.94 ± 0.33	46.1 ± 4.36

Statistics

The t-tests to compare the conditional variables and the Mann-Whitney U-Tests to analyse diet differences were performed using SPSS 17 (SPSS, Inc., Chicago, IL, USA). All statistic morphological analyses were performed with Integrated Morphometrics Package (IMP) developed by Sheets (which is available at: http://www2.canisius.edu/~sheets/morphsoft.html; Buffalo, NY, USA).

Results

Morphology

In none of the two years did perch hatched in the pond or in the wild differ in length, weight or condition factor (Table 1; t-Test: length: df=6, p=0.43; weight: df=6, p=0.14; condition factor df=6, p=0.27), nor did perch originating from Lake Ängersjön or Lake Fisksjön differ in length, weight or condition factor (t-Test: length: df=6, p=0.64; weight: df=6, p=0.45; condition factor: df=6, p=0.51). Thus, the following observed morphological analysis is not biased by any conditional differences between the groups.

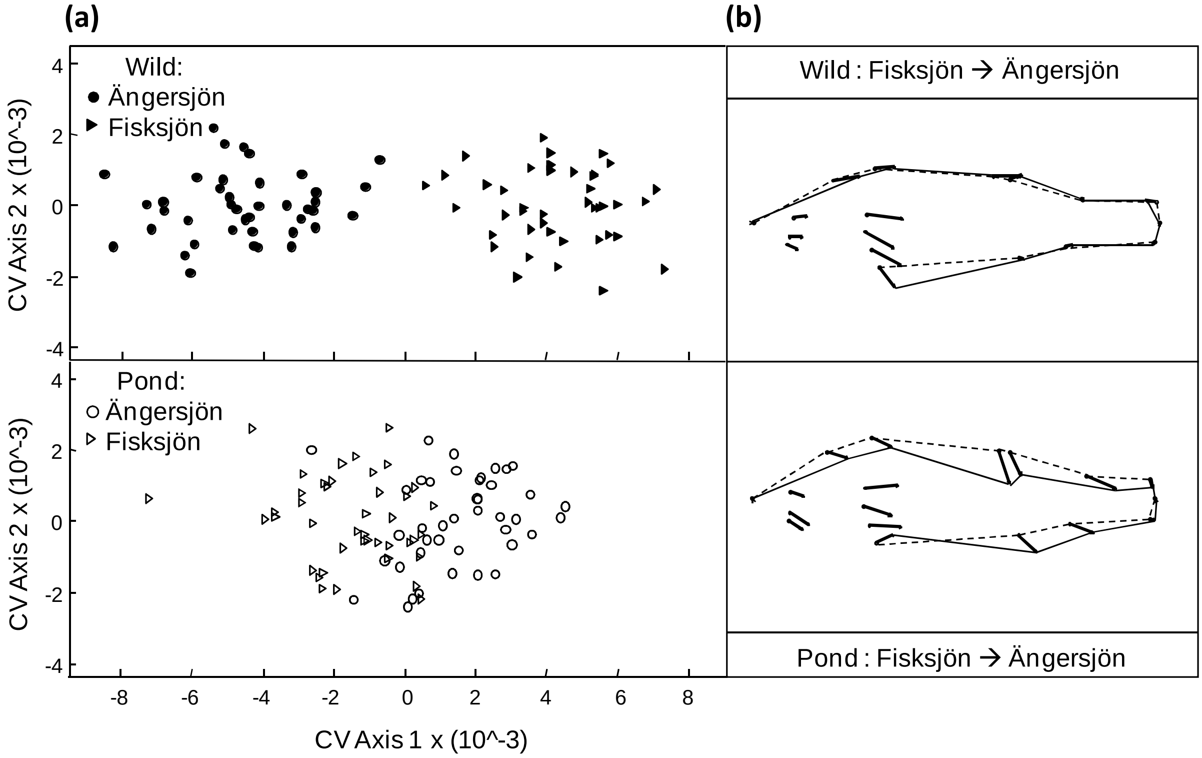

Figure 1: a) Canonical variate scores of perch hatched in the pond (white) or the wild (black) in 2007 and 2008, originating from Lake Fisksjön (triangles) or Lake Ängersjön (circles), depicted along the first (significant for Wild, n.s. for Pond) and the second (n.s.) canonical variate axes. b) Shape difference correlated with the first canonical variate axis of perch from the pond and the wild originating from Lake Ängersjön or Lake Fisksjön. The shape changes were obtained by regressing the shape on the CVA scores, depicted as growth vectors starting from the perch originating from Lake Fisksjön (dotted line) to the perch originating from Lake Ängersjön (solid line).

The CVA analyses found one significant axis discriminating the perch hatched in Lake Ängersjön from those hatched in Lake Fisksjön (Fig.1, Wild, $\lambda=0.13$, $\chi^2=135.5$, df=32, $p<0.001$, eigenvalue=6.8). The wild perch from Lake Ängersjön developed a relatively longer head and gape and a slightly deeper ventral body than the YOY perch caught in Lake Fisksjön. However, we did not find any significant differences between the pond-reared perch originating from Lake Fisksjön and Lake Ängersjön (Fig.1, pond; CVA not significant, eigenvalue=0.958). Comparing the wild and pond perch from Lake Ängersjön or Lake Fisksjön, always revealed one significant CV axis ($p<0.0001$).

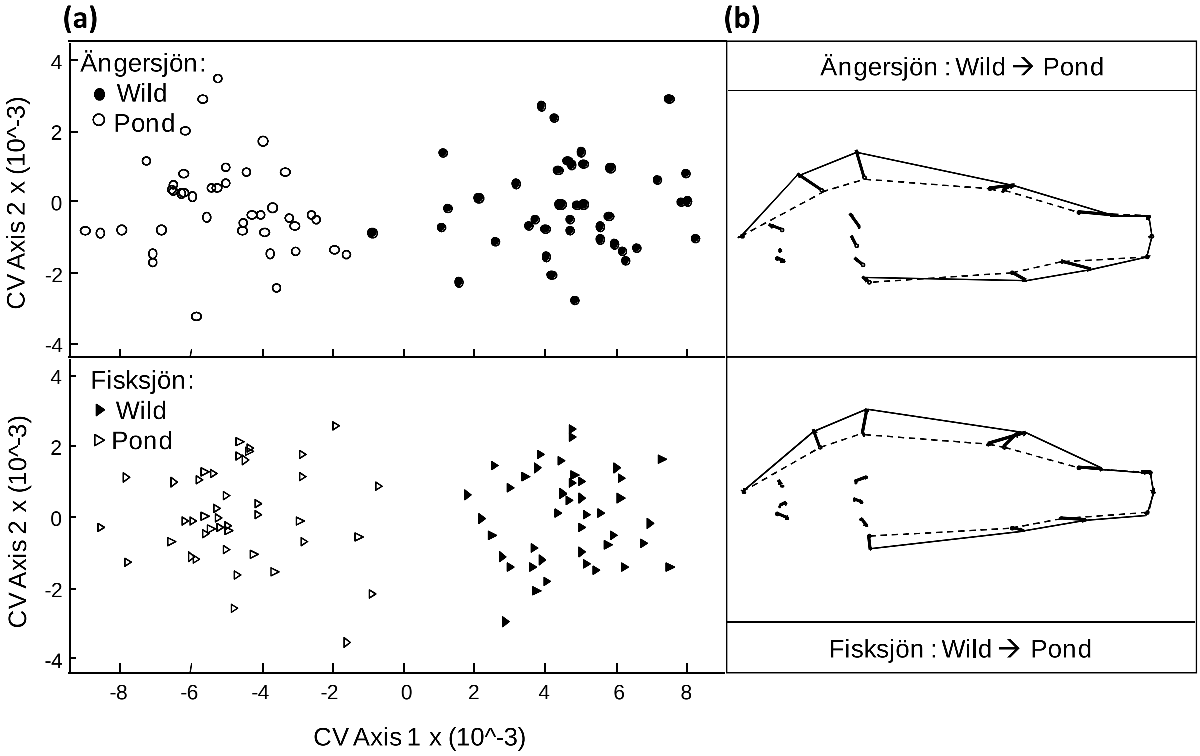

Figure 2: a) Canonical variate scores of perch from Lake Fisksjön (triangles) and Lake Ängersjön (circles) hatched in the pond (white) or in the wild (black) in 2007 and 2008, depicted along the first (significant) and the second (n.s.) canonical variate axes. b) Shape difference correlated with the first canonical variate axis of perch from each population (Lake Ängersjön and Lake Fisksjön) that hatched in the pond or the wild. The shape differences were obtained by regressing the shape on the CVA scores, depicted as growth vectors starting from the perch hatched in the wild (dotted line) to the perch hatched in the pond (solid line).

The pond-perch originating from Lake Ängersjön developed an overall deeper body and a relatively shorter head than their conspecifics from the wild (Fig.2, Ängersjön; CVA: λ=0.13, χ^2=130.01, df=32, p<0.001, eigenvalue=6.6). We found similar differences between the pond and wild perch from Lake Fisksjön (Fig.2, Fisksjön; CVA: λ=0.1, χ^2=152.5, df=32, p<0.001, eigenvalue=8.7). The pond perch originating from Lake Fisksjön were also deeper bodied, but had a relatively longer head than their wild conspecifics.

Diet

The pond fish that were used for stomach content analyses were of similar size and condition as the pond perch used for the morphological analyses. Unfortunately, as the original stomach samples for the wild fish were accidentally destroyed, we used samples from slightly different dates (Table 1). Thus, the wild perch used for the stomach content analyses were smaller than their conspecifics used for the morphometric analyses (Table 1). The stomach content analyses showed clear differences between the diet in the pond and the lakes.

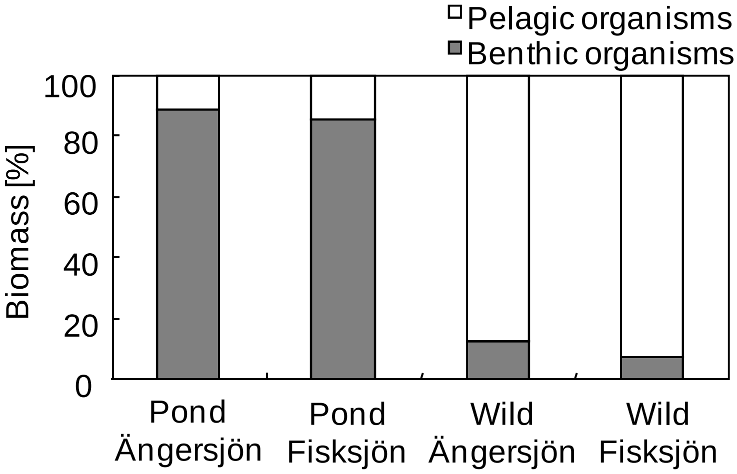

Figure 3: Stomach content (% biomass, bars) of perch hatched in the wild or the pond, originating from Lake Fisksjön and Lake Ängersjön, caught between mid-August and mid-September 2008.

The perch hatched in the pond fed nearly exclusively on benthic prey, mainly insect larvae and benthic cladocerans, while the wild perch primarily consumed zooplankton (proportion of benthic prey: Mann-Whitney U-test, z = 6.78, p<0.001) (Fig.3). There were no significant differences in diet utilisation within the two habitats, as the wild fish from the two lakes (proportion of benthic prey: Mann-Whitney U-test, $z = 1.32$, $p=0.19$), as well as the pond fish originating from lake Ängersjön and lake Fisksjön (proportion of benthic prey: Mann-Whitney U-Test, $z = 1.06$, $p=0.29$) consumed equal proportions of benthic prey, respectively.

Discussion

The results of the present study indicate that the morphological differences of juvenile perch under different predator regimes are primarily driven by phenotypic plasticity. As expected, the morphological differences between the wild perch are in accordance with the results from previous studies (Borcherding & Magnhagen 2008), but the morphological differences were not maintained under common garden rearing.

The most striking morphological differences between the wild YOY perch from the two lakes occurred in the anterior part of the body, with the perch from Lake Ängersjön having a relatively larger head and gape than the fish from Lake Fisksjön. Wild YOY and 1+ perch studied in 2006 (Borcherding & Magnhagen 2008) showed the same morphological head differences between the juvenile perch from the two populations, indicating relatively stable differences between the two lakes. However, the morphological differences we found between the wild YOY perch from the two populations were not maintained under common hatching and rearing conditions. Direct comparison of the wild and pond perch from the two lakes seems to further confirm phenotypic plasticity as the major source of the differences in head morphology. While the pond perch from Lake Fisksjön developed a larger head and gape than their wild conspecifics, the pond perch from Lake Ängersjön had a smaller head and gape than their conspecifics caught in the wild.

Previous studies showed that differences in morphology of the head in fish are often related to differences in diet (Heermann et al. 2007; Heynen, M. & Borcherding, J. unpubl. data) and/or status of nourishment (Borcherding & Magnhagen 2008). For example, under-nourishment and/or a planktivorous diet have been shown to induce phenotypes with relatively large heads and gapes (Borcherding & Magnhagen 2008; Heynen, M. & Borcherding, J. unpubl. data). We did not find any differences in diet between the wild perch that would explain the difference in head morphology, nor were there any differences in length, weight or condition factor between the analysed groups of wild or pond perch in our study. However, we can not rule out the possibility that the size differences between the group used for morphometric analysis and the group used for stomach content analysis influenced the results, e.g. that fish of different size consumed different prey.

Direct comparison between the wild and pond perch from the two populations also revealed plastic effects associated with rearing environment (pond vs. lake). Most obviously the pond-reared perch had a deeper body than the wild-caught perch, independent of origin. These differences were probably driven by resource and structural differences between the shallow pond with its mainly littoral habitats and the pelagic-dominated lakes. Correspondingly the diet of the pond perch was dominated by benthic prey, while the perch caught in the lakes mainly consumed plankton. In many field studies and experiments juvenile perch were shown to exhibit a trophic polymorphism with a more slender pelagic form feeding mainly on zooplankton and a deeper-bodied littoral form feeding mainly on macroinvertebrates (Hjelm et al. 2001; Eklöv & Svanbäck 2006). However, in aquaria it was shown that perch also react morphologically to predator presence in that individuals that were exposed to a pike predator became more deep-bodied than control fish held in predator absence (Eklöv & Jonsson 2007). The pond and lakes in our study differ in their predation intensity, with the pond being free from predators, but producing the deeper-bodied fish. These findings indicate that the plastic morphological response to different resources is much stronger than the response to predator cues and even seems to overrule potential predator-induced responses, supporting the results of Eklöv & Svanbäck (2006). They demonstrated that differences in predation risk in different habitats may cause shifts in behaviour of the prey which, in turn, affect the prey’s morphology. The authors suggested that differences in body depth between habitats with different predation risks do not primarily stem from a direct reaction to predator cues, but more likely reflect an indirect effect of habitat and resource shifts (Eklöv & Svanbäck 2006). In the present study we found some differences in body depth between the wild YOY perch, but not between the pond perch from the two populations. In accordance, Magnhagen & Heibo (2004) found that body depth and dorsal fin ray length in YOY perch were positively correlated with the relative predation risk from pike. Similar body depth patterns were also found in 2006, when perch also slightly differed in their resource use, with wild perch from Lake Ängersjön having a more benthic diet than the YOY perch from Lake Fisksjön (Borcherding, J. and Magnhagen, C. unpubl. data). Although body depth differences of YOY perch seem to be constant between the two lakes, our results indicate that they do not have a heritable basis, but seem to stem mainly from a plastic response to different environmental conditions in the two lakes. It is clear that the morphological traits are not influenced by direct reaction to the risk of cannibalism, but probably stem from a combination of different environment characteristics, including resource and habitat use, and the density of other piscivores, such as pike.

In conclusion, the results of the present study indicate that the morphological differences between the YOY perch from two lakes with different size-specific predation risk are primarily attributed to phenotypic plasticity. Furthermore, our results indicate that resource utilization seems to contribute to morphological development to a greater extent than direct response to predation risk.

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CHAPTER II

Does the consumption of divergent resources influence risk taking behaviour in juvenile perch (*Perca fluviatilis* L.)?

Martina Heynen, Lisa Heermann and Jost Borcherding

Zoological Institute of the University of Cologne, Department of General Ecology & Limnology,
Ecological Field Station Grietherbusch, D-50923 Cologne, Germany

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Letter

The exploitation of different habitats and resources by members of the same population is called resource or trophic polymorphism and seem to be especially common in fishes (e.g. Ruzzante et al. 2003). Although behaviour has been considered to be even more plastic than morphology (e.g. Price et al. 2003) and has been shown to vary with predation risk (e.g. Christensen & Persson 1993), habitat complexity (e.g. Snickars et al. 2004) or nutrition level (e.g. Borcherding & Magnhagen 2008), relatively few studies focused on the behavioural consequences of resource polymorphism. In this study, we reared young-of-the-year (YOY) perch on different prey to investigate whether the utilisation of divergent resources changes the reaction to a novel surrounding and the behaviour under the threat of predation.

We expected that the perch trained on either fish larvae or zooplankton will react differently in the behavioural experiments with or without predation risk and that their reaction will additionally be influenced by the presence of their familiar or a novel prey.

In June 2006, 104 YOY perch (average size 44.4 ± 1.6 mm total length, T_L) were caught with a beach seine net in Lake Speldrop (51°46´N, 6°22´E), Germany (Beeck et al. 2002) and stocked into four tanks lacking any internal structure ($2 \times 1{,}8$ m^2, $2 \times 1{,}1$ m^2, density: 18 perch/m^2). The perch in one small and one large tank were fed *ad libidum* with living cyprinid larvae (9 – 21 mm T_L), while the remaining tanks were fed with the same weight (later same percentage of fish body weight) of living zooplankton (mainly daphnids) each day, for 6 weeks. The T_L of each perch was measured to the nearest 1 mm after 3 weeks and at the end of the experiment.

Growth was calculated as thermal-unit growth coefficient (TGC) (Iwama & Tautz 1981): $TGC=1000(W_t^{1/3}-W_i^{1/3})(T\times\Delta t)^{-1}$, were W_t = weight at time t (g), W_i = initial weight (g), T = water temperature (°C) and Δt = duration of the experiment ($T\times \Delta t$ is the thermal sum in degree-days). Fulton’s condition factor (K; Bagenal & Tesch 1978) was calculated for each experimental group as indicator for the perch’s nutritional status: $K = 10^5 \, W \, T_L^{-3}$ (W in g and T_L in mm).

After 6 weeks, the planktivorous (T_L mean ± SD; 54.9 ± 4.3 mm) and piscivorous (T_L mean ± SD; 93.1 ± 6.2 mm) perch from the tanks were transferred into 20-l (23.5 × 20 × 45 cm) and 40-l aquaria (33 × 30 × 45 cm) (T 21 ± 1 °C, 9h L: 15h D). To shorten the time of irritated behaviour after handling, all fish were accustomed to be moved to another aquarium every day during the next week. Perch were fed with zooplankton and fish according to the pre-treatment in the tanks, but starved at least 12 h prior the start of a trial.

The behavioural trials were carried out in Y-shaped aquaria as described by Borcherding (2006). One arm of the aquarium contained artificial vegetation, food was presented in the second arm (chosen randomly for every trial), and the predator (pike, *Esox lucius*, 27 ± 2 cm) was placed in the third arm, excluded from the experimental arena through a transparent perforated Perspex pane.

The experiments were conducted as repeated measurements. The behaviour of a group of four perch was assayed in the absence of a predator. Following the same procedures, the behaviour of the group was re-assayed the subsequent day (approximately same time) in the presence of a predator. To evade pike odours in the predator free runs, two identical Y-shaped aquaria (plus equipment) were used, never mixing equipment or water from predator and predator-free-Y-tank. Each group, planktivorous and piscivorous perch was tested with each food, living fish larvae and living zooplankton (presented in a transparent perforated box). Every combination was tested with and without predator and had six replicates.

At the start of a trial, four perch were introduced to the vestibule (triangle where the three arms of the Y meet). To diminish disturbance, all further measures were handled from outside the room. After an orientation time of 10 min. the barrier that enclosed the perch to the vestibule was lifted. The trial ended after another 30 min, by putting the barrier back in place.

Every behavioural experiment was recorded with a video camera. The videos were analysed with a computer program, which recorded one behavioural unit every second (Borcherding & Magnhagen 2008). Received data were used to calculate the time perch spent in the vegetation and open water compartment. Activity was calculated as the number of changes between the experimental areas (vegetation, open water and vestibule) per time (30 min). Predator inspection was defined as a fish directly approaching the predator and dwelling in a right angle within two body length to the predator compartment. Data were recorded and calculated for the whole group and then expressed as average-behaviour per perch.

Behavioural changes because of predator presence were analysed with a repeated measurement ANOVA (predator as factor of the repeated design) with “time in vegetation", "activity" or "predator inspection" as dependent factors and presented food and pretreatment as independent factors. All statistics were conducted with SPSS (15.0).

The divergent pretreatments in this study induced crucial phenotypic changes in juvenile perch. The perch receiving zooplankton (35% body weight per day) grew steadily over the hole pretreatment period (planktivores: $\text{TGC}_{\text{week1-3}} = 0.30 \pm 0.04$; $\text{TGC}_{\text{week3-6}} = 0.43 \pm 0.07$), while the fish fed with fish larvae (23% body weight per day) grew predominantly during the last 3 weeks, where their TGC value increased nearly fourfold (piscivores: $\text{TGC}_{\text{week1-3}} = 1.52 \pm 0.26$, $\text{TGC}_{\text{week3-6}} = 5.19 \pm 0.92$). A diet of fish supplies much more energy per consumed gram than a diet of zooplankton (cf. Borcherding et al. 2007), thus at the end of the dietary pretreatment in the tanks piscivorous (mean ± SD; $T_L = 101.0 \pm 6.0$ mm; weight = 12.7 ± 2.4 g) and planktivorous perch (mean ± SD; $T_L = 60.5 \pm 4.0$ mm; weight = 2.2 ± 0.5 g) differed in length and weight with planktivores being smaller (t-test, d.f. = 97, $p < 0.01$) and lighter (t-test, d.f. = 97, $p < 0.001$). Additionally, piscivorous perch ended up with a slightly higher condition factor than planktivorous perch (t-test, d.f. = 97, $p < 0.001$; condition factor: piscivores = 1.22 ± 0.07, planktivores = 0.97 ± 0.06).

Beside the physiological divergence, the planktivorous and piscivorous YOY perch in this study showed clear behavioural differences, where dietary pretreatment, predator presence and the presented prey species in the trials affected the behaviour of the YOY perch (Table 1). In absence of a predator, planktivores displayed an overall high activity, while piscivores stayed primarily in the vegetation.

Table 1: Results of the analyses of variance testing the effects of predator presence, pre-treatment and presented food on the variables time in vegetation, activity and predator inspection.

	Predator Inspection			**Activity**			**Time in Vegetation**		
	d.f.	F	>P	d.f.	F	>P	d.f.	F	>P
Food	1	1.43	0.25	*1*	*7.04*	*0.02*	1	0.28	0.60
Predator	1	5.73	0.03	1	0.77	0.39	1	3.87	0.06
PreTreat	1	2.13	0.17	1	5.77	0.03	1	0.51	0.49
Food x Predator	1	5.06	0.04	1	0.63	0.44	1	0.11	0.74
Food x PreTreat	*1*	*11.89*	*0.001*	1	0.04	0.84	1	2.11	0.16
Predator x PreTreat	1	2.96	0.11	*1*	*17.73*	*0.001*	*1*	*10.38*	*0.001*
Food x Predator x PreTreat	1	5.57	0.03	*1*	*7.29*	*0.01*	1	1.83	0.19

Previous experiments showed that suboptimal nourished fish behave differently in the trade-off between the need to forage and the seek for shelter and were observed to intensify activity and their search for food (e.g Olsson et al. 2007).

As planktivorous perch had a slightly lower condition factor than piscivorous perch, the different activity patterns of the two groups documented when perch were introduced to the novel aquaria for the first time might be influenced by nutritional effects.

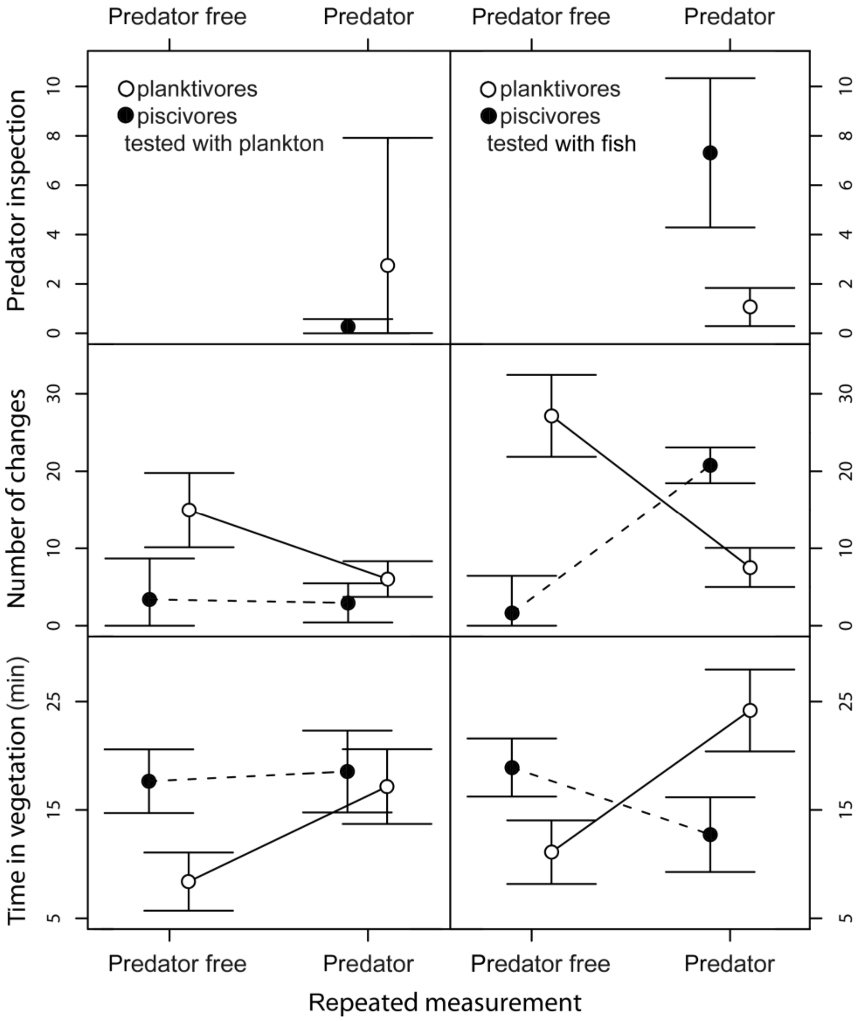

Fig. 1: Behaviour of piscivorous (black circles) and planktivorous (white circles) perch, in the absence and presence of a predator (pike), when the presented prey during the trials was plankton (left plots) or fish (right plots). Behavioural patterns were defined as predator inspection (number of times a perch started predator inspection, mean ± SD), activity (number of changes between compartments during a 30 min trial; mean ± SD) and time in vegetation (min a perch spend in the vegetated habitat; mean ± SD).

When in the aquaria for the second time and in the presence of a predator, planktivorous perch decreased their activity, as was shown in many other studies on young perch where predator avoidance caused decreasing activity levels (e.g. Bean & Winfield 1995). The piscivorous perch, however, increased their activity in the trials with a predator, but only with fish larvae as presented prey (Table 1, Fig. 1). Correspondingly, the behaviour defined as predator inspection of piscivores almost exclusively occurred in the trials with predator and fish larvae as attractor (Fig. 1). Planktivorous perch in this study also inspected the predator compartment, but mostly in the trials with plankton as presented prey species, although less frequently than piscivores (Table 1, Fig. 1). Prey size as well as prey condition was shown to promote predator inspection (Kulling & Milinski 1992). This indicates that the physiological condition, the previous experience (fish might feel safer, when in the aquaria for the second time) as well as the presence of their familiar resource might influence the divergent behaviour of the pretreated fish.

Although not significant, these tendencies are also visible in habitat use. In the presence of a predator both groups spent on average less time in the vegetated habitat, when their familiar resource was presented. Especially under predation hazard, fish are generally known to carefully adjust their behaviour, trying to maximize the risk/reward relationship, where the attractiveness of a presented food ration might influence the willingness to take risks (Skalski & Gilliam 2002). Fish have been shown to be more effective in consuming a familiar resource (Hughes et al. 1992) and need to learn to utilise a novel food type efficiently (Magnhagen & Staffan 2003). This suggests that fish in this study react primarily to the familiar prey, because they can consume it quickly and effectivly, whereas utilising a novel prey under predation hazard might be considered as too risky. However, in predator absence, fish did not positively react to their familiar prey, indicating that the predator presence triggers the affinity/reaction to the familiar resource.

To conclude, this study showed that the consumption of different resources lead to specific physiological and behavioural changes in juvenile perch, suggesting that resource polymorphism might induce complex behavioural consequences, leading to phenotypic differences in the behaviour during predator encounter. Under natural conditions, these divergences might be mediated or even promoted by additional factors influencing the individual reaction like e.g. phenotypic ability, maternal effects or earlier experiences.

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CHAPTER III

Experienced predation risk affects behavior and morphology of juvenile perch

Martina Heynen, Ingo Rentrop and Jost Borcherding

Zoological Institute of the University of Cologne, Department of General Ecology & Limnology,
Ecological Field Station Grietherbusch, D-50923 Cologne, Germany

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Abstract

Empirical field studies have shown that boldness of perch differed between lakes and age. The present study used juvenile perch in an experimental approach to vary the factor predation risk. Predators were able to feed on perch during a mesocosm period. Perceived predation risk affected the behavior and the morphology of both age classes of perch. Boldness decreased with the intensity of predation, while morphology of perch changed towards deeper bodied individuals. Analysis of the length-frequency distributions from the start and the end of the mesocosm period indicated some size selective effects of the predator. Although it remains unanswered if the predator induced these changes by selective predation, or if perch adjusted their behavior and morphology in response to the predator. The latter explanation is assumed to be conclusive because there was no correlation between the changes in the length-frequency distributions and the predation risk of perch.

Introduction

Phenotypic plasticity seems to play an important role in the ontogenetic success of animals (Ancel 2000; Robinson et al. 2008; Minderman et al. 2009) and is assumed to be a driving force for allopatric speciation (Stauffer & Gray 2004; Ostbye et al. 2005; Whiteley 2007). Those animals with the ability to adjust their behavior and morphology to the environment are assumed to have a higher chance at contributing their alleles to the next generation (Gabriel et al. 2005). Whether this ability is a result of plasticity or has an underlying genetic basis, or both, is widely discussed in literature (Mittelbach et al. 1999; Marcil et al. 2006; Leimar 2009). It was found that morphological differences across rainbow trout (*Oncorhynchus mykiss*) populations have a genetic basis (Keeley et al. 2007), while a common garden experiment on European perch (*Perca fluviatilis*) revealed that differences in perch morphology could be attributed to phenotypic plasticity only (Heynen et al. 2010).

Morphological adaptations can play an important role in ecological performance of an organism (Langkau 2008). Recent studies showed that the habitat choice of perch and the accompanied adjustment to swimming modes and food resources varied the morphology from streamlined to a deeper one (Eklöv & Svanbäck 2006). Deeper bodied morphs also developed in response to a predator: when the predator pike (*Esox lucius*) inhabited experimental ponds, crucian carp (*Carassius carassius*) developed a deeper body morphology compared to those from ponds without predators (Brönmark & Miner 1992). However, body morphology may be also influenced through feeding strategies: in a mesocosm experiment perch that were fed with fish became much deeper bodied than their plankton fed streamlined conspecifics (Heermann et al. 2007).

Besides phenotypic plasticity with respect to morphology, effects contributing to the behavior of fish are well studied, again depending on factors like habitat structure, predation risk, food type and food availability. A survey on rainbowfish (*Melanotaenia eachamensis*) revealed that the habitat structure in three fresh water environments had significant influence on anti-predator responses (Brown & Warburton 1997). A further study showed that perch in different states of nourishment developed different degrees of boldness concerning foraging strategies. Undernourished individuals spent more time in a risky area than did their well fed conspecifics (Borcherding & Magnhagen 2008). Anti-predator behavior is also correlated to the individual body length of the prey (Krause et al.

1998a). A study on the shoaling behavior of juvenile roach (*Rutilus rutilus*) revealed that the body length and nutritional state are factors influencing the positioning of roach at risk prone positions in the shoal (Krause et al. 1998b). Further, individual experience can influence behavior of animals (Galef & Laland 2005). Young-of-the-year (0+) perch from a lake with relatively low cannibalistic predation risk on this age class were bold compared to those from other lakes with a higher predation risk on 0+ perch (Magnhagen 2006). Boldness of perch from two of the aforementioned lakes differed between lakes and age classes and their behavioral patterns were not consistent but were connected to the actual experience of cannibalistic predation (Magnhagen & Borcherding 2008). The results of this empirical field study clearly indicated a connection between the boldness and the calculated predation risk in the two lakes for 0+ and 1+ perch. The disadvantage of such empirical field studies is, however that numerous factors like different habitat structures (Brown & Warburton 1997; Svanbäck & Eklöv 2002), different hunger levels (Heermann et al. 2007; Borcherding & Magnhagen 2008) or different water transparencies (Skov et al. 2007) may influence the results. Thus, basic functionality of a certain ecological adaptation should be analyzed additionally with a defined experimental approach, in which only the independent factors of interest are varied and all other possible factors are kept constant.

Based on the results outlined by Magnhagen & Borcherding (2008), the present study used also 0+ and 1+ perch. Groups of perch were held in mesocosms with and without predatory perch. As these predators were able to feed on the prey perch, the analyses could only use the remaining juvenile perch of the mesocosm experiments. It was hypothesized that these surviving perch would differ in their behavior and their morphology according to the perceived predation risk. With increasing risk of predation we expected (1) that perch should become shyer in the tradeoff between food and shelter (Magnhagen 2006), and (2) should develop a deeper body morphology (Eklöv & Jonsson 2007). Finally we tried to give a initial estimate of the extent that selective predation could influence not only the size distribution of the experimental populations, but the extent that such prey selection might affect observed differences in behavior and morphology.

Methods

Field samples

For all mesocosm treatments about 500 young-of-the-year (0+) perch (size: up to 88 mm L_T) and 250 perch of age 1 (1+, size: 100 – 135 mm L_T) were necessary. Experimental 0+ and 1+ perch were caught in Lake Speldrop (latitude: 51°46´50.70’’ N, longitude: 6°22´42.11’’ E), a gravel pit lake at the Lower River Rhine (Beeck et al. 2002), Germany, using either a Bretschneider direct current device for electrofishing, a beach seine net (mesh size: 4 mm, length: 10 m), a dipnet (size: 1m x 1m, mesh size: 4 mm) or by angling. Until the start of treatments the fish were held in two large mesocosms (2 × 1.17 m^3). Predatory perch were caught by gillnet fishing in Lake Speldrop and in Lake Reeser Meer (latitude: 51°45´01.03’’ N, longitude: 6°27´27.37’’ E; Borcherding et al. 2007). The exposure time for the nets never exceeded two hours to ensure high survival rates for the fishes. Until the introduction of the predators to the treatments the fishes were held in two mesocosms (2 × 0.7 m^3).

Mesocosm treatments

All treatments were set up in round mesocosms (diameter of 1.8 m, content approximately 1.65 m^3) filled with water from the nearby Lake Reeser Meer. The bottom of the mesocosms was covered with gravel, to ensure a closer natural environment and to complicate the search for food for the prey fish. A filter with a recirculation pump was placed into the center of every mesocosm to ensure cleaning and the supply of oxygen (at least always > 8 mg l^{-1}). The temperature in the mesocosms never exceeded 19.6 °C (mean 16.0 °C) and the conductivity ranged between 534 and 758 µS cm^{-1}. The mesocosms were equally divided into an open water zone and an artificial vegetation zone with no physical barrier between them. The artificial vegetation was made with green PVC strips (1.5–2 cm wide, 248 strips m^{-2}) attached to a wire-net which was then placed on top of the mesocosms.

To generate different levels of predation pressure in the mesocosms, prey perch were stocked with either a small predatory perch (i.e., a predator near the size limit to prey on the stocked fish, cf. Persson et al. 2004), a large predatory perch (i.e., a predator that certainly could prey on all perch), or without a predator. Each treatment on both age classes was replicated three times, making a total of 18 mesocosms (Tab. 1). The predators were introduced four weeks before treatment start in order to become well accustomed to the conditions in the mesocosms and were not fed in that time so they reached a high hunger level. As differences were expected for the threat the individual predators exert to the prey, the actual experienced intensity of predation was calculated for each mesocosm as the number of consumed prey fish divided by the number of stocked fish, divided by the number of days in treatment. This real experienced daily predation was then classified (no predator, low risk < 0.006, high risk; Tab. 1), and used in the following analyses as the independent variable “predation risk”.

Before adding prey perch to the mesocosms, size of all individuals was measured to the nearest mm (total length, L_T). During the mesocosm period, prey perch were fed daily with deep frozen red chironomid larvae. Perch biomass was calculated via length distribution and an amount of 15 % of perch wet weight was administered daily in three to six randomly distributed daytime feeding instances to ensure the perch did not become accustomed to feeding times or instances. The amount of food was not adjusted during treatments, because it was not possible to determine the number of remaining prey perch before the end of the treatments. During mesocosm treatments mortality of prey fish (i.e., observed dead fish, thus not eaten by the predator) was low (3.9 %), and only occurred during the first ten days of the treatment. Dead prey fish were removed from mesocosms.

Because it was not possible to work on all fish from the 18 mesocosms in the behavior experiments in parallel and to reduce time and sequence effects to a minimum, the mesocosms have been pseudo randomly distributed in a block design that each of the six different treatments was represented in one of the three blocks: perch in block 1 (mesocosms 1–6) stayed for 42 days in treatment, those in block 2 (mesocosms 7–12) for 48 days and those in block 3 (mesocosms 13–18) for 54 days (Tab. 1). It was considered that after an experimental period of up to 54 days at least twelve prey individuals should remain in each mesocosm, to form three groups of four perch for the behavioral experiments. This was achieved for all but one mesocosm with only ten surviving juvenile perch, resulting in two groups of four perch for the behavioral experiments and 10 instead of 12 individuals for the morphological analyses (Tab. 1).

Table 1: Scheme of mesocosm stocking with sizes of prey perch and predators, numbers of prey perch at the start and the end of the mesocosm treatment (duration), and mean values of condition, growth and daily predation on the prey perch. The latter values were used to obtain predation risk classes (-=no predator, +=low risk < 0.006, ++=high risk)

Age class	**Prey L_T±*SD* [mm]**	**Treatment**	**Predator L_T [mm]**	**Prey to predator ratio**	**N_{start}**	**Duration [days]**	**N_{end}**	**Condition factor**	**Growth [mm day^{-1}]**	**Daily predation**	**Predation risk class**
0+	75.8 ± 7.0	no pred			30	42	30	0.95	0.14	0.000	-
0+	75.8 ± 7.1	no pred			29	48	29	1.04	0.20	0.000	-
0+	75.1 ± 6.8	no pred			30	54	30	0.99	0.17	0.000	-
0+	80.8 ± 6.4	small pred	198	0,41	39	42	17	1.11	0.17	0.013	++
0+	77.2 ± 7.4	small pred	185	0,42	50	48	43	1.06	0.17	0.003	+
0+	73.0 ± 8.0	small pred	198	0,37	50	54	36	1.02	0.22	0.005	+
0+	78.4 ± 7.3	large pred	250	0,31	70	42	58	1.07	0.18	0.004	+
0+	77.8 ± 7.6	large pred	247	0,31	70	48	59	1.06	0.22	0.003	+
0+	77.1 ± 8.1	large pred	242	0,32	68	54	31	1.05	0.26	0.010	++
1+	113.9 ± 10.2	no pred			16	42	16	1.10	0.19	0.000	-
1+	123.6 ± 7.6	no pred			14	48	14	1.17	0.16	0.000	-
1+	116.5 ± 9.7	no pred			17	54	17	1.14	0.19	0.000	-
1+	114.6 ± 8.4	small pred	205	0,56	15	42	10	1.12	0.21	0.008	++
1+	115.6 ± 11.0	small pred	200	0,58	17	48	15	1.15	0.24	0.002	+
1+	120.0 ± 10.0	small pred	215	0,56	17	54	17	1.14	0.17	0.000	+
1+	112.7 ± 8.7	large pred	360	0,31	37	42	24	1.10	0.22	0.008	++
1+	115.6 ± 10.0	large pred	345	0,33	32	48	15	1.13	0.20	0.011	++
1+	113.4 ± 9.9	large pred	360	0,31	36	54	28	1.12	0.21	0.004	+

Behavioral studies: Aquaria Experiments

The experimental aquaria were 80 x 42.5 x 34 cm in size, containing 100 liters of fresh water from the nearby Lake Reeser Meer. The light:dark cycle was set at 14:10 h, similar to natural conditions. The aquaria were partitioned in three equal compartments (cf. Magnhagen & Borcherding 2008 for details of the whole experimental set-up). One third on the left side of each aquarium was used for the predator and the rest, in the middle and on the right side remained as compartments for the groups of juvenile perch. Artificial vegetation was placed on top of the right side of each aquarium, making a refuge for the prey fish. The middle of the aquaria remained free of vegetation, making an open area next to the predator. A plastic net with a mesh size of 5 mm was placed between the predator compartment and the prey perch. All aquaria were provided with gravel on the bottom. During acclimatization and between observations, a non-transparent plastic plate was placed next to the net to prevent visual contacts of the small fish with the predator.

Every individual of each group of four prey perch was marked with a different color of tattoo-ink on the base of its caudal fin to make it easier for the observer to distinguish between group members. Before being handled the perch were always anaesthetized in a suspension of 5 mg L^{-1} metomidate hydrochloride (Aquacalm). The weight of the fish was measured to the nearest 0.01 g and the length to the nearest mm. Fulton’s condition factor ($K = 10^5 m L_T^{-3}$, where m = biomass in g and L_T = total length in mm; (Bagenal & Tesch 1978) was calculated for every individual perch. The perch were allowed to acclimatize in the aquaria for three days and were fed three times a day with red chironomid larvae in the open area. Before the observations started, the opaque plate was moved from its position towards the section on the right side of the aquarium, to enclose the small perch in the vegetation area.

Approximately 60–70 red chironomid larvae were poured into the middle of the open area and allowed to sink to the bottom. The observations started after carefully lifting the opaque plate, making the predator visible to the small perch.

The computer program “Perchmon” was used to record every second the different activities for every individual fish. As in former studies (e.g. Borcherding & Magnhagen 2008), three parameters were recorded: time spent in the open area, latency to start feeding, and duration of first feeding bout. Each observation lasted for 10 minutes, and was terminated with moving the opaque plate back next to the predator compartment. The 18 experimental aquaria were observed for three successive runs, two on the first day and a third on the second day, where the order of observations was the same for each run, balancing the time between observations for each aquarium. To equalize possible olfactory cues from the predatory perch for every experimental period, the water in the aquaria was replaced after every third run of experiments. After the last round of observations all experimental perch were killed with an overdose of metomidate hydrochloride and deep frozen for further morphological analyses.

Morphometric analyses

For morphometric analyses juvenile perch were grouped according to age class and predation risk (0+ no, 0+ low, 0+ high, 1+ no, 1+ low and 1+ high predation risk). The fish were defrosted, placed on a scooped piece of polystyrene and the fins were erected with needles to prevent deformation. After photographing the fish together with a ruler using a digital camera, the software tpsDigit from Rohlf (available at: http://life.bio.sunysb.edu/morph/; NY, USA) was used to digitize 18 homologous landmarks (12 defined points to describe the outer shape, two for the pectoral fin, two the mouth and one for the eye and the operculum spine respectively) on the left side of each specimen.

All further analyses were performed with the Integrated Morphometrics Package (IMP), developed by Sheets (available at: http://www2.canisius.edu/~sheets/morphsoft.html; Buffalo, NY, USA). The IMP software CoordGen6 was used to transform all specimens to the same standard length and baseline orientation. To minimize the sum of squared errors between landmarks of the individuals within each group, the non-shape-variations were removed by using the procrustes superimposition option of CoordGen6. Shape differences between groups were analyzed with a Canonical Variant Analysis (CVA), using the IMP software CVAGen6, tested with a MANOVA (Wilk´s lambda value $p<0.05$) and CV scores from the significant axes were extracted for further analyses. CVAGen6 was also used to visualize the CVA-plot on the first two CVA axes and to depict the associated shape differences as vectors on landmarks.

Statistics

As the experimental design in the mesocosms allowed predation and, thus the loss of single individuals within the group of prey fish, size-specific effects on the groups of prey fish cannot be excluded that may bias the behavioral and morphological data. These effects can be either direct predation of a certain size class of the prey fish or indirect effects, like size related changes in boldness and accompanied changes in food uptake and growth. To analyze possible changes in the length-frequency distribution (LFD) in single mesocosms, and, thus size-specific effects of the predator on the prey population, the normal quantile-quantile-plots (Q-Q-plots, Tsai & Yang 2005) from the start and the end of the mesocosm experiment of the prey fish were computed. This was done using the qqnorm and qqline commands from the free software pack R (R Development Core Team 2009). Here, the slopes of the lines were used as a measure of variation in length distributions.

Prior to analysis and to understand the effects of a size-selective predation, prey fish were theoretically removed from the LFD in several ways: a) The lower edge of the distribution was removed, b) the upper edge was removed, and c) randomly chosen individuals were removed from distribution (repeated eight times). Artificially amended upper and lower distributions revealed that a predator, who feeds on the lower or the upper edge of a distribution of prey fish, induced a negative Δslope, which equals the difference in slopes of the Q-Q-plots from LFDs at the start to the end of the mesocosm treatments. In contrast, slopes of random amended distributions showed only small differences in Δslope. In addition, the coefficient of variance (CoV) of the start and the end distributions was calculated (Huss et al. 2008). The CoV is a measure of dispersion of a distribution. It is defined as the ratio of the standard deviation to the mean. Residual CoV ($\Delta CoV = CoV_{end} - CoV_{start}$) was compared as response variable with the residual slopes ($\Delta slope = slope_{end} - slope_{start}$) in a linear model. This analysis was restricted to the 0+ perch (n=9), because the numbers of individuals in the 1+ treatment, especially at the end of mesocosm experiments, were to low for meaningful LFDs.

The three recorded behavioral variables, namely time spent in the open area, latency to start feeding and duration of first feeding bout, where duration of feeding was defined as the time the fish spent oriented towards the bottom, attacking the food without checking for potential threats, were used as measurements representing boldness (Magnhagen & Borcherding 2008). The mean over the three runs of the three behavioral variables for each individual were included into a principle component analysis, to derive a more general measure of boldness (Magnhagen & Borcherding 2008). The first component, explaining most of the variation (eigenvalue >1) was extracted and used as boldness score (PC1) for all further analyses.

For the morphological analyses, CV axis 1 explains more of the variation present in the morphological data, however as we are interested in the effect of perceived predation risk, represented by the CV axis 2, we retained CV axis 2 for further analyses. In the final step we correlated the mean boldness score (PC1) and mean morphology score (CV2) per mesocosm with the actual perceived predation risk, separately for both age classes.

To analyze the effect of predation risk on the behavioral and morphological data in detail, two separate linear mixed effect models were set up. To avoid pseudoreplication (Hurlbert 1984), a nested design was created. Between-individuals within group was added as random effect at the innermost level, between-groups in one mesocosm was added at the following level and between-mesocosms was added as random effect at the outer level. To analyze the effects of predation risk on body shape, we included the morphology score (CV2) as response variable and age class, predation risk and their interaction as fixed effects. The equivalent model was setup to analyze the behavioral data, using the boldness score (PC1) as response variable and condition factor, age class, predation risk and the interaction of age class and predation risk as fixed effects.

A variance component analysis was carried out to be able to decompose the variation explained by the different random factors between individual, between group and between mesocosms (Pinheiro & Bates 2000; Magnhagen & Bunnefeld 2009) and to compare their importance for the examined traits (morphology and behavior) and the different age classes (0+ and 1+). Keeping the above described structure of the random effects (between individual/ between groups/ between mesocosms), four additional mixed models were set up, analyzing the morphological and behavioral data separately for the two age classes. Including the morphology score (CV2) as response variable and age class, predation risk and their interaction as fixed effects, two separate models were run for the 0+ and 1+ perch, respectively. Additionally, two separate models were set up, one for the 0+ and one for the 1+ perch, using the boldness score (PC1) as response variable and condition factor, age class, predation risk and the interaction of age class and predation risk as fixed effects. The most parsimonious models for the separate behavioral and morphological data for the two age classes, 0+ and 1+ perch, were derived by testing the fixed effects using Wald statistics (Pinheiro & Bates 2000). The free software R for statistical computing (R Development Core Team 2009) was used for all analyses. The PCA (prcomp) and the correlation (lm) were calculated with the standard stats library. The library nlme v.3.1.-90 was used to run the mixed effect models.

Results

Condition, growth and length-frequency distributions

At the end of the mesocosm period 1+ perch were in a better condition compared to their younger conspecifics (Mann-Whitney U-test, z=3.407 p<0.001), while growth rates during the experimental period were similar (Mann-Whitney U-test, z=0.444 p=0.666, Tab. 1). Within the two age classes and with respect to experienced predation risk as independent variable, however, no significant differences were observed, neither for condition nor for growth (Kruskal-Wallis H-test: all p at least >0.1)

The calculations of the slopes from the Q-Q-plots of the 0+ perch revealed for some mesocosms a reduction in slope from the LFD at the start to the end of the experiment (negative Δslope). Large negative values of Δslope can be quoted as a hint that either some small or some large individuals from the LFD of the start were removed until the end of the experiments (or other size specific indirect factors affected either the small or the large individuals). In contrast, other mesocosms showed a slight increase (positive Δslope) or nearly no changes in slope comparing the LFD at the start with the one from the end of the mesocosm experiment.

A predator who feeds randomly over the entire LFD would induce only small differences in slopes from the start to the end LFD, and in correlation only small differences in the coefficient of variation (CoV) of the LFD are expected. In contrast, when a predator feeds only on the small or on the large prey fish, then a decrease in the slope should be accompanied with a decrease in the CoV. Thus, an overall clear correlation between the Δslope and the ΔCoV is expected. And indeed, the linear model from ΔCoV against Δslope was highly significant (Fig. 1).

While the CoV of the LFDs of nearly all mesocosms decreased between the start and the end of the mesocosm treatment (i.e., the LFDs were more narrow at the end of the mesocosm period), the Δslope was either negative or positive. In total, at least in four mesocosms both indicators of size-specific effects (i.e., Δslope and ΔCoV) were clearly negative and, thus give evidence that some size-specific effects during the mesocosm period occurred. However, the observed size-specific effects on the population in the mesocosms did probably not depend on the predation risk, as the different risk classes for predation were mixed up along the linear regression (Fig. 1).

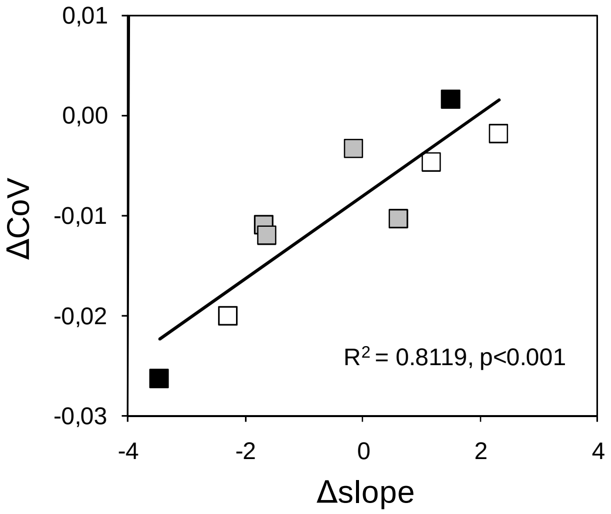

Figure 1: Linear model of the response variable ΔCoV built against Δslope from mesocosm prey length distributions for all mesocosms with 0+perch. The three predation risk classes are indicated as no (white), low (light gray) and high (black) predation risk.

Behavior

The PCA resulted in one principle component with an eigenvalue >1, explaining alone 82% of the variation (Tab. 2). Positive scores on PC 1 indicate a longer time spend in the open, a longer duration of first feeding bout and shorter latency to start feeding, signifying a high degree of boldness (Tab. 2).

Table 2: Factor loadings, proportion of the total variance explained and eigenvalues of the first three axes, extracted from a PCA over the three different measures of boldness (time in the open, latency to start feeding and duration of first feeding bout)

	PC1	**PC2**	**PC3**
Time in the open	0.597	0.264	-0.757
Latency to start feeding	-0.583	-0.505	-0.636
Duration of first feeding bout	0.551	-0.821	0.147
Proportion of total variance	0.821	0.123	0.056
Eigenvalue	2.462	0.369	0.168

The two age classes differed in boldness, with 0+ perch being significantly bolder than their 1+ conspecifics. Whereas, there were no overall differences between predation risk classes or the interaction between age class and predation risk class or an effect of condition factor (Tab. 3). However, the mean boldness score per mesocosm significantly decreased with increasing actual predation risk for the 1+ perch only, while this tendency was not significant for the 0+ perch (Fig. 2).

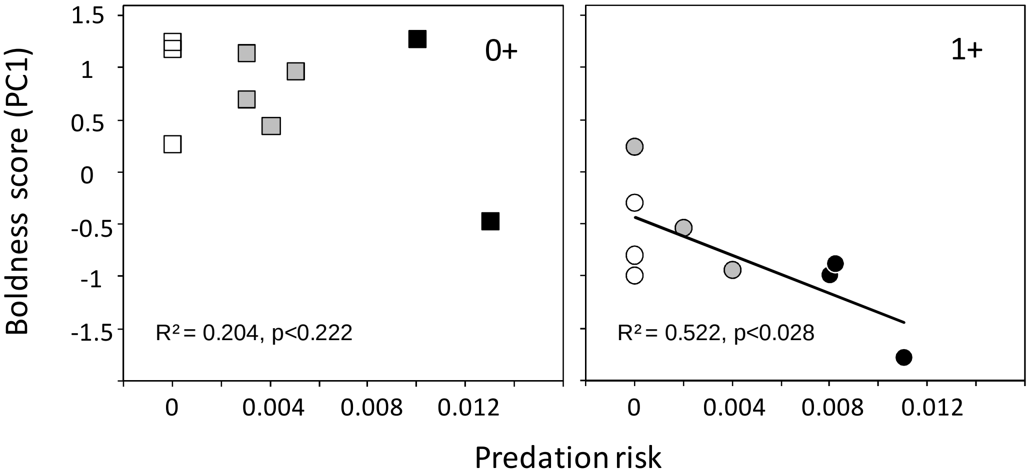

Figure 2: Mean boldness score (PC1) per mesocosm in relation to the actual predation risk for both age classes. The three predation risk classes are indicated as no (white), low (light gray) and high (black) predation risk.

Table 3: Wald statistic for the fixed effects age class, predation risk class (no, low and high predation risk) and their interaction for the morphology score (CV2), and additionally the condition factor (without interactions) for the boldness score (PC1), tested with two mixed effect models

		F	**df,dfden**	**P**
Morpho - CV2	Age class	1.37	1,12	0.264
	Pred risk class	48.24	2,12	0.0001
	Age class x pred risk class	10.31	2,12	0.003
Behav - PC1	Age class	19.87	1,12	0.0008
	Condition factor	0.19	1,158	0.662
	Pred risk class	1.12	2,12	0.359
	Age class x pred risk class	0.16	2,12	0.856

Morphology

The CVA revealed two significant CV axis, discriminating the two age classes from another and the high- from the no- and low-predation risk class (Fig. 3). Independent of perceived predation risk, 1+ perch were considerably deeper bodied than their 0+ conspecifics (Fig. 3, CV axis 1: $\lambda=0.08$, $\chi^2=494.7$, df=160, $p<0.001$, eigenvalue=3.8). In addition, individuals experiencing a high predation risk level had a deeper bodied and more downward bended appearance, compared to the individuals experiencing no or a low predation risk level (Fig. 3, CV axis 2: $\lambda=0.38$, $\chi^2=189.8$, df=124, $p<0.001$, eigenvalue=0.6).

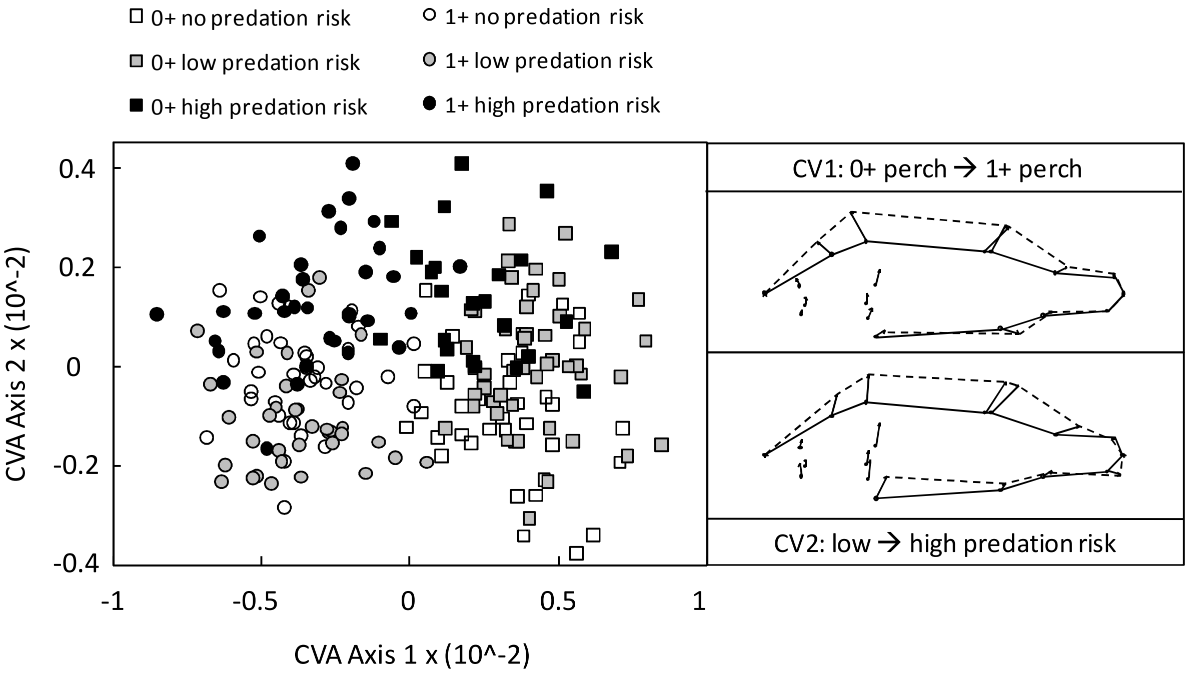

Figure 3: Canonical variate scores of 0+ (squares) and 1+ perch (circles) experiencing no (white), a low (light gray) and a high (black) predation risk level during the mesocosm treatment, depicted along the first and the second canonical variate axes. CV1: Shape difference correlated with the first canonical variate axis, obtained by regressing the shape on the CVA scores and depicted as growth vectors starting from the 0+ perch (solid line) to the 1+ perch (dotted line). CV2: Shape difference correlated with the second canonical variate axis, obtained by regressing the shape on the CVA scores and depicted as growth vectors starting from perch experiencing a no/low predation risk level (solid line) to the perch experiencing a high predation risk level (dotted line).

As we focused our analysis on the effect of perceived predation risk represented by the CV axis 2, additional analyses were carried out, taking into account the pseudoreplications within each mesocosm. We found no significant differences between the 0+ and 1+ perch along CV axis 2, while predation risk was confirmed to have a significant effect on body depth and bending (Tab. 3). In addition, there was a significant interaction of age class and predation risk class for CV 2, as 0+ fish from the low predation risk class were in relation to the 0+ no predation risk class deeper bodied than he 1+ fish from the low predation risk class in relation to the 1+ no predation risk class (Fig. 4). Correspondingly, we found a positive correlation between mean morphology score (CV 2) per mesocosm and actual predation risk for both age classes, which was, however, more pronounced for the 0+ perch (Fig. 4).

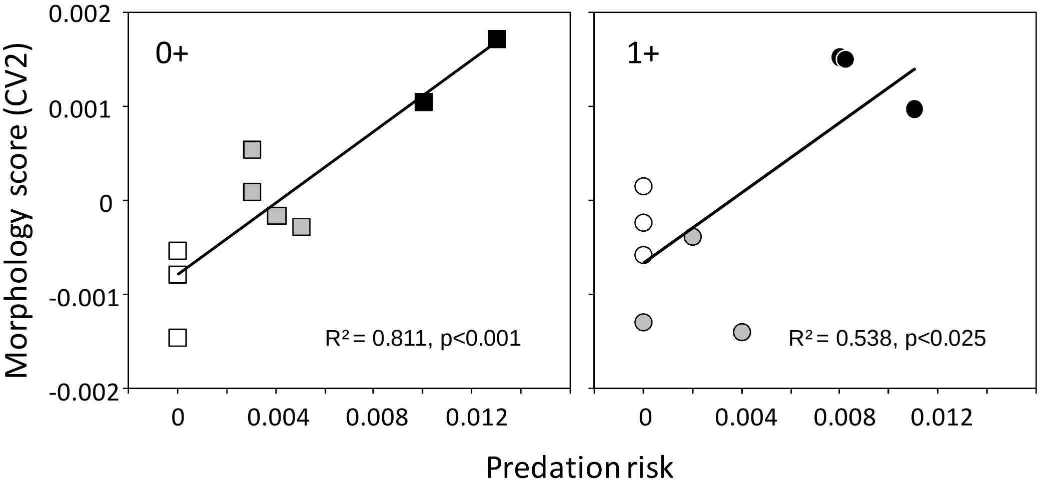

Figure 4: Mean morphology score (CV2) per mesocosm in relation to the actual predation risk for both age classes. The three predation risk classes are indicated as no (white), low (light gray) and high (black) predation risk.

Variance component analysis

The separate models on the morphological data for the 0+ and 1+ fish that included only predation risk as fixed effect, were the most parsimonious. While, including the boldness score (PC1), the most parsimonious models for the two age classes were the ones without any fixed factors and thus used to extract the variance explained by the random effects. For both age classes the highest explained variance, analyzing the morphology score (CV2), was between individuals (Tab. 4), indicating that individuals differed greatly in body shape. Whereas on the behavioral data, the highest explained variance was, for both age classes, between groups, followed by between individuals (Tab. 4). This shows that individuals adjusted their behavior to the group they have been tested with, but still expressed some individual differences, where 0+ perch behaved slightly conformer within groups than 1+ perch (Tab. 4).

Table 4: Percentage of the explained total variance of the nested random effects between individuals, between groups, between mesocosms and the residual variance, extracted from the most parsimonious linear mixed effect models using the behavioral (PC1) and morphological (CV2) data for the 0+ and 1+ perch, respectively

		between individuals (%)	**between groups (%)**	**between mesocosms (%)**	**residuals (%)**
Morpho - CV2	0+ perch	99.99	<0.01	<0.01	<0.01
	1+ perch	97.68	<0.01	2.26	0.06
Behav - PC1	0+ perch	19.05	80.82	<0.01	0.14
	1+ perch	27.17	67.94	0.98	3.91

Discussion

In accordance to the hypothesis, the results of the present experimental study revealed clear relationships between perceived predation risk, the behavior and the morphology, respectively, and this for both age classes of surviving prey perch. The mean boldness score in the tradeoff between food and shelter for both age classes decreased with the intensity of predation, while morphology of prey perch changed especially under high predation pressure towards deeper bodied individuals.

Excluding several environmental factors that may be responsible for changes in behavior or morphology in the field (e.g., habitat structure: Brown & Warburton 1997; Svanbäck & Eklöv 2002; food: Heermann et al. 2007; hunger level: Borcherding & Magnhagen 2008; water transparency: Skov et al. 2007), the changes in behavior and morphology of the prey perch populations in our experimental approach were only induced by the predatory risk. This suggests that experience with a predator influences the establishment of anti-predator responses within a few weeks. Thus, our results give evidence that prey fish adjusted their phenotype according to the actual predation risk within short periods, or, in other words, reveal a high level of phenotypic plasticity (Smith & Skúlason 1996). Consequently, our experimental data, in which only the predation risk changed between treatments, are a proof for some recent suggestions based on empirical field data that described behavioral changes in perch of some lakes in northern Sweden in response to predation risk (Magnhagen 2006; Magnhagen & Borcherding 2008).

Other possible explanations for the observed mean changes on the population level after the treatment in the mesocosms, however, cannot be totally excluded. These are mainly based on size-specific effects of predation (cf. Magnhagen & Heibo 2004). Selective predation, either in relation to certain types of behavior, morphology or in relation to individual prey length, could also lead to a postponement in behavior or morphology of the total population. The investigations on LFDs seem to suggest that the predator had influential effects on the size distributions of the prey perch, at least in some of the experimental mesocosms. Due to gape-limited constrains it is most likely that in some mesocosms the predator preyed only on the smaller individuals (Lundvall et al. 1999), instead of preying randomly across the entire distribution. This would be a direct effect from the predator on the population that removed individual prey fish with certain characteristics. However, indirect effects from the predator can also be suggested. When a predator removes a part of the population, the remaining individuals are expected, e.g., to gain less intra-specific competition, as they can feed on relatively higher amounts of unchanged food resources. This may result in some growth advantages for smaller individuals within the population, as smaller individuals are expected to exhibit a higher degree of boldness in the trade-off between predation risk and feeding, in order to compensate the higher risk of starvation compared to their larger conspecifics (Krause et al. 1998a; 1998b). Such a scenario would lead to a lower coefficient of variation within the population (Huss et al. 2008). Further, vulnerability to predators changes with size (Lundvall et al. 1999; Claessen et al. 2002), and a high feeding rate would enable the prey to minimize the time spent in a vulnerable size window (Persson et al. 2004). Such size-specific effects of predation on a given population, independently if they are direct or indirect, can be suggested in some of the mesocosms when comparing the length-frequency distributions of the start and the end of the experimental period. However, as the observed changes in the length-frequency distribution of the prey perch were independently from the perceived predation in each mesocosm (as shown in Fig. 1 for the 0+ perch) and may depend on individual differences between the predators, we assume that the observed changes in behavior and morphology on the population level are more likely a result of the individual phenotypic responses.

In our behavioral experiments boldness scores differed between age classes, of which 0+ perch were significantly bolder than their 1+ conspecifics. This is in contrast to other studies that have shown behavioral consistency across age classes. For example, two populations of three-spined sticklebacks (*Gasterosterus aculeatus*) differed in boldness across ontogeny (Bell & Stamps 2004). Differences were also found in aggressive behavior between three strains of grayling (*Thymallus thymallus*) that were consistent over age classes (Salonen & Peuhkuri 2004). It is discussed that smaller individuals are bolder than their larger conspecifics because of a higher risk of starvation: for example, small three-spined sticklebacks spent more time in a risky area than did larger ones (Krause et al. 1998a). Similar, body length was strongly correlated with boldness scores in tropical poeciliid (*Brachyraphis episcopi*), where smaller fish were bolder than their larger conspecifics (Brown et al. 2005). Additionally it must be considered that undernourished perch spent more time in the open area and extended their first feeding bout, thus were bolder in the tradeoff between food and shelter than satiated perch (Borcherding & Magnhagen 2008). As size and condition factor were lower for the 0+

perch compared to their 1+ conspecifics, the higher degree of boldness of the 0+ compared to the 1+ perch in our experiments may thus be assumed to depend primarily on condition and size differences between the age classes of prey perch.

Despite these effects of size and condition, and because the perch's individual size differences were minor within each age class and between treatments (Tab. 1), the linear model of our analysis revealed a significant dependency between the actual perceived predation risk and the mean boldness scores of the 1+ perch. The model for 0+ perch showed a similar trend, although it was not significant. Thus, our experimental results are in good correlation to recent field studies that revealed the effect of predation risk on the behavior of perch (e.g., Magnhagen 2006; Magnhagen & Borcherding 2008). Similar, three-spined sticklebacks from an area with low predation risk showed less pronounced anti-predator behavior than did sticklebacks originating from a high predation risk site (Bell & Stamps 2004; Bell 2005). Further, predator inspection was more pronounced in three-spined sticklebacks from predator-experienced populations compared to populations with no earlier predator exposure (Walling et al. 2004). Previous studies in fish have shown relations between predation risk and differences in anti-predator behavior between populations (Walling et al. 2004; Brown et al. 2005) and within populations (van Oers et al. 2004; Westerberg et al. 2004; Ward et al. 2004). Such effects within populations may originate from personality (Gosling 2001), individual coping (Koolhaas et al. 1999) or behavioral syndrome (Sih et al. 2004), and may depend on a variety of environmental factors.

Beside these changes in behavior with respect to predation risk, the results of our experimental study also revealed changes in morphology for both age classes, where individual 0+ and 1+ perch developed a clearly deeper and more downward bent body under high predation risk. Under low predation risk this development was still obvious for the 0+ perch, whereas 1+ perch from no and low predation risk classes showed no morphological differences. This may be a hint that significant changes only occurred after reaching a certain level of predation intensity, which is underpinned by the linear models on the mesocosm means of response CVA scores against the actual predation risk that revealed highly significant dependencies for both age classes in which perch's body depth increased with the actual predation risk. Fish with an increased body depth have a higher chance to escape predation attacks due to the difficulties of gape limited predators in handling and swallowing deep-bodied individuals (Nilsson & Brönmark 2000). However, developing a deeper body morphology was also shown to be a normal trait during the ontogeny of perch (Svanbäck & Eklöv 2002; Heermann et al. 2007), and is strongly correlated to the niche shifts of juvenile perch (Persson 1988). Perch feeding on benthos develop a deeper body compared to their plankton feeding conspecifics (Hjelm et al. 2001; Olsson & Eklöv 2005; Svanbäck & Eklöv 2006). Studies on morphology influenced by predation also revealed differences within or between fish populations (Brönmark & Miner 1992; Eklöv & Svanbäck 2006; Eklöv & Jonsson 2007), whether these differences are induced directly (Rundle et al. 2003; Langerhans et al. 2004) or indirectly by the predator (Abram 2000). In a recent study, prey perch shifted their habitat according to predation risk. They chose the habitat with the lower risk of predation and fed on resources specific to this habitat, resulting in habitat-specific morphology (Eklöv & Svanbäck 2006). The adaption to habitat-specific swimming modes resulted in the development of different morphologies, improving the maneuverability in vegetated areas of deeper bodied perch (Eklöv & Svanbäck 2006). In the present study all experimental perch used the vegetation areas of the mesocosms as preferred habitat, at least during daytime (personal observation). In order to minimize effects of different food resources on morphology (e.g., Heermann et al. 2007), all perch were fed with frozen red chironomid larvae that represent natural benthic diet. Further, food abundance is known to affect morphology of perch: perch that were fed to excess developed a deeper body compared to fish from a low food level (Borcherding & Magnhagen 2008). All these experimental conditions could have lead to deeper body morphology at the end of the mesocosm period. However, as the level of morphometric change was clearly correlated to the factor predation risk (the only factor that varied substantially between the treatments), we are confident that the observed changes in morphology of prey perch within each age-class depended to a greater extent on the perceived predation risk. Whether these changes depended on indirect effects or direct effects like size-specific predation is, however, difficult to assess.

In an aquarium experiment perch increased body depth within six weeks in the presence of pike predators, underlining that induced defenses in aquatic organisms are generally triggered by waterborne chemical cues released in the interaction between a predator and a prey (Eklöv & Jonsson 2007). In crucian carp, chemical cues from pike were also sufficient to induce an increase in body depth (Brönmark & Pettersson 1994). It is further known that such substances can also be released from the epidermis of ostariophysan fish as alarm signals in the case of an injury (Stabell & Lwin 1997). Perch-experienced common bullies (*Gobiomorphus cotidianus*) responded to chemical cues from a predatory perch, indicating the probable presence of an alarm substance ("Schreckstoff", Kristensen & Closs 2004). Yellow perch (*Perca flavescens*) showed an increased swarm cohesion and bottom bound behavior as responses to alarm cues of injured conspecifics, and further, individual juvenile yellow perch increased shelter use and froze more when exposed to chemical alarm cues from both juvenile and adult yellow perch (Mirza et al. 2003). Until now there are no results whether adult, piscivorous perch excrete olfactory, predatory cues that affect juvenile conspecifics or whether the occurrence of alarm substances released by perch that were attacked by a predator are responsible for observed predatory effects. And of course, this question cannot be answered with the results of the present study as in our experimental set-up both types of chemical cues were possible as a trigger for the observed morphometric alterations.

In conclusion, it was shown that perch became shyer with the intensity of predation, and when a certain level of predation risk was exceeded prey perch from the mesocosms also became deeper bodied. However, while changes in morphology were somewhat more pronounced in the 0+ age group, the 1+ age group exhibited significantly stronger changes in behavior. This can be quoted as a first hint that age-specific responses towards predation risk exist in perch, assuming that phenotypic plasticity on different response levels may be inconsistent over age-classes in European perch. However, it remains unanswered if individual prey perch adjusted their behavior and morphology in response to the predator, or if the predator induced these changes by selective predation or if further size-specific indirect effects existed in the experiments that resulted in the observed mean changes of morphology and behavior. Although the analysis of the changes in the length-frequency distributions indicated some size-specific effects, the observed changes in the length-frequency did not depend on the predation risk of the different treatments. Nevertheless, these size-specific effects should be examined more intensively in the future, by testing the prey perch for boldness and morphology before and after the treatment with individually marked specimens. Such comparisons would allow further insights on individual responses in behavior and morphology towards the effects of predation risk.

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CHAPTER IV

Facing different predators: Adaptiveness of behavioral and morphological traits in juvenile perch under predation of adult perch and pike

Martina Heynen[1], Nils Bunnefeld[2] and Jost Borcherding[1]

[1]Zoological Institute of the University of Cologne, Department of General Ecology & Limnology,
Ecological Field Station Grietherbusch, D-50923 Cologne, Germany
[2]Department of Life Sciences, Imperial College London, Silwood Park, Ascot SL5 7PY, UK

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Abstract

Predation is thought to be one of the main structuring forces in animal communities. However, selective predation is often measured on isolated traits in response to a single predatory species, but only rarely are selective forces on several traits quantified or even compared between different predators naturally occurring in the same system. In the present study, we therefore measured behavioral and morphological traits in young-of-the-year Eurasian perch and compared their selective values in response to the two most common predators, adult perch and pike. Using mixed effects models and model averaging to analyze our data, we quantified and to compared the selectivity of the two predators upon the different morphological and behavioral traits. We found that selection on the behavioral traits was higher than on morphological traits and perch predators preyed overall more selectivly than pike predators. Pike tended to positively select shallow bodied and non-vigilant individuals (i.e. individuals not performing predator inspection). In contrast, perch predators selected mainly for bolder juvenile perch (i.e. individuals spending more time in the open, more active), which was most important. Our results are to the best of our knowledge the first that analyzed behavioral and morphological adaptations of juvenile perch facing two different predation strategies. We found that relative specific predation intensity for the divergent traits differed between the predators, providing some additional ideas why juvenile perch display such a high degree of phenotypic plasticity.

Introduction

Predation is thought to be one of the main structuring forces in freshwater communities (e.g. Eklöv & Hamrin 1989; Langerhans et al. 2004; Sharma & Borgstrom 2008), influencing not only species assemblage through selective predation but also the distribution and abundance of phenotypes within a population (e.g. Kishida & Nishimura 2005; Bell & Sih 2007). Typically a prey faces multiple predators (Sih et al. 1998), which might differ in size (Scharf et al. 2000), density (Magnhagen & Heibo 2004), habitat use (Krupa & Sih 1998), diel activity (Turesson & Bronmark 2004) and/or hunting strategy (Kishida & Nishimura 2005), imposing different predator specific selection forces on the shared prey. In single predator systems a predator specific defense would decrease a prey`s vulnerability to predation (Matsuda et al. 1996; Krupa & Sih 1998; Sih et al. 1998). In contrast, facing equally abundant predators with different strategies, a more plastic and general defense might be advantageous (Matsuda et al. 1996; Krupa & Sih 1998; Sih et al. 1998). Many defense strategies of the prey were shown to bear costs, confronting the prey with time and/or resource allocation trade-offs (e.g. Steiner & Pfeiffer 2007). Individual sticklebacks (*Gasterosteus aculeatus*) that behave less actively (Moodie et al. 1973) or more vigilantly (Godin & Davis 1995) are less likely to fall victim to predation, but simultaneously lose foraging opportunities. Watching out for potential threats and inactivity are largely incompatible with foraging (Treves 2000; Lind & Cresswell 2005). In crucian carp (*Carassus carassus*) increased body depth, though effective against gape limited predators (Nilsson et al. 1995), was also found to reduce competitive abilities (Pettersson & Bronmark 1997) and increase swimming costs (Pettersson & Brönmark 1999). Throughout many fish species, different defense strategies have been described such as group living (Godin et al. 1988), vigilance (Pitcher 1992), reduced activity (Bean & Winfield 1995), or seeking shelter (Snickars et al. 2004), armor (Vamosi 2002), spines (Zimmerman 2007), camouflage (Ryer et al. 2008) or increased body depth (Brönmark & Pettersson 1994). The variety of behavioral and morphological defenses were sometimes shown to compensate or augment each other (Steiner & Pfeiffer 2007). In goldfish (*Carassius auratus*), deep bodied individuals displayed lower intensity of anti-predator response than shallow bodied ones, thus individuals with morphological defenses exhibited less behavioral modification than those lacking such defenses (Chivers et al. 2007). However, selective predation is often measured on isolated traits in response to a single predatory species, but only rarely are selective forces on several traits quantified (e.g. Bell & Sih 2007; Holmes & McCormick 2009; Smith & Blumstein 2010) or even compared between different predators naturally occurring in the same system (e.g. Botham et al. 2006; Holmes & McCormick 2009). Comparing the fitness consequences of just one axis of variation may, however, overestimate the importance of one trait and lead to a fractioned view on the operating selective forces (Steiner & Pfeiffer 2007).

Eurasian Perch (*Perca fluviatilis* L.) is a common freshwater species throughout Europe (e.g. Thorpe 1977; Collette & Banarescu 1977), where juveniles (like most fishes), suffer the highest predatory mortality during their first year (Sogard 1997; Claessen et al. 2002; Huss et al. 2010). For juvenile perch the two most common predators are adult perch and pike (*Esox lucius*) (Byström et al. 1998; Magnhagen & Heibo 2001; Persson et al. 2003), that differ in habitat use and hunting strategies (Turesson & Bronmark 2004). While perch predators hunt and search actively (Christensen 1996), pike is a sit-and-wait predator, ambushing the prey from shelter (Bean & Winfield 1995). Juvenile perch were found to show consistent variation in morphology (Borcherding & Magnhagen 2008) and behavior (Magnhagen & Bunnefeld 2009; Magnhagen 2006). The evidence suggests individuals adapt their behavior to the experienced level of predation risk (Magnhagen & Borcherding 2008) and increase their body depth in the presence of pike (Eklöv & Jonsson 2007). However, to our knowledge, the relative selection advantages of morphological and behavioral traits with respect to predation have not yet been quantified, or even compared for different predators.

In the present study, we therefore measured behavioral and morphological traits in young-of-the-year Eurasian perch and compared their selective values in response to the two most common predators, adult perch and pike. Boldness towards a predator and morphological features describing the body shape were measured before groups of juvenile perch faced either a piscivorous pike or perch. To analyze the selective value of the different phenotypic traits, we compared the initial morphological and behavioral characteristics of the juvenile fish that survived with the characteristics of the juvenile fish that were preyed upon. Using mixed effects models and model averaging to analyze our data, we quantified and compared the selectivity of pike and perch predators upon the different morphological and behavioral phenotypes within the population of juvenile perch. In order to reduce size selective predation in our set-up, we applied a relatively high predator-prey size ratio. This would suggest that morphological traits, like a slightly deeper body should be of minor importance, despite indications that predators regularly prefer shallow bodied prey to reduce handling time. In contrast, behavioral defense strategies should then be of higher importance in our analysis, and we hypothesize relatively risk prone prey individuals to suffer higher mortality rates. As an actively searching and hunting predator like perch depends to a greater extent on the behavior of its prey, we finally hypothesize that adult perch would prey more selectively than the sit-and-wait predator pike.

Material and Methods

In July and August 2010, in total 152 young-of-the-year perch (total length, $T_{L,}$ X ± SD, 61.9 ± 6.3 mm; weight, X ± SD, 2.1 ± 0.7 g) were caught by beach seining in a gravel pit lake near the city of Rees (51°46´N, 6°20´E), Germany. The fish were directly transported to the Field Station Grietherbusch of the University of Cologne nearby and stocked to an outdoor tank (1,8 m^3) to acclimate to captivity (6-12 days). Fish were fed daily with pre-frozen chironomid larvae (6% of total body mass). The adult piscivorous perch used as predators (T_L, X ± SD; 231.5 ± 18.3 mm, N = 17), were caught in another gravel pit lake (51°45´N, 6°28´E) and were fed with fish daily. Predatory pikes (T_L, X ± SD; 146.7 ± 14.9 mm, N = 5) were caught in a small oxbow near the laboratory, and fed daily with fish.

Experimental design

Before being handled all fish were sedated with MS222. The juvenile perch were measured, weighed and carefully placed on wet thin towel, laying on a carved piece of Styrofoam to prevent deformation. Using a digital camera perch were photographed together with a ruler and then randomly marked with an individual color code on the caudal fin. Subsequently, the juvenile fish were transferred to small aquaria to recover from narcosis (30 min) and assigned to groups of four in an experimental aquarium. Each group participated in one behavioral experiment, with two repeated observations (see below). After the behavioral observations were conducted, 4 groups of four perch each (16 individuals) were added to one outdoor tank (60 cm high, 0.47 m^2, 50% cover with artificial vegetation), containing a piscivorous pike or perch. As adult perch are more sensitive to handling than pike (pers. observation), the adult perch were stocked to the outdoor tanks 10 days and the pikes 3 days prior adding the juvenile fish. During this time the predators were not fed, to reach a high hunger level. In total, ten circular outdoor tanks with recirculation pumps were used (X ± SD, 17.5 ± 1.5 °C, natural light regime), to set up 5 replicates per predator species. Aiming to examine the predator specific selection for different behavioral and morphological phenotypes and to ensure the comparability between the replicates, prey size was chosen to result in a constant predator prey size ratio (prey predator size ratio, perch: X ± SD, 0.30 ± 0.02; pike: X ± SD, 0.39 ± 0.02), which was slightly higher for pike, because they are less gape size limited than perch (Nilsson & Brönmark 2000). The study was conducted in four successive experimental blocks (with two tanks stocked with fewer prey fish, 12 individuals instead of 16). During the tank treatments the juvenile perch were fed twice per day equal to 15% body weight, which was equally distributed over the whole tank.

The tanks were checked every second day, visually counting the remaining prey fish and each treatment ended, when about 50% of the prey fish were consumed. Tank treatments were on average ended after 11 days, recovering between 18.7%-66.6% of the prey and all the predators alive (survival prey, perch: X ± SD, 42.4 ± 15.5 %; pike: X ± SD, 49.9 ± 12.8 %). After the experiments the juvenile fish were sacrificed with an overdose of MS222 and frozen for further morphological analyses. The perch and pike predators were released at the same location they were caught.

Behavioral experiments

The experimental aquaria were 100 l (85 x 42 x 34 cm) and the bottom was covered with gravel (X ± SD, 20.8 ± 1.2 °C, light regime 13L:11D). One-third of each aquarium was used for the predator separated with a plastic net and the remaining part for the group of perch. An opaque plastic screen was placed close to the net, to prevent the juvenile fish habituating to the predator. Artificial vegetation and aeration was provided in the predator compartment and in the half of the space for the perch group that was furthest away from the predator. After each set of behavioral experiments one third of the water in each aquarium was renewed.

Prior to the behavioral experiments the small perch were acclimatized to the aquarium for two days and fed daily with red chironomid larvae in the open area. On the third day the behavioral experiments were conducted, during which juvenile fish were observed twice. Before each observation the juvenile perch were enclosed by the opaque screen in the half of their section that also contained the vegetation. Chironomid larvae (approx. 75 larvae, corresponding to 3% of the total fish weight) were poured into the open space produced between the net and the opaque screen and allowed to sink to the bottom. The observation started by lifting the opaque screen, making a large perch visible to the juvenile perch though the net. Each aquarium was observed for 10 min, in which an observer recorded four different activities for each individual fish: occurrence in the vegetation, occurrence in the open, feeding, and predator inspection. The activities were entered into a computer program, which recorded one behavioral unit every second. After each observation the opaque screen was put back next to the net.

Morphological Analyses

For morphometric analysis 12 homologous landmarks (9 defined points to describe the outer shape, two for the pectoral fin and one for the eye respectively) were digitized on the left side of each specimen using tpsDigit and tpsUtility software from Rohlf (available at: http://life.bio.sunysb.edu/morph/; NY, USA). All following analyses were performed with Integrated Morphometrics Package (IMP) developed by Sheets (which is available at: http://www2.canisius.edu/~sheets/morphsoft.html; Buffalo, NY, USA). All specimens were transformed to the same baseline orientation and length, using IMP software CoordGen6 and nonshape-variations were removed, using the Procrustes superimposition option of the IMP software. Using the software PCAGen6n, a PCA was conducted and PCA scores were computed for the pike and perch data, respectively. PCAGen6n was also used to visualize the morphometric distinction along the selected PCA axes as vectors on landmarks.

Statistical analyses

The recorded behavioral data were used to calculate the average behavior per fish over the two observations for seven behavioral variables: time spent in the open area, total time spent feeding, latency to start feeding, duration of the first feeding bout, activity (number of changes between open area and vegetation), latency until first change of habitat, and time spent with predator inspection. Using a principal component analyses (PCA) the calculated behavioral parameters were combined to behavioral scores, reducing the number of behavioral variables but retaining the variation present in the recorded data. Two PCA´s were conducted for the perch and pike treatment data, respectively.

To analyze the factors influencing the survival of a prey individual, two separate linear mixed effect models were set-up for perch and pike. Survival (0/1) was used as binominal response variable. To avoid pseudo-replication in the analysis, a nested design was created. `Between-groups in one tank´ was added as random effect at the inner level and `between-tanks´ was added as random effect at the outer level. The following variables were added as fixed effects: principal component 1 and 2 from the behavioral analysis

(B-PC1 and B-PC2), principal component 1 and 2 from the morphological analysis (M-PC1 and M-PC2), the average amount of prey consumed per day (PCPD) in each tank and the predator-prey size ratio (PPSR).

The dredge function in the MuMIn package in R was used to run all possible combinations of the fixed effects and ranked the resulting models according to the associated AIC´s, to find the most parsimonious combinations of the fixed effects. Instead of focusing on a single minimum best model, the model.avg function in the MuMIn packed in R was used to average the models identified to best support our data (Johnson & Omland 2004), where models with Akaike difference < 2 were considered important (Burnham & Anderson 2002). Parameter estimates were averaged according to Akaike´s weights. This resulted in robust parameter estimates and predictions, and helped to avoid to focusing on or rejecting a special hypothesis, where multiple alternative hypotheses may be relevant (Johnson & Omland 2004). This approach is especially useful, allowing us to identify and present the relative contributions of the different important factors in explaining our data.

Results

Behavior

For the behavioral data from the juvenile fish used in the pike treatments the PCA produced two behavioral principle components with eigenvalues > 1 (B-PC1 and B-PC2), explaining together 79.5% of the variation (Tab. 1). Whereas the PCA on the behavioral data from juvenile prey perch used in the perch treatments resulted in only one axes with an eigenvalue > 1 (B-PC1), however we retained the first two axes for comparability, explaining together 80.6% of the variation (Tab. 1).

Table 1: Factor loadings, eigenvalues, and proportion of the total variance explained by the first behavioral principle components (B-PC) extracted from the two PCA´s over the seven different measures of behavior, for the perch and pike treatments, respectively

	Perch		**Pike**	
	B-PC1 Boldness	B-PC2 Vigilance	B-PC1 Boldness	B-PC2 Vigilance
Time in the open	**0.402**	0.211	**0.426**	-0.041
Total time spent feeding	**0.403**	-0.340	**0.407**	-0.342
Latency to start feeding	**-0.424**	0.038	**-0.439**	-0.097
Duration of first feeding bout	0.318	**-0.523**	0.362	**-0.406**
Number of changes	**0.403**	-0.063	**0.384**	0.268
Time spent with predator inspection	0.256	**0.725**	0.060	**0.748**
Latency until first change	**-0.405**	-0.185	**-0.418**	-0.271
Proportion of total variance	0.693	0.113	0.598	0.196
Eigenvalue	4.848	0.790	4.190	1.375

The loadings on the two axes were similar between the behavioral data from the juvenile perch used in the different predator treatments, resulting in comparable scores, where positive scores on B-PC1 indicated more time in the open, more time feeding, a lower latency to start feeding, a higher activity (number of changes between open area and vegetation) and a lower latency to leave the vegetation, which would signify a fish with ahigh degree of boldness. Positive scores on B-PC2 indicated more time spent with predator inspection and a lower duration of the first feeding bout, signifying vigilance.

Morphology

From the two morphometric analyses we retained the first two principal components for further analyses, explaining together 52.8% and 56.7% of the morphological variation between the juvenile perch used for the perch and pike treatments, respectively. The shape difference associated with the first morphological principal component (M-PC1) were similar for the pike and perch population subsamples, where positive scores on M-PC1 indicated a more downward bended body shape and a deeper bodied appearance (Fig. 1). For the juvenile prey perch used in the perch treatments positive scores on M-PC2 indicate a larger head, while for the juveniles used in the pike treatments positive scores on M-PC2 are associated with smaller head morphology (Fig 1).

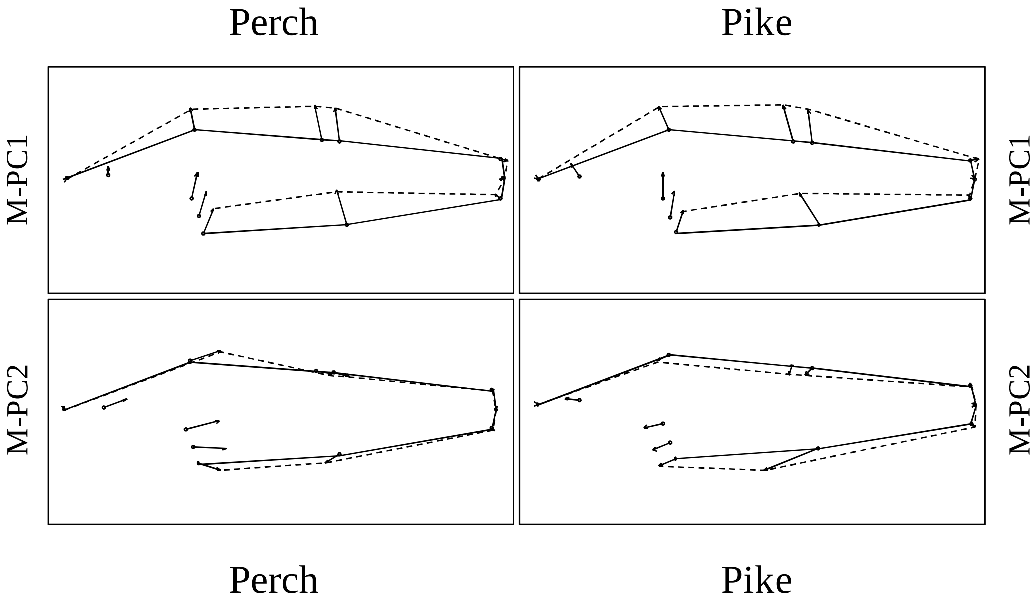

Figure 1: Shape difference associated with the first and second morphological principle component (M-PC1 and M-PC2) from the juvenile perch that participated in the perch and the pike tank treatment. The shape differences are depicted as growth vectors starting from the perch with small M-PC scores (solid line) to the perch with high M-PC scores (dotted line).

Mixed effect models – fixed factors

Testing for the most parsimonious combinations of fixed effects resulted in seven models with Akaike differences < 2 per predator model, including a mean of 2 terms for the models for the perch data and 0.85 terms for the pike models, respectively (Tab. 2). Multi-model inference from the subsets of important models indicates that only the fixed factor behavioral component 1 (B-PC1 boldness) showed a trend different from zero (Tab. 3). This is also reflected by the relative variable importance of the fixed factors, indicating that between individual variation in behavioral component 1 (B-PC1 boldness), but also variation in behavioral component 2 (B-PC2 vigilance) and morphological component 1 (M-PC1 body shape) contribute to the likelihood that a juvenile perch is preyed upon in a perch treatment (Tab. 3, Figure 2). For the pike treatments all examined factors had a relative low contribution in explaining the survival of prey individuals, among which the between individual variation on behavioral component 2 (B-PC2 vigilance) seems to be most important (Tab. 3, Figure 2).

Table 2: Akaike weights (ω_i), Akaike differences (Δ_i), Akaike information criteria, k values and the terms (behavioral component 1 and 2 (B-PC), morphological component 1 and 2 (M-PC), prey consumed per day (PCPD) and the predator prey size ratio (PPSR)) and associated coefficients in each model for the perch and pike treatment models

	Intercept	B-PC1 Boldness	B-PC2 Vigilance	M-PC1 Body shape	M-PC2 Head size	PCPD	PPSR	k	AIC	ΔAIC	AIC weight
Perch	-0.328	-0.246						4	112.0	0.000	0.098
	-0.332	-0.258		13.91				5	112.8	0.811	0.065
	-0.332	-0.242	-0.301					5	102.9	0.885	0.063
	-0.328	-0.252			15.04			5	113.4	1.416	0.048
	-0.335	-0.255	-0.293	13.57				6	113.7	1.740	0.041
	-0.527	-0.263				0.147		5	113.8	1.815	0.039
	0.391	-0.247					-2.39	5	114.0	1.975	0.036
Pike	-0.111							3	105.6	0.000	0.094
	-0.112		0.221					4	106.4	0.847	0.061
	-3.834						9.39	4	106.9	1.282	0.049
	-0.113			10.68				4	106.9	1.356	0.047
	0.453					-0.912		4	107.0	1.425	0.046
	-0.112				-11.18			4	107.2	1.561	0.043
	-0.111	0.032						4	107.5	1.925	0.036

Table 3: The coefficient, standard error, lower and upper 95% confidence interval and the relative variable importance (Rel var importance) for each term (behavioral component 1 and 2 (B-PC), morphological component 1 and 2 (M-PC), prey consumed per day (PCPD) and the predator prey size ratio (PPSR)) of the averaged model for pike and perch treatment data

		Coefficient	SE	Lower CI	Upper CI	Rel var importance
Perch	Intercept	-0.283	0.679	-1.640	1.070	
	B-PC1 Boldness	*-2.251*	*0.114*	*-0.479*	*-0.023*	**1.00**
	B-PC2 Vigilance	-0.079	0.155	-0.384	0.226	0.27
	M-PC1 Body shape	3.740	7.140	-10.300	17.800	0.27
	M-PC2 Head size	1.850	4.560	-7.140	10.800	0.12
	PCPD	0.014	0.050	-0.084	0.114	0.10
	PPSR	-0.223	1.620	-3.450	3.000	0.09
Pike	Intercept	-0.531	1.240	-2.970	1.910	
	B-PC1 Boldness	0.031	0.116	-0.200	0.264	0.10
	B-PC2 Vigilance	0.221	0.209	-0.196	0.637	**0.16**
	M-PC1 Body shape	10.700	13.400	-16.000	37.400	0.13
	M-PC2 Head size	-11.200	16.900	-45.000	22.600	0.11
	PCPD	-0.912	1.210	-3.320	1.490	0.12
	PPSR	9.390	11.100	-12.800	31.600	0.13

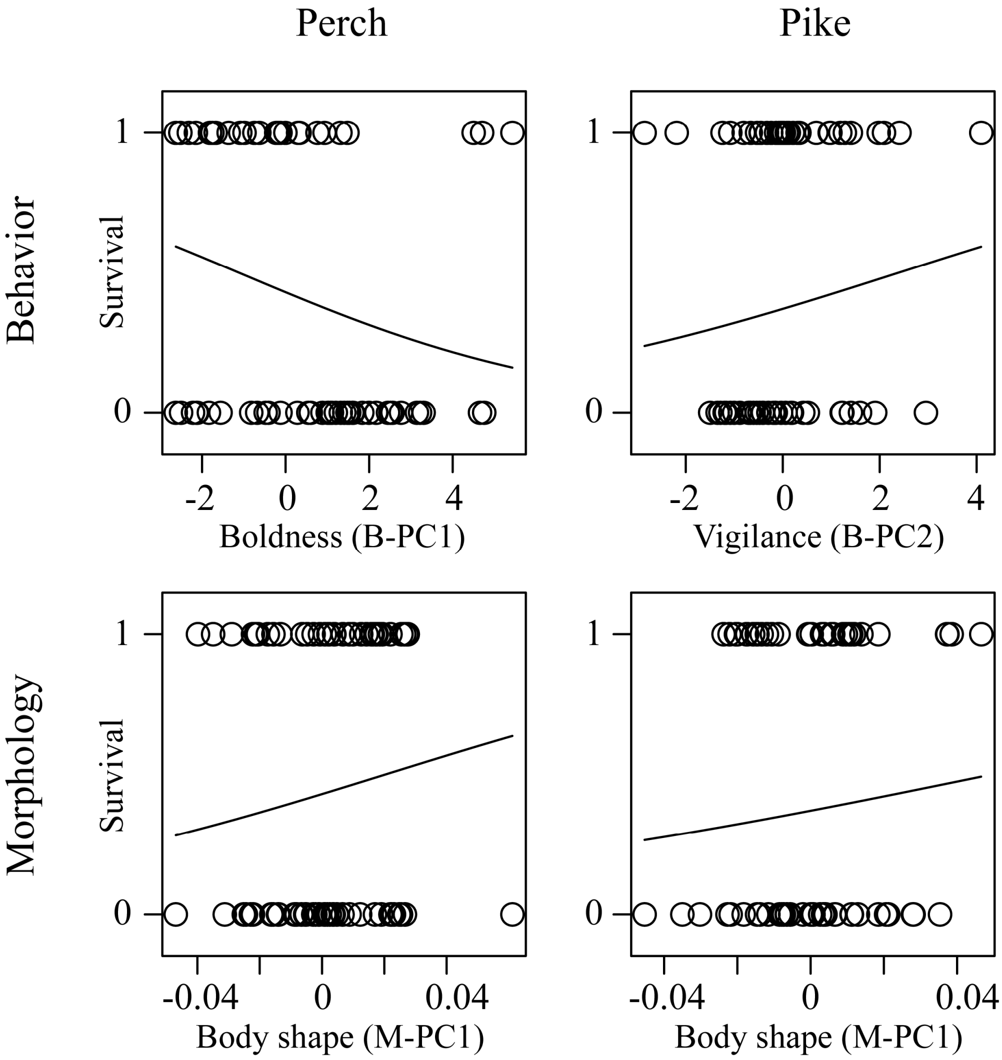

Figure 2: Vulnerability function for the divergent behavioral (B-PC1 and B-PC2) and morphological (M-PC1) phenotypes in response to adult pike and perch, extracted from the averaged model for pike and perch treatment data. Multi-model inference indicate that only the behavioral component 1 (B-PC1 boldness) shows a trend different from zero.

Discussion

In the sets of the most parsimonious models the numbers and combinations of the fixed effects differed between the pike and perch data. The most parsimonious pike models contained zero or only one fixed effect, however without any consistency. In contrast the best perch models contained on average more fixed effects and the factor boldness appeared consistently in all models. Consequently, the fixed effect boldness had the highest relative variable importance in the perch models, in which between individual prey variation in boldness was negatively related to survival. These results indicate that perch predators preyed selectively on bolder juvenile perch and overall considerably more selectively than pike.

We kept size variation between the prey individuals small in the present study, to focus purely on the effects of morphological and behavioral variation. In consequence, the predator-prey-size-ratio and accordingly size selective predation was of only minor importance. Size-biased predation is, however, a common phenomenon in fish populations (Juanes & Conover 1994; Lundvall et al. 1999) and may substantially configure a cohorts size distribution in juvenile stages (e.g. Beeck et al. 2002; Borcherding et al. 2010). A number of mechanisms can induce size selective patterns, which might complicate disentangling the causal effects of selective predation (Sogar 1997). Size selective consumption may be attributed to gape limitations, in which a fish´s vulnerability to predators changes with size (e.g. Lundvall et al. 1999) and body depth (e.g. Nilsson & Brönmark 2000). Size selective consumption may also result from size associated variation in preys escape ability (Webb 1978; Taylor & Mcphail 1985), conspicuousness (e.g. Lundvall et al. 1999) or behavior (e.g. Biro et al. 2004). For example, larger prey individuals might suffer from increased mortality, because they allocate their time in the trade-off between feeding and anti-predator behavior to maximize growth (Mangel & Stamps 2001; Biro et al. 2004; Dibattista et al. 2007). These findings are in correlation to our results that revealed relatively bolder juvenile perch (i.e. spending more time in the open, more active) less likely to survive during the perch treatments. These results are consistent with previous studies on three-spined sticklebacks (*Gasterosteus aculeatus*), in which individuals that were more active (Moodie et al. 1973) and fed more (Bell & Sih 2007) had a higher mortality risk. In meta-analyses across several species Smith & Blumstein (2008) found bolder individuals to have an increased reproductive success, but a shorter life span due to selective predation. Boldness that decreases refuge use and increases activity, increases the risk to encounter (Scharf et al. 2003) and to attract primarily visual oriented predators (Ware 1973; Martel & Dill 1995), increasing predation risk. Additionally, Turesson & Bronmark (2004) found that solitary perch predators need to separate single individuals from a school to successfully attack them. Therefore bold individuals that more often occupy front positions (Ward et al. 2004) and keep greater distance from the school (Wilson et al. 1993), might be more vulnerable to predation. In contrast, Smith & Blumstein (2010) recently reported that more active, bold and exploratory trinidadian guppies (*Poecilia reticulate*) survive longer when exposed to a cichlid predator. The authors suggested that these traits might be advantageous, if an individual´s behavior under predation risk is an honest signal of escape ability. However, they also found that the relative selective advantage depends on the individual predator (Smith & Blumstein 2010).

In contrast to perch, pike predators in the present study did not selectively prey upon bold individuals. Our results indicate a lower tendency for pike predators to hunt vigilant individuals that perform predator inspection. Although predator inspection is generally thought to increase an individual’s vulnerability (e.g. Dugatkin 1992), Godin & Davis (1995) demonstrated that acara cichlids (*Aequidens pulcher*) as predators were less likely to attack guppies that inspected them than those that did not. In sticklebacks, predator inspection was correlated with prey condition and escape ability (Kulling & Milinski 1992) and Pitcher (1992) suggested that predator inspections signals the predator that the prey is aware of its presence. Pike is a highly effective ambush predator (Eklöv & Diehl 1994; Bean & Winfield 1995; Turesson & Bronmark 2004), typically attacking its prey from a hideout in littoral vegetation (Savino & Stein 1989). This tactic was suggested to be highly successful in piscivores, since predators mostly attack unaware prey (Turesson & Bronmark 2004). However, this strategy might be less effective once detected by the prey, hence inspection might deter the predator from attacking (Pitcher 1992; Godin & Davis 1995).

Compared to the behavioral traits, selection on morphological traits was relativly low in the present study. Slightly downward bended individuals, with a deeper bodied appearance were more likely to survive than fish with a more slender appearance. Increased body depth is generally interpreted as an adaptive morphological prey characteristic that decreases a fish´s vulnerability to gape size limited pisivores (e.g.

Nilsson & Brönmark 2000). Indeed, Nilsson et al. (1995) could show that pike needs longer to process deep bodied crucian carp and preferably attacks slender bodied individuals. Magnhagen & Heibo 2001 found juvenile perch from pike dominated lakes to be deeper bodied than juvenile perch from lakes with a lower pike population, which they suggested to stem from a mixture of selective predation and phenotypic adaptation. We found pike predators to exhibit less morphological selection than perch predators. However, morphological variation in natural perch populations might be much more pronounced, suggesting that in the present study morphological variation might have been too small to be a selection criterion. Pike predators in the present study preyed less selectively than perch. Pike had a slightly lower starvation period and faced prey relatively larger than perch. Starvation is suggested to decrease selectivity in fish (Turesson et al. 2006), while increased relative prey size is assumed to pronounce morphological selection for shallow bodied prey in pike (Nilsson & Brönmark 2000). Hence, it might be suggested that the lower starvation period and the higher predator-prey-size ratio would rather increase selectivity in pike, i.e. indicating that differences in selectivity between the divergent predators observed in our study are probably not an experimental artifact. We found pike and perch to select differently on the different behavioral and morphological traits. Pike tended to positively select shallow bodied and non-vigilant individuals, while perch predators selected for shallow bodied and bolder juvenile perch. This supports the idea that different anti-predator defenses may not be independent from each other (e.g. Lind & Cresswell 2005). In previous studies different anti-predator behaviors (e.g. Lind & Cresswell 2005), but also morphological and behavioral defense traits were found to compensate or augment each other, depending on the ecological circumstances (Steiner & Pfeiffer 2007). For example goldfish (Chivers et al. 2007), anural tadpoles (*Rana pirica*) (Kishida et al. 2009) and largemouth bass (*Micropterus salmoides*) (Brown et al. 2002) were found to decrease anti-predator behavior with increasing body depth. However, we found no correlation between boldness, vigilance and body depth for juvenile perch. Vigilance, i.e. predator inspection is generally interpreted as an act of boldness. In our study boldness (time in unsheltered habitat, activity, latency to leave the shelter) (as defined by e.g. Sneddon 2003, Magnhagen & Borcherding 2008) and vigilance (predator inspection) loaded on different PC axes, giving some evidence they are not connected. Similar results were obtained in previous studies on juveniles from Swedish (Hellström et al. unpubl. data, Heynen et al.

unpubl. data) and German perch populations (Goldenberg et al. unpubl. data), fathead minnows (*Pimephales promelas*) (Pellegrini et al. 2010) and sticklebacks (Huntingford 1976). The reaction of sticklebacks towards a predator is summarized along a "precaution-investigation" (or predator inspection as defined by Pitcher 1992) and a "boldness-timidity" axis (activity, jerky swimming, spine rising) (Huntingford 1976). In fathead minnows, activity and predator inspection were also uncorrelated (Pellegrini et al. 2010). However, behavioral trait correlation or behavioral syndromes might also be species specific (Sih et al. 2004; Conrad et al. 2011). Our results on juvenile perch indicate that boldness and vigilance might represent uncorrelated alternative anti-predator tactics, as suggested for shoaling (Ward et al. 2002) and predator inspection (Walling et al. 2004) in sticklebacks (Bell & Sih 2007). This is assumed to be advantageous for juvenile perch, as we found pike and perch to select differently on the two behavioral traits. Furthermore, defense strategies might bear costs, confronting the prey with time and/or resource allocation trade-offs (Wolf et al. 2007; Steiner & Pfeiffer 2007). This can be also assumed in the present study, in which vigilance and shyness was associated with a lower foraging rate, as watching out for potential threats, inactivity, hiding and foraging are largely incompatible (e.g. Lind & Cresswell 2005). In previous studies, fishes were found to display a high degree of phenotypic plasticity (e.g. Smith & Skúlason 1996; Lima 1998; Robinson & Parsons 2002; and references therein). Juvenile perch were found to adapt their behavior on a long-term basis to the experienced level of predation risk (Magnhagen & Borcherding 2008), but also to short term changes of predation risk (Bean & Winfield 1995; Snickars et al. 2004; Borcherding 2006), by reducing activity and foraging, while intensifying the use of shelter. Furthermore, juvenile perch were found to increase in body depth in the presence of pike (Eklöv & Jonsson 2007). Hence, the results of the present study indicate that these plastic reactions are adaptive and might decrease an individual's vulnerability to predation. This might be particularly an advantageous in the natural environment, where predation risk is not a fixed constant factor (Lima 2002). Predation risk for juvenile perch is expected to be variable due to population size structure, density and distribution of pike and adult perch, and might change through harvesting (Lewin et al. 2006; Arlinghaus et al. 2009; van Kooten et al. 2010), natural population circles (Persson et al. 2003) and/or interactions between predators (Eklöv & Diehl 1994). In a recent study, Svanbäck and Persson (2009), suggested that the intrinsically driven population dynamics in perch may favor morphological plasticity in perch over genetic diversification. Bearing in mind the results of the present study, i.e. that the two most common predators select with different intensity on different traits, clearly supports the idea of Svanbäck and Persson (2009). Thus our results give some further hints, that not only the specific behavioral or morphological phenotypic reactions that individuals were found to display in response to predator risk (e.g. Bean & Winfield 1995; Eklöv & Jonsson 2007; Magnhagen & Borcherding 2008), but also the intrinsic ability to respond plastically to predation risk might be adaptive for perch (Svanback & Persson 2009; Kishida et al. 2010).

In conclusion, our results emphasized the importance of looking at more than one predator and more than one trait (e.g. Kishida & Nishimura 2005; Steiner & Pfeiffer 2007). This is mainly due because relative specific predation intensity for the divergent traits differed between the predators, in which the positive selection of perch predator on bold individuals was the most important. Thus, the results of the present study support the assumption that behavioral and morphological reactions of juvenile perch in response to a predator might be advantageous, as it was suggested in several previous studies (e.g. Snickars et al. 2004; Eklöv & Jonsson 2007; Magnhagen & Borcherding 2008). Furthermore, our results are, to the best of our knowledge, the first that analyzed behavioral and morphological adaptations of juvenile perch facing two different predation strategies. We provide some additional ideas as to why juvenile perch display such a high degree of phenotypic plasticity.

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ABSTRACT

Predation is one of the major structuring forces in animal communities, because most predators hunt selectively. This favors characteristics in prey that facilitate the avoidance of predation. Correspondingly, various and often plastic morphological and behavioral defense strategies have been found throughout numerous taxa. However, the expression of defense traits often confronts prey with time and/or resource allocation trade-offs. Thus behavioral defenses, like watching out for potential threats, inactivity and hiding largely are incompatible with foraging and the energy expenditure to build up morphological defenses cannot be allocated into e.g. growth, storage or reproduction. Therefore, additional effects, like an individual’s nutritional status, resource use, size and/or age balance, often influenced of these trade-offs. Furthermore, theoretical results indicate that the plasticity of a trait is *per se* determined by the adaptiveness of a given phenotype, its associated costs and the variability of the selective environmental agent. This illustrates the complexity of patterns shaping animal behavioral and morphological defense expression under predation risk. Eurasian perch (*Perca fluviatilis* L.) is a common freshwater species throughout Europe, where juvenile perch display consistent variation in morphology and behavior and both traits are sensitive to the environment and especially to predation risk. Therefore perch is an optimal model organism to study the complex defense trait dynamics in predator-prey interactions.

In the first study, a common garden setup was used to examine the genetic and environmental components of the morphological variation from two lake populations with differences in size-specific predation risk. We found differences in head and jaw length and slight differences in body depth between the wild young-of-the-year perch from Lake Ängersjön and Lake Fisksjön. The differences found between the wild fish from the two lakes were, however, not maintained under common garden rearing. The observed morphological divergence between the wild juvenile perch from Lake Ängersjön and Lake Fisksjön seems to stem mainly from a plastic response to different conditions in the two lakes. Morphological traits are not influenced by direct reaction to the size-specific risk of cannibalism, but probably stem from a combination of different environmental characteristics, including resource and habitat use, and the density of other piscivores such as pike.

In the second chapter young-of-the-year perch were reared on either fish larvae or zooplankton to investigate whether the use of divergent resources changes the reaction to a novel surrounding and the behavior under the threat of predation. Both phenotypes reacted differently under predation risk and inspected the predator more frequently when their familiar prey was presented during the trials, indicating that resource polymorphism may influence risk-taking behavior in juvenile fish.

The third study used juvenile 0+ and 1+ perch in an experimental approach to vary the factor of predation risk. Predators were able to feed on perch during a mesocosm period. Perceived predation risk affected the behavior and the morphology of both age classes of perch. Boldness decreased with the intensity of predation, while morphology of perch changed towards deeper bodied individuals. Although it remains unanswered if these changes are a result of selective predation or phenotypic response of the prey, the latter explanation is assumed to be conclusive because there was no correlation between the observed changes in the length-frequency distributions and the predation risk of perch.

In the final study, we measured behavioral and morphological traits in 0+ perch and compared their selective values in response to the two most common predators, adult perch and pike. Selection on behavioral traits was higher than on morphological traits and perch predators preyed overall more selectively than pike. Pike tended to positively choose shallow bodied and non-vigilant individuals. In contrast, perch predators selected mainly for bolder juvenile perch. These results indicate that shyness and increased body depth might be adaptive for juvenile perch under predation risk. However, the relative specific predation intensity for the divergent traits differed between the predators, providing some additional ideas why juvenile perch display such a high degree of phenotypic plasticity.

KURZZUSAMMENFASSUNG

Prädation wirkt auf Beutepopulationen stark strukturierend, da die meisten Räuber selektiv jagen. Dabei werden bei den Beutetieren solche Eigenschaften selektiv gefördert, die das Risiko mindern gefressen zu werden. Entsprechend finden sich im Tierreich zahlreiche Beispiele oft plastischer Körperform- und Verhaltensmerkmale, welche eine Vulnerabilität gegenüber Räubern senken. Allerdings ist die Merkmalsausprägung für ein Beutetier oft mit Kompromissen in der Zeit- und/oder Energieverteilung verbunden. So schließen sich Nahrungsaufnahme und Räuber-vermeidende Verhaltensweisen (wie Achtsamkeit, Inaktivität, Schutzsuchen) gegenseitig aus und die Energie, welche beim Aufbau körperlicher Verteidigungsstrukturen verwendet wird, kann nicht in Wachstum oder Reproduktion investiert werden. Folglich beeinflussen auch Faktoren wie Ernährungsstatus, Nahrungsnutzung, Größe und Alter eines Tieres die Investition von Zeit und Energie in Feindvermeidungsverhalten und Verteidigungsstrukturen. Des Weiteren deuten Ergebnisse aus theoretischen Studien an, dass die Plastizität eines Merkmals *per se* abhängig ist von der Adaptivität eines gegebenen Phänotyps, den assoziierten Kosten und der Variabilität des wirksamen Selektionsfaktors. Die Einflüsse, die bei einem Tier unter Räuberdruck die Ausprägung bestimmter Körperform- und Verhaltensmerkmale beeinflussen, sind also äußerst komplex.

Der Flussbarsch (*Perca fluviatilis* L.) ist einer der häufigsten Süßwasserfische Europas. Juvenile Flussbarsche zeigen stabile individuelle Unterschiede in Körperform und Verhalten, aber auch ein hohes Maß an phänotypischer Plastizität, wobei sie morphologisch und im Verhalten auf die gegebene Prädationsintensität reagieren. Damit erweist sich der Flussbarsch als ein geeignetes Modelltier, um die Ausprägung verschiedener Verteidigungsmerkmale und ihre komplexe Dynamik in Räuber-Beute Interaktionen zu untersuchen.

In einer ersten Studie wurden die Unterschiede in der Körperform zwischen zwei Seepopulationen mit unterschiedlichen größenspezifischen Prädationsrisiko untersucht, um deren genetische und plastische Komponenten zu bestimmen. Dafür wurden in einem Common-garden-Design Tiere beider Barschpopulationen im gleichen Habitat herangezogen, ihre Körperformen analysiert und sowohl miteinander als auch mit denen von Freilandtieren verglichen. Zwischen den 0+ Freilandbarschen aus den Seen Fisksjön und Ängersjön wurden Unterschiede in Kopf- und Kieferlänge und leichte Unterschiede in der Hochrückigkeit gefunden. Diese Unterschiede waren jedoch zwischen den Tieren beider Populationen, die im gleichen Habitat heranwuchsen, nicht mehr vorhanden.

Die im Freiland gefundenen Körperformunterschiede zwischen den juvenilen Flussbarschen aus den Seen Fisksjön und Ängersjön scheinen sich hauptsächlich aus plastischen Anpassungen an die verschiedenen Umwelteinflüsse der beiden Seen zu ergeben. Dabei scheinen diese Unterschiede in der Körperform keine direkte Folge des unterschiedlichen größenspezifischen Prädationsrisikos zu sein, sondern vielmehr eine plastische Reaktion auf eine Kombination von seespezifischen Umwelteinflüssen, wie Nahrungs- und Habitatnutzung und die Dichte anderer Raubfische, wie zum Beispiel Hecht.

In zweiten Teil der Studie wurden 0+ Barsche über 6 Wochen mit Fischlarven oder Zooplankton ernährt, um herauszufinden, inwieweit die Nutzung unterschiedlicher Ressourcen einerseits die Reaktion auf eine unbekannte Umgebung und andererseits das Verhalten unter Räuberdruck verändert. Beide Phänotypen reagierten unterschiedlich auf Räuberrisiko und beobachteten den Räuber öfter, wenn ihnen eine vertraute Ressource angeboten wurde. Diese Ergebnisse lassen vermuten, dass Nahrungspolymorphismus die Risikobereitschaft von juvenilen Flussbarschen unter Räuberdruck beeinflussen kann.

In der dritten Studie wurden 0+ und 1+ Flussbarsche in einem experimentellen Ansatz unterschiedlich starkem Räuberdruck ausgesetzt. Die Raubfische konnten die Beutetiere während des Mesokosmos-Experiments jagen und fressen. Das erlebte Räuberrisiko beeinflusste Körperform und Verhalten in beiden Altersklassen. Die Risikobereitschaft der Flussbarsche sank mit steigendem Räuberrisiko und die Tiere hatten eine hockrückigere Körperform. Allerdings bleibt es ungeklärt, ob diese Veränderungen selektiven Fraß der Räuber oder eine plastische Anpassung der Beutetiere reflektieren. Die letztere Erklärung scheint hierbei wahrscheinlicher, da keine Korrelation zwischen den beobachteten Veränderungen der Längenvariabilität und dem Räuberrisiko gefunden wurde.

In der letzten Studie, wurden Verhaltens- und Körperformmerkmale von 0+ Barschen gemessen, um den artspezifischen, selektiven Fraßdruck von adulten Hechten und Barschen auf bestimmte Morphen oder Verhaltensphänotypen zu untersuchen. Die Selektion auf Verhaltensmerkmale war ausgeprägter als jene auf Körperformmerkmale, und adulte Barsche jagten deutlich selektiver als Hechte. Hechte tendierten dazu, flachere und weniger achtsame Individuen zu jagen. Adulte Barsche dagegen fraßen bevorzugt risikobereite juvenile Barsche.

Diese Ergebnisse lassen vermuten, dass Schüchternheit und Hochrückigkeit für juvenile Barsche unter Räuberdruck vorteilhaft sind, wobei sich die relative spezifische Selektionsintensität auf die verschieden Merkmale zwischen den Räubern unterscheidet, was wiederum eine weitere Erklärung für die außerordentliche phänotypische Plastizität juveniler Barsche darstellt.

ERKLÄRUNG

Köln, den 28.04.2011

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Abbildungen und Tabellen – die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzellfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie – abgesehen von den auf der folgenden Seite angegebenen Teilpublikationen – noch nicht veröffentlicht worden ist sowie, dass ich solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von PD Dr. habil Jost Borcherding betreut worden.

Martina Heynen

TEILPUBLIKATIONEN

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