Intraspecific variation in phenotypic plasticity

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Academic dissertation

To be presented for public examination with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki in Auditorium 3, Viikki Building B, on November 30th 2012 at 1 p.m.
So long, so long and thanks
for all the fish

Douglas Adams
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V. Välimäki K, Herczeg G & Merilä J. Intraspecific variation in energy storage traits in ecologically divergent nine-spined stickleback populations. *Manuscript.*


### Author contributions to the articles

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Abstract

Understanding the causes and consequences of phenotypic divergence among natural populations is one of the fundamental goals of evolutionary biology. Phenotypic differentiation among populations can arise through divergent selection leading to local adaptation, phenotypic plasticity, or a combination thereof. Selection can also influence the expression of plasticity in different environments resulting in divergence in phenotypic plasticity. It is therefore essential to know how plasticity evolves under divergent ecological conditions when aiming to understand the mechanisms that underlie phenotypic differentiation.

In this thesis I have explored the extent of variation in phenotypic plasticity across a range of locally adapted nine-spined stickleback (*Pungitius pungitius*) populations from different habitats. Pond and marine populations have diverged in a suite of morphological, life-history and behavioural traits. This divergence has been thought to stem from the absence of piscine predators and interspecific competitors combined with the high intraspecific competition in pond habitats. My aims were to establish if and how predator cues and variation in resource levels induce phenotypic plasticity in the nine-spined stickleback, and whether phenotypic plasticity has also diverged as a response to divergent selective pressures in different environments and between sexes. I quantified phenotypic plasticity in a set of traits that form a continuum from very labile to developmentally fixed ones.

The results show that plasticity was induced by both predator presence and food availability. Fish responded to perceived predation risk with reduced growth rates, decreased body condition and by behavioural changes. Food restriction resulted in slower growth and reduced investment in energy storage, but increased feeding activity and risk-taking. The results were largely in accordance with my predictions of increasing plasticity from morphology through life history traits to behaviour. The results also show that population divergence in phenotypic plasticity is habitat dependent. Pond populations responded more strongly to food treatment in terms of behaviour and growth, while marine fish exhibited stronger responses to predation treatment. However, in the case of brain size and lateral line system, predation-induced plastic responses were detected only in pond fish that had evolved in the absence of piscine predation. I also detected strong sexual dimorphism in both trait means and phenotypic plasticity, uncovering a very important determinant of within population variation in phenotypic plasticity.

Taken together, the results of my thesis demonstrate how natural and sexual selection do not only affect phenotypic traits themselves, but also the plasticity of the traits. The resultant adaptive variation in phenotypic plasticity is present both between and within populations. In some traits, plasticity was greater whenever the selective pressure was stronger, while in other traits the increased plasticity was coupled with relaxed selection due to the lack of piscine predation in ponds. My thesis demonstrates that the response of phenotypic plasticity to natural selection is context dependent. The results also work to advance our knowledge on the maintenance of phenotypic variation.
Introduction

Evolutionary biologists are fascinated about the phenotypic variation surrounding us, and this interests spans from individual to taxonomic level diversity. In fact, principles of evolutionary thinking were based on research which aimed essentially to understand ultimate causes of variation in different life-forms (Darwin 1859). Indeed, phenotypic variance, how it emerges and how it is maintained, is still one of the most fundamental questions in evolutionary biology. Knowledge about causes and consequences of phenotypic variation are even more important now when anthropogenic changes are threatening survival of the species in form of climate change, habitat loss and pollution (Hanski 1999). In many regards the study of if and how organisms adapt to changing environmental conditions has gained great interest (e.g. Hoffmann and Willi 2008; Merilä 2012). Apart from evasione (e.g. range shifts), the two most important mechanisms behind response to varying environmental conditions are (i) adaptation (shifting the allele frequencies at the population [deme] level to maximise fitness; e.g. Kawecki and Ebert 2004) and (ii) phenotypic plasticity (production of new superior phenotypes from a single genotype; Schlichting and Pigliucci 1998; West-Eberhard 2003; Miner et al. 2005; Pigliucci 2005).

While local adaptation and the genetic changes it entails have been the cornerstones of the study of evolution since the formation of modern synthesis (Fisher 1930; Huxley 1942), phenotypic plasticity was long treated as a nuisance or considered to be unimportant for evolutionary studies. Phenotypic plasticity was thought to confound the results of experiments (Falconer 1952), and to damper the effects of selection (reviewed in Pfennig et al. 2010). However, partly thanks to the unifying review papers by Schlichting (1986), Sultan (1987), West-Eberhard (1989), Stearns (1989) and Scheiner (1993), the misunderstandings concerning the role of environmental variation in evolutionary change have been revised. In modern thinking, phenotypic plasticity is one of the central concepts of evolutionary biology, which brings together developmental biology and genetics. It is now generally accepted that phenotypic plasticity can be adaptive and beneficial (Gomulkiewicz and Kirkpatrick 1992; Moran 1992; Gotthard and Nylin 1995; Sultan and Spencer 2002; Ernande and Dieckmann 2004; Beldade et al. 2011), but it can also be neutral or even maladaptive (Price et al. 2003; Ghulambor et al. 2007). Nevertheless, discussion on the nature of phenotypic plasticity and its role in evolution is still ongoing (DeWitt and Scheiner 2004; de Jong 2005; Pigliucci 2005; Pigliucci et al. 2006; Ghulambor et al. 2007; Lande 2009; Pfennig et al. 2010; Moczek et al. 2011; Wund 2012). According to some authors (e.g. de Jong 2005), plasticity itself is a quantitative trait that can be under selection, while others have suggested that plasticity can participate in evolutionary processes by bringing populations closer to adaptive peaks (Price et al. 2003; Ghulambor et al. 2007), or that plasticity can be the starting point for novel evolutionary inventions (West-Eberhard 2005; Pigliucci et al. 2006; Pigliucci 2007). Based on this discussion, it is easy to understand why there are still many open questions about the role of phenotypic plasticity in adaptive evolution.

In the end, phenotypes are products of both environmental and genetic effects. What we see in the wild might also be the result of either co- or countergradient variation (Conover and Schultz 1995; Conover et al. 2009). These two phenomena reflect the interaction and direction of phenotypic change as a response to genetic and environmental effects. Covariation refers to the case in which genetic and environmental influences on a trait act towards the same direction, and countergradient variation refers to the case where genetic and environmental effects are opposing each other, sometimes equalling each other and resulting in the apparent lack of phenotypic change (e.g. Arendt and Wilson 1999; Craig and Foote 2001; Laugan 2003). Similarly, phenotypic plasticity can increase phenotypic variation in populations under divergent selection, or it can create convergence of phenotypes within genetically diverse
populations exposed to the same selective pressure. Hence, to understand the underlying causes of phenotypic variation, we need to be able to tell apart its genetic and environmentally induced components. This is usually done in form of common garden experiments in laboratory, or sometimes, by using reciprocal transplant experiments in the wild (Conover and Schultz 1995; Gotthard and Nylin 1995).

What is phenotypic plasticity?

Phenotypic plasticity is defined as the ability of one genotype to express multiple phenotypes as a response to different environmental cues (West-Eberhard 2003). Common examples of phenotypic plasticity include the formation of pigment in the skin of humans as a response to ultraviolet radiation (i.e. tanning), and the ability of plants to grow longer stems in shady places to capture more sunlight. Phenotypic plasticity is found in almost every trait, from the level of gene-expression to behaviour and morphology (reviewed in Whitman and Agrawal 2009). Often a separation is made between how plasticity in traits is expressed. Some traits can only be modified during certain developmental period and can be irreversible, while some are flexible - even reversible - like many of the behavioural and physiological traits (Gabriel 2006; Crispo 2008). Environmentally induced variation can be expressed as distinct-stage polyphenisms or as a continuum of different phenotypes between extremes. Plasticity can also be restricted to one generation, to one developmental state, but it can also be present in several generations via transgenerational maternal effects (Schlichting and Pigliucci 1998; West-Eberhard 2003). Often variation in individual phenotype in different environments is described in form of a reaction norm. These are typically visualized as lines or curves where environmental gradient is plotted against variation in phenotype (Box 1; Schlichting and Pigliucci 1998).

Phenotypic plasticity can facilitate acclimatisation and possibly adaptation to new environmental conditions (Stearns 1989; Scheiner 1993; Giannapp et al. 2008). Often there is also variation in the level of phenotypic plasticity between populations. What then explains when phenotypic plasticity is expressed, and how does it evolve? Evolution of phenotypic plasticity is dependent on the relative costs and benefits of plasticity in a given environment. Plasticity

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**Box 1.** The relationship between phenotypic plasticity and genetic variation

![Diagram](https://via.placeholder.com/150)

The lines represents two genotypes and their expression in relation to variation in environment. a) The phenotypes of the two genotypes are different, but they are insensitive to environmental variation. Phenotypic plasticity is absent. b) The two genotypes are sensitive to environmental variation, but the magnitude of plastic response is similar in both genotypes. Plasticity is present, but genetic variation in plasticity is absent. c) The slopes of the reaction norms are different for both genotypes, thus there is plasticity and genetic variation in plasticity (genotype × environment interaction).
Introduction

is favoured in environments characterized by temporal or fine grained spatial heterogeneity (Sultan and Spencer 2002; Bathyavong 2011). Predictability of environmental variation and gene flow (Via 1993; Scheiner 1998; Sultan and Spencer 2002; Crispo 2008) are also beneficial for the evolution of phenotypic plasticity. Plasticity is also favoured when different phenotypes have selective advantage in different environments and none of the phenotypes is superior in all environments. The main factors constraining evolution of plasticity are the costs of plasticity and the lack of genetic variation in plasticity. The costs of plasticity can be defined as the extra costs (loss of fitness) that an individual has to pay when expressing a plastic phenotype in comparison to having a fixed phenotype (De Witt 1998; Pigliucci 2005; Auld et al. 2010). Genotype × environment interaction in the expression of a phenotypic trait can be detected by reaction norms in two or more populations showing divergent response to the same environmental factor (Gillespie and Turelli 1989; Schlötterer and Pigliucci 1998; Box 1). In a spatially or temporally invariant environment plasticity can be reduced through the process of genetic assimilation (Waddington 1953). Genetic assimilation is a process where a previously environmentally induced phenotype becomes canalized and environmental cues are no longer needed for its expression (Waddington 1953; Crispo 2007, 2008).

In his review of phenotypic plasticity, Pigliucci (2005) emphasized the need for studies that would increase our understanding on which ecological conditions favour stabilizing or directional selection on reaction norms. In particular, studies where phenotypic plasticity is examined using populations/individuals originating from divergent selective environments can help us to better understand the interplay between local adaptation and phenotypic plasticity. Since that review (Pigliucci 2005), the number of studies examining phenotypic plasticity in different populations with divergent selective pressures has increased. Some studies have compared phenotypic plasticity between ancestral and derived populations (Day et al. 1994; Messler et al. 2007; Wund et al. 2008), or after colonisation of new habitats (Edgell et al. 2009; Karlsson et al. 2010; Harris et al. 2011). How variation in abiotic environmental factors influences plasticity has been studied for instance in frogs (pool desiccation: Laurila et al. 2002; Lind and Johansson 2007, 2009; Lind et al. 2011) and fish (anoxia: Chapman et al. 2000; Crispo and Chapman 2010a,b). There are also examples of variation in phenotypic plasticity in populations that have experienced variation in predation pressure (De Meester 1993; Trussell 2000; Relyea 2002; Van Buskirk and Arioli 2005; Latta et al. 2007; Dowdall et al. 2012). However, studies comparing phenotypic plasticity induced by certain biotic environmental factors between populations that are locally adapted to different levels of those biotic environmental factors are still scarce (e.g. Rogell et al. 2012).

**Predator- and food-induced plasticity**

Predation is an important environmental factor affecting life-history and population dynamics of the prey (Roff 1992). There is a number of constitutive antipredatory traits that have evolved as a response to predation pressure in various species (Tollrian and Harwell 1999). Although fixed within populations, these traits often vary between populations as a response to the heterogeneity in the type of predators and the predation pressure that individuals from different populations are likely to encounter. However, predation regimes might change abruptly, in which case fixed antipredatory traits (or a fixed lack thereof) will come with a cost (Harvell 1990; Baker et al. 2010). Therefore many organisms have developed plastic antipredatory traits which are turned on only under predation risk and might be either irreversible (like many morphological traits) or reversible (e.g. behaviour) (West-Eberhard 2003).

Predator-induced plasticity has been observed in several different traits. Classic examples are morphological defensive traits, like spines and helmets in cladocerans (Black 1993; Tollrian 1995; Riessen 1999; Weber 2001; Hammill et al. 2008; Pauwels et al. 2010), and deeper body form in tadpoles (Van Buskirk and Schmidt 2000; Relyea 2001; Laurila et al. 2002, 2008; Teplitsky et al. 2005). In fish, induced morphological antipredatory responses include deeper body size and longer spines (Brönmark and Miner 1992; Januszkiewicz and Robinson
Besides morphology, predator-induced plasticity is common in behaviour and life history traits. Exposure to predators is often decreased through reduced activity and exploration (Werner et al. 1993; Gotthard 2000; Biro et al. 2004). Similarly, predation can modify individual personality traits like boldness, aggression and risk-taking (Sih et al. 2004). The behavioural shifts induced by the presence of predators can also be reflected in life-history traits. Reduced activity makes individuals less vulnerable to predators, but at the same time it can decrease energy intake, which can further affect growth, energy storage or reproductive success negatively (Werner et al. 1993; Gotthard 2000; Biro et al. 2004). In spite of the general interest towards predator-induced plasticity, information on predator-induced plasticity in neuroanatomical and sensory systems is still lacking.

When organisms colonize new habitats, they often face shifts in predation regime (Harris et al. 2011). A typical case would be the loss of predators in habitats where migration becomes restricted, such as in ponds after they become isolated. Such relaxed selection often causes decrease in the expression of antipredatory traits (Blumstein and Daniel 2003; Messler et al. 2007; Lahti et al. 2009) due to stochastic processes like mutation accumulation or gene flow. However, it is difficult to predict how plastic responses will evolve under relaxed selection. If plasticity is not expressed due to the lack of environmental induction, it cannot be under direct selection. In this case, selection can only act through the costs associated with maintaining the capacity for phenotypic plasticity (Masel et al. 2007; Hall and Colegrave 2008; Lahti et al. 2009). Non-functional plasticity can thus be retained in the population, depending on the costs that were originally involved. Hence, the capacity to express traits that are non-functional in the given environment can be hypothesised to persist longer if the trait is more labile in nature. One could assume for instance that non-functional phenotypic plasticity of behavioural traits can persist longer than that of life-history traits, non-functional phenotypic plasticity in life-history traits can persist longer than in morphological traits, etc. For instance, populations of California ground squirrel (Spermophilus beecheyi) have retained antipredatory responses after isolation from rattlesnakes for approximately 70 000 years, while the arctic ground squirrels (S. parryii) have lost their antipredatory responses towards rattlesnakes after 3 000 000 years in isolation (Foster 1999).

Availability of food resources obviously has a strong impact on many life history traits. Low food availability often results in reduced growth, energy storage, size at maturity and reproductive output (Wootton 1973). Intraspecific food competition can influence behavioural patterns and even influence formation of species pairs (reviewed in Ward et al. 2006). Food availability can also affect behaviour: scarcity of resources can force organisms towards higher activity to obtain food, which exposes them to greater risk of predation (Biro et al. 2004, 2005). Predators and food availability can also have combined effects: for instance development of predator-induced morphological traits are known be facilitated by abundant food resources (Noonburg and Nisbet 2005; Teplitsky et al. 2005; Chivers et al. 2007), while growth can be suppressed by predation risk alone, even in the presence of abundant and nutritious food (Nicieza et al. 2006). However, interactions between food and predation are often context-dependent and unifying patterns have been difficult to find (reviewed in Bolnick and Preisser 2005). Further, plastic responses in traits are known to be generated not just by the amount but also by the type of food eaten. For instance, perch (Perca fluviatilis) will develop either deeper or slimmer body, depending whether they are on littoral or pelagic diet, respectively (Svanbäck and Eklöv 2006). Similarly, three-spined stickleback (Gasterosteus aculeatus) head size exhibits a plastic response to food type (Day et al. 1994; Wund et al. 2008).

**Study system**

Nine-spined stickleback (Pungitius pungitius) is a small teleost fish occurring throughout the Northern hemisphere. The Latin name pungitius means prickly, pointy and accurately describes the visual appearance of nine-spined stickleback with 8-12 dorsal spines and two pelvic spines (Banarescu and Paepke 2001). It occupies a wide variety of habitats ranging from saline sea waters, through streams and large lakes into...
isolated pond environments (Banarescu and Paepke 2001). In ponds, nine-spined stickleback can persist as a single fish species. Research on nine-spined stickleback in Fennoscandia has established strong patterns of habitat-specific intraspecific differentiation in the pond – marine habitat axis. The two types of stickleback (Box 2), pond and marine, are divergent in number of fitness related traits (behaviour: Herczeg et al. 2009b,c, brain architecture: Gonda et al. 2009, 2011; Gonda 2011, lateral line system: Trokovic et al. 2011, 2012, reproductive output: Herczeg et al. 2010a; Ab Ghani et al. 2012, body size: Herczeg et al. 2009a, 2010a, growth: Shimada et al. 2011; Herczeg et al. 2012 and morphology: Herczeg et al. 2010b; Box 2). The two important selective factors in this system appear to be variation in intensity of piscine predation and the consequent variation in degree of intraspecific competition. In the absence of piscine predation in ponds, success in intraspecific competition becomes the key factor in fitness, and this is the reason for the repeated, independent evolution of the giant, competitive phenotype (Herczeg et al. 2009a,b,c, 2012, Box 2). In the marine environment harbouring the ancestral morph, predation and interspecific competition are likely to be important factors affecting fitness and these environments are inhabited by the commonly found small, shy phenotype.

Nine-spined stickleback are excellent models for studying predation and food induced phenotypic plasticity, and the variation of that plasticity between populations adapted to different predation / competition scenarios using various traits. Thinking about evolution of plasticity the two environments, pond and marine have different selective pressures. Predation by piscivorous fish is either absent or present, though predation pressure can fluctuate both temporarily and spatially between populations in marine habitats. Nine-spined stickleback are vulnerable to almost any sympatric predatory fish (e.g. salmonids, *Salmo sp.*; perch; lake *Esox lucius*; pikeperch *Sander lucioperca*), which all can be considered as gape-unlimited predators of the nine-spined stickleback, because the defence value of the nine-spined stickleback bony armour is way lower than that of the closely related three-spined stickleback (Hoogland et al. 1957). Food is obviously present in both habitat types, but stochasticity in food availability is likely to be larger in pond habitats with sometimes very low resource levels. While there is no quantitative proof for this, in the sampling sites of this thesis, extreme density of fish in very poor condition has repeatedly been observed during some springs in the ponds, while there have been no such observations from the marine sites (Välimäki, Herczeg, Gonda personal observation). This might happen due to the small size of the ponds and the resulting environmental stochasticity. Further, success in intraspecific competition seems to be a key to high fitness in the ponds without piscine predation or interspecific competition.

**Box 2. Two types of stickleback**

The Fennoscandian pond type (upper fish) can reach giant body size. Body weight of adult marine nine-spined stickleback are around 1-2g with a total length of 5-6cm, while extremely large pond sticklebacks can be heavier than 8g and longer than 12cm (Herczeg et al. 2009a). They have an extended growth period and delayed maturation (Shimada et al. 2011 Herczeg et al. 2012). Giant pond females can produce 2-3 times larger clutches than marine females but egg production is linearly related to body length (Ab Ghani et al. 2012). Fish in ponds are bold and aggressive and they have larger costs from group-living than marine fish (Herczeg et al. 2009b,c). Body armour is reduced and the whole pelvic complex with spines can be missing in ponds (Herczeg et al. 2010b, Mobley et al. 2011). Relative telecephalon and bulbous olfactory size is smaller in pond fish (Gonda 2011) and they have lower number of neuromasts, with high intrapopulation variation in neuromast numbers (Trokovic et al. 2011).

The marine ecomorph (lower fish) is small (Herczeg et al. 2009a) and while growth rate (mass/time unit) is slower, they reach their asymptotic size and mature faster (Ab Ghani et al. 2012, Herczeg et al. 2012). It has full armour with several lateral plates and long spines (Herczeg et al. 2010b). It is also shy, not aggressive or explorative (Herczeg et al. 2009b). It has larger bulbous olfactoryus and telencephalon size (Gonda 2011) and higher number of neuromasts than pond fish, but intrapopulation variance in neuromast number is lower (Trokovic et al. 2011).
(e.g. Herczeg et al. 2009a,b,c; 2012). In theory, the situation outlined above might lead to a larger range of food-induced phenotypes in pond populations together with canalisation (in this case, loss) of predator-induced responses. Along the same lines, variation in predation pressure could generate larger variation of phenotypes in marine populations, while the food-induced variation should be lower.

**Aims of the thesis**

Although geographic (interpopulation) variation in phenotypic plasticity has been already demonstrated (e.g. Kishida et al. 2007; Edgell et al. 2009; Lind and Johansson 2009; Dowdall et al. 2012), our knowledge on this topic is still limited. This is especially true with regard to variation in phenotypic plasticity among populations locally adapted to different levels of environmental factors actually inducing the plasticity. Such studies are essential for our understanding of the interplay between local adaptation and phenotypic plasticity induced by the same environmental factors at different (evolutionary vs. ontogenetic) levels. Finally, it can be informative to compare phenotypic plasticity of different phenotypic traits (morphological, life-history, neuroanatomical, behaviour, etc.) in the same study system both within and between locally adapted populations to seek for general vs. individual patterns.

In this thesis, my aim was to investigate the following main questions:

1. Is there predation- or food-induced phenotypic plasticity in nine-spined stickleback?

2. Does the presence/absence and strength of phenotypic plasticity vary across different trait types?

3. How adaptation to local selection pressures has influenced the expression of phenotypic plasticity, with special focus on how relaxed selection might have shaped expression of plasticity?

4. Does the degree of phenotypic plasticity in different traits differ between the sexes?

I studied food- and predation-induced plasticity using manipulative factorial common garden experiment in six different fitness-related traits which can be viewed to represent a cline from very constitutive morphological traits to very flexible behavioural traits. In chapter (I), I studied the plasticity in body shape and defensive armour. Bony armour is costly and plays a central role in predator defence of stickleback. Reduction and loss of armour is related to predator absence in nine-spined stickleback (Hoogland et al. 1957; Herczeg et al. 2010; Mobley et al. 2011). Plasticity in the size of different brain parts was the target of the chapter (II). Lateral lines are important sensory organs found in aquatic vertebrates that are little studied from a microevolutionary perspective. Chapter (III) represents the first study to investigate predator and food induced plasticity in lateral line system. Chapter (IV) focused on plasticity in body size and growth, traits under strong directional selection which has resulted in evolution of gigantism and distinct growth strategies (Herczeg et al. 2009a, 2012, Shimada et al. 2011) in the studied pond populations. In chapter (V), I targeted energy storage traits, important fitness components which are likely to trade-off with growth rates, which in turn are divergent among my study populations (Ab Ghani et al. 2012; Herczeg et al. 2012). Finally, chapter (VI) focuses on behavioural plasticity.

**Materials and Methods**

**Experimental setup**

The results presented in my thesis are based on a large common garden experiment performed between June 2009 - April 2010. I caught parental fish from six populations (Fig. 1) at the onset of reproductive season in early summer, with minnow traps and seine nets. 6-10 artificial crosses per population were performed at the aquacultural facilities in University of Helsinki. Breeding conditions are described in the chapters of this thesis. The hatched fry were placed in to their individual 1.4l containers in four Allentown Zebrafish Rack Systems (Fig. 2a).
Treatments

I applied two treatments: food and predation. All racks (Fig. 2a) were fitted with an extra 150l container and water in the closed rack system circulated through these extra tanks before it entered individual containers. I chose two racks randomly for predation treatment and placed two perches into each of their extra tanks. Perch is a common predator in the low salinity Baltic Sea and also the most common predatory fish in Fennoscandian freshwaters (Ådjers et al. 2006). Hence, it is an excellent species to be used for producing predatory stimulus for nine-spined stickleback. For the food treatments, fish were assigned randomly within population/family/rack into high and low food treatments. The high food group received food twice a day in excess (assumed to represent ad libitum feeding given the amount of uneaten food I had to remove from the tanks regularly), whereas the low food group were only fed once every second day. Feeding was started with live brine shrimp (*Artemia salina*) nauplii and after 80 days gradually changed to frozen bloodworms (Chironomidae sp).

**Figure 1.** Map of the sampling sites of the parental generations.

**Figure 2.** A) One of the four zebrafish racks used in this experiment. B) Over-anaesthetised nine-spined stickleback is being prepared for the last photograph where both body size and shape are measured. C) Brain size and architecture was analyzed taking pictures from three different angles. D) Close up on neuromasts under fluorescense microscope.
Measured traits

Body armour and morphology
Sticklebacks have protective armour which consists of lateral plates and dorsal and pelvic spines, and is effective against piscine predators (Hoogland et al. 1957; Gross 1978). Variation in body shape is connected to performance in either benthic or pelagic environments. Streamlined body with long and narrow caudal peduncle is optimal for prolonged steady swimming in pelagic environments, while in benthic environments, a deep body with short caudal peduncle is favoured as it results in increased manoeuvrability (Webb 1983; Walker 1997; Bergstrom 2002). Occupation of predator-free ponds after the most recent glaciation has resulted in significant loss of body armour and divergence in body shape in the nine-spined stickleback (Herczeg et al. 2010b; Mobley et al. 2011). Predator-induced plasticity in body shape has been previously shown for three-spined stickleback (Frommen et al. 2010). Morphology (armour and shape, see Fig. 2b) was measured at the end of the experiment using over-anaesthetised fish. Digital photographs were made of the lateral side of each fish, lateral plates were counted, and length of pelvic girdle and pelvic spine was measured. Body shape was analysed using landmark-based geometric morphometrics (Bookstein 1991; Rohlf 1999; Zelditch et al. 2004). Lateral plates were counted under stereomicroscope. Length of pelvic girdle and pelvic spine was measured with digital calliper.

Brain
Variation in brain size and size of different brain parts is common between species and higher taxa (Kotrschal et al. 1998; Day et al. 2005). Brain size has large potential for both ontogenetic and environmentally induced plasticity (Wagner 2003; Lowsley et al. 2007). It is often found that brain parts that are important in some context are larger than the less important parts (Kihlström and Nevitt 2006). While environmentally induced plasticity in brain size variation is often detected, studies testing how biotic environmental stressors can influence brain development are scarce, as well as studies comparing brain plasticity between populations (Gonda 2011). Brain size and architecture was measured from digital photographs taken from formalin fixed brains (Fig. 2c). The volume of total brains and different brain parts were estimated using the ellipsoid model (Huber et al. 1997; Pollen et al. 2007).

Lateral line system
Fish use their lateral line system to detect subtle variations in water flow (Dijkgraaf 1963; Bleckmann 1993). It is an important organ in predator (Blaxter and Fuiman 1990; Fuiman 1993) and prey detection (Hoekstra and Janssen 1985; Montgomery and Macdonald 1987) and schooling (Partridge and Pitcher 1980). Intraspecific variation in lateral line system has rarely been addressed, but few recent studies have shown that variation in biotic and abiotic conditions have led differentiation in number of neuromast in the lateral line system (Michel et al. 2005; Wark and Peichel 2010; Yoshizawa et al. 2010; Trokovic et al. 2011; 2012). Neuromasts are the functional units of lateral line system (Fig. 2d), and consists of bundles of hair cells within a protective cupula, which can locate in separate canals or on skin surface (canal and superficial neuromast, respectively). Neuromasts are distributed throughout the body surface in separate groups (Fig. 3). Neuromasts were dyed with DASPEI vital fluorescent dyes and counted under fluorescence microscope using the over-anaesthetised fish at the end of the experiment.

Body size
Body size is a fundamentally important trait of biological interest as it is often directly connected to individual fitness (Peters 1983). In fish, larger body size correlates positively with fecundity (Wootton 1998), competitive ability (Rowland 1982; Johnsson 1993), mating success (Andersson, 1994) and life expectancy (Hutchings 1994). Nine-spined stickleback in ponds can reach gigantic sizes (twice as long as the adult marine fish) and the size divergence has genetic basis (Herczeg et al. 2009a, 2012, Ab Ghani 2012). In fish, body size and growth are strongly affected by competition and food availability; higher density, food competition and low food availability often lead to suppressed
growth and body size (Dmitriew 2010). Threat of predation can either suppress growth and body size through restricted movement and feeding (Biro et al. 2005, 2006; Strobbe et al. 2010; Thaler et al. 2012) or increase growth rates as a response to gape-limited predation (Werner and Gilliam 1984; Abrams and Rowe 1996; Urban 2007, 2008). Body size was measured at 60 days intervals from lateral side photographs (Fig. 2b).

**Energy storage**

Fish store energy in form of lipids in the body cavity (as a distinct fatbody), in muscle tissue and liver (Chellappa et al. 1989). Glycogens are stored in muscle tissue and liver. Energy stores are extremely important for fitness, especially in temperate areas where hibernation is common and energy for reproduction is often limited during spring. Local adaptation in energy storage to different lengths of activity season has been observed along latitudinal and altitudinal clines (Schultz and Conover 1997; Finstad et al. 2009; Jönsson et al. 2009; Takahashi and Pauley 2010; Berg et al. 2011), whereas adaptation to biotic conditions like predation has been rarely addressed. Experimental studies have shown that fish grown together with predators had lowered their energy storages (Pratt and Fox 2002; Garvey et al. 2004; Laakonen 2006). Predation can influence patterns of energy storage either directly or through altered growth rates. In this thesis energy reserves were estimated from lean body weight (eviscerated body mass), fatbody weight and liver weight.

**Behaviour**

Population variation in behaviour is common (Foster 1999; Foster and Endler 1999). Several studies have found genetic basis for observed variation between different habitat types (e.g. Brown et al. 2007; Shaw et al. 2007). Predation is an important selective factor influencing behaviour and it can often impact several behaviours at the same time (Magurran and Seghers 1991, 1994; Herczeg et al. 2009a). Behaviour is often very plastic and shows ontogenetic responses to both predation and food stress (Tollrian and Harwell 1999; Relyea 2001; Biro et al. 2005; Steiner 2007). However, predation and food induced variation in behaviour is rarely studied in populations that have adapted to varying level of predation and competition. Behaviour was assessed between 30-34 weeks old fish. I measured feeding activity, risk-taking, exploration and aggression following Herczeg et al. (2009b). Briefly, feeding activity was estimated as the time needed till the first bite attempt during a normal feeding event, risk-taking was assessed as the time needed till the first bite attempt after a stimulated predatory attack, exploration was assessed as the time needed to leave a dark start box and exploring a simple maze (Fig. 4), while aggression was estimated based on time spent orienting towards and number of attacks against a smaller stimulus fish presented in a tank next to the focal fish.
Results and Discussion

The most important questions and answers included in this thesis are summarized in Table 1. In what follows I will discuss the importance of those results in relation to the study of nine-spined stickleback adaptive evolution following the invasion of small ponds, and in wider perspective, to the study of adaptive phenotypic variation in the wild. The detailed discussion of the results can be found from the individual chapters. The following section will have three main parts: first, I will present and discuss my results regarding phenotypic plasticity induced by presence/absence of perceived predation risk represented by olfactory cues from perch, and high/low food availability. Second, I will discuss habitat-dependent population divergence in the observed phenotypic plasticity. Third, I will discuss the results in respect to sex differences in phenotypic plasticity and finally shortly describe the results concerning pure habitat specific population divergence. By addressing these questions with a suit of fitness-related traits differing in their expected level of plasticity (West-Eberhard 2003), I believe that I have been able to gain good insight into both the within- and among population components of intraspecific variation in phenotypic plasticity.

Plasticity from different angles

Previous research on nine-spined stickleback in Fennoscandia has shown that the biotic factors of predation and competition are important forces driving adaptive divergence in the species. One of the aims of this thesis was to establish what kind of role phenotypic plasticity can play in this system. This was especially interesting because previous studies have implicated that when wild-caught and common-garden individuals are compared, the variation in the former tended to be greater indicating that in addition to genetically-based local adaptations, direct environmental induction is also affecting the phenotypes (Herczeg et al. 2009; Gonda et al. 2011; Trokovic et al. 2011). My results show that both predation and food treatments induced plastic responses. However, the patterns were highly incongruent among traits. Body armour and body shape – even though the evolutionary significance of these traits is highly recognized and nine-spined stickleback show great divergence in them (Gross 1979; Herczeg et al. 2010b; Mobley et al. 2011) – were practically unaffected by the treatments (I). Predation had major effects on olfactory bulb development (II), growth (IV) and behaviour (VI) and lesser effects on lateral lines and energy reserves (III and V, respectively). While one could have predicted that available energy has a major effect on all traits, the response was completely lacking in the brain (II), lateral line system (III) and even in some behavioural traits (VI).

Predator-induced plasticity was present in behavioural traits (VI): fish decreased risk-taking and aggression under predation risk. However, a response was absent in feeding activity and exploration. Considering that predation risk also resulted in reduced body size (IV) and relative body weight (V) of the fish, it was interesting that feeding activity was unaffected by predation. Secretive behaviour and reduced feeding are commonly suggested reasons for smaller body size under predation threat (Lima and Dill 1990; Werner et al. 1993). Here, the larger body size in the absence of perceived predation risk might be due to some behavioural traits that were not measured, like time spent feeding after the initial feeding attempt. Or smaller body size can be a physiological response to predator stress reflecting maladaptive plasticity (Ghalambor et al. 2007). Krause and Liesenjohann (2012) obtained similar results in guppies (Poecilia reticulata). They suggested that reduced growth under predation threat despite high food availability might be a result of
Results and Discussion

Table 1. Schematic overview of the main questions and answers of this thesis.

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
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<tbody>
<tr>
<td>Does perceived predation risk induce phenotypic plasticity in nine-spined stickleback?</td>
<td>Yes. Under high perceived predation risk stickleback grew slower and some stickleback (see below) had decreased body condition. The number of neuromasts in some parts of the lateral line system was elevated under predation risk. The size of the hypothalamus was decreased in all, while the size of the olfactory bulb increased in some (see below) populations. Predation risk decreased aggression and risk-taking.</td>
</tr>
<tr>
<td>Does available energy affect nine-spine stickleback phenotypes?</td>
<td>Yes. Under food restriction stickleback grew slower, were in worse body condition and had relatively larger livers. Neuromast number was elevated in one neuromast group but result was habitat, predator and sex specific (see below). Food-restriction increased feeding activity and risk-taking.</td>
</tr>
<tr>
<td>Does available energy alter the effect of perceived predation risk?</td>
<td>Yes. Stickleback grew faster in the absence of predation only when they were fed in excess.</td>
</tr>
<tr>
<td>Is phenotypic plasticity sex-dependent?</td>
<td>Yes. Female stickleback exploited the advantage of excess food in terms of growth and energy reserves better than males. Sex (as part of a complex interaction) affected the number of neuromasts in one neuromast group. Males in the low food treatment, whereas females in the high food treatment had higher feeding activity.</td>
</tr>
<tr>
<td>Is phenotypic plasticity dependent on the populations’ evolutionary history?</td>
<td>Yes. Growth of pond fish was less suppressed by perceived predation risk than that of marine fish, but only pond fish had decreased body condition under predation risk. Pond fish exploited excess food in terms of growth better than marine fish. Pond stickleback had elevated number of neuromasts in one lateral line under predation risk, while neuromasts from another lateral line were affected by habitat in a complex interaction. Pond stickleback developed larger olfactory bulbs under predation risk, but marine fish did not. Pond fish were more risk-taking under food shortage than marine fish.</td>
</tr>
</tbody>
</table>

altered physiology or activity, rather than feeding behaviour per se. Physiological costs caused by predators have been tested for instance in tadpoles which only showed short term elevation in oxygen consumption under perceived predation stress, which cannot explain reduced growth under longer time-spans (Steiner and Van Buskirk 2009).

Both the hypothalamus and bulbus olfactorius (II) responded to perceived predation risk: the bulbus olfactorius was larger (but only in pond fish) and the hypothalamus smaller in the predation treatment. As the bulbus olfactorius is the center for olfaction, the increase in size is not surprising, given that the fish in this experiment could only perceive information about predators and conspecifics through olfactory cues, and the size of certain brain part is indicative of its importance (Striedter 2005). The hypothalamus on the other hand has a strong regulatory role in the nervous system, and why the predation treatment resulted in smaller hypothalamus size is not self-evident. The hypothalamus regulates feeding behaviour (Kuczykowska et al. 2010),
and since predator presence often decreases activity and feeding, it can be that decreased activity under perceived predation risk can affect hypothalamus size. Certain parts of the lateral line system (III; opercular, dorsal head & otic) also responded to perceived predation risk with an increase in neuromast number. Higher number of neuromasts can increase the resolution of lateral line system (Coombs et al. 1988; Yoshizawa et al. 2010; Yoshizawa and Jeffery 2011). Hence, higher number of neuromasts under predation risk can be adaptive. While the lateral line system is known to be important in predator avoidance (Blaxter and Fuiman 1990; McHenry et al. 2009), the role of predator presence in the lateral line development has not been tested before. All in all, population variation in plasticity of sensory organs is rarely studied (Dangles et al. 2009), and intraspecific comparisons of plasticity induced by biotic factors in brain size and architecture are underrepresented (Gonda 2011). Here, I have demonstrated that a common biotic factor, predation, is capable of inducing plasticity in both sensory organ and neural architecture development of vertebrates.

Food treatment did not affect the relative size of the total brain or size of its parts (II). Considering the high energy needs of brain tissue (Mink et al. 1981; Aiello and Wheeler 1995), and the four-fold differences in the food availability between food treatments, the result was surprising. It suggests that brain size is prioritized during development. Fish in the high food treatment had higher lean body weight (V). Lean body weight might indicate investment on locomotive performance or energy storage, but body composition analysis would be necessary to separate between the two. In contrast liver weight was smaller in the high than the low food treatment, also a pattern which was somewhat counterintuitive as usually higher rations result in higher liver weights.

I was also interested in a possible interaction between food and predation treatments. Several studies have investigated this connection but the results are often context-dependent (e.g. Bolnick & Preisser, 2005). However, in many cases, a lack of resources (or high competition) leads to negative antipredatory performance of the prey (Bolnick and Preisser 2005). I observed an interaction between food and predation treatment in body size (IV) and the lateral line system (III). Nine-spined stickleback could only utilize the extra food for growth and body size in the high food treatment when the predator was absent. Perceived predation risk can suppress growth rates when resources are high, but when resources are scarce and growth is already reduced, predation risk had no effect (Fig. 5). The treatment interaction effects on the lateral line system (III) were more complex and restricted only to the anterior trunk group of neuromasts. They were also dependent on habitat and sex of the fish: in pond males, fish in the high food treatment were able to develop more neuromasts than fish in the low food treatment. However, in marine females predation resulted in a higher number of neuromasts in the low food treatment and a lower number in high food treatment. This indicates that in certain cases the fish can invest more on developing neuromasts, but the pattern is obscure.

Taken together, nine-spined stickleback were phenotypically plastic in that both the presence/absence of olfactory cues from predator and variation of food supply induced plasticity in a number of fitness-related traits. Overall, the patterns reported here – divergent responses to environmental cues in different traits – are in line with my predictions. Behaviour and life history (predominantly size/growth) were the most plastic, morphology the least plastic and the rest in between. It also demonstrates that drawing general conclusions about the nature of environmentally induced variation based on measuring variation in one or two traits can give a biased view. Furthermore, even though one intrinsically expects that there is a tight connection between behaviour, growth/body size and energy storage, this is not always detectable.

How evolutionary history shapes plasticity

Perhaps the most important question of my thesis was how biotic environmental variation shapes expression of phenotypic plasticity in different populations. While local environmental conditions can define the genotype of an individual via local adaptation, they can also cause shifts in slopes of reaction norms, resulting in changes in
the range of phenotypes a population can express from the given genetic material (Gotthard and Nylin 1995; Ghalambor et al. 2007). On the other hand, if there is no environmental variation within a population, canalisation of phenotypes through a process of genetic assimilation can occur (Waddington 1953; Crispo 2007). Variation in predator-induced plasticity among populations that have locally adapted to different predator regimes have been shown for instance in the antipredatory morphology of tadpoles (Van Buskirk and Arioli 2005; Kishida et al. 2007), and fish (Januszkiewicz and Robinson 2007); and in behaviour of isopods (Harris et al. 2011) and fish (Magurran 1990; Dingemanse et al. 2009; Kozak and Boughman 2012). While there are examples of studies where predator-induced phenotypic plasticity is examined using several variables simultaneously, those usually tend to focus on particular type of traits like behaviour or life history traits (Dennis et al. 2011). Studies which examine predator-induced plasticity of populations locally adapted to certain predator regimes in a wide variety of traits are scarce. However, selection by predators is not likely to target single traits, but several traits in combination (Svensson et al. 2001; Sinervo and Svensson 2002) and possible genetic correlations between traits would result in complex phenotype shifts even if selection acted on only one trait.

One of my original assumptions was that the marine populations would show a stronger response to predation and pond populations to food manipulation. However, the results I received were mixed. While the expected patterns were found both in body size (IV) and behaviour (VI), the opposite responses were present for brain architecture (II) lateral line (III) and lean body weight (V) variation. Marine fish restricted their growth more in the predation treatment (Fig 6a), whereas pond fish had stronger responses towards the food treatment (Fig 6b). Size-unlimited predation selects against large body size (Blanckenhorn 2000; Biro et al. 2004, 2006) and fish communities with strong interspecific competition decreases the chances of body size shifts (Wilson 1975; Lomolino 2000; Simberloff et al. 2000). Hence, in ponds where predation and interspecific competition are relaxed, large body size has evolved in order to gain superiority in intraspecific competition.

**Figure 5.** Interaction between food and predation treatment in body size. Fish could only utilize the extra food in body size when predator cues were absent.

**Figure 6.** Habitat specific phenotypic plasticity in body size in a) predator treatment and b) in food treatment. Least Squares means (± standard error) are shown.
(Herczeg et al. 2009c, 2010a). Therefore, pond fish aimed for maximal growth, while marine fish for avoiding predation. Similarly, pond fish were more risk-taking in the low food treatment than marine fish, which is understandable as it allows pond fish to obtain more energy for growth. Both results are indicative of the important role that resource availability is likely to have in pond environments and predation avoidance in the marine sites.

The results from the chapters that studied plasticity in brain architecture (II), lateral line system (III) and energy storage (V) were both against my original expectations of greater predator-induced plasticity in marine populations. I found that while marine fish in general have a larger bulbus olfactorius than pond fish, only pond fish responded to the predation treatment by increasing their bulbus olfactorius volume (Fig. 7a). Similarly to the above patterns, marine fish had a generally higher number of neuromasts in the opercular lateral line (III), but only pond fish showed a plastic response to predation treatment by increasing the number of neuromasts (Fig. 7b). The bulbus olfactorius is the center for olfactory recognition and it transmits cues of conspecifics and predators. The lateral line is an important organ in recognizing conspecifics, predators and prey (Coombs et al. 1988; Bleckmann 1993; Bleckmann and Zelick 2009). It is possible that stabilizing selection towards optimal bulbus olfactorius size and neuromast number has led to the canalisation of the phenotype with high antipredator capacity in marine environments. Relaxed selection in ponds has resulted in the decrease of these traits, while somehow regaining the ability for phenotypic plasticity. Considering that it is likely that predation adapted marine stickleback represents the ancestral form for which the pond phenotypes are descendants of, results suggest that plasticity has appeared parallel to a general reduction in the traits. Similar patterns have been found from periwinkles (Littorina obtusata) where old world populations living in sympatry with green crabs (Carcinus maenas) show canalized behavioural response (soft tissue withdrawal always present) to predator cues (Edgell et al. 2009). In North America, where the green crab is a recent invasive species, periwinkles show a plastic response to green crab cues (Edgell et al. 2009). The response is also dependent on the time since invasion and stronger canalization is observed in populations with a longer history of co-existence with crabs. The two systems (periwinkles and nine-spined stickleback) are different in the sense that while plasticity seems to be the ancestral state in periwinkles of North America and selection for antipredatory behaviour led to canalization, in nine-spined stickleback the fixed phenotype represents the ancestral state and plasticity is the result of relaxed selection. This finding relates to the theory of decanalization in developmental biology, which predicts that perturbations can force the canalized phenotype

\[ \text{Figure 7. Habitat x predation risk treatment interaction in the in a) relative brain volume and b) Opercular neuromast group. LS means ±S.E are shown.} \]
out from its developmental channel (Rice 1998, 2008; Gibson 2009). This could lead to the release of cryptic genetic variation which has accumulated in the robust phenotype (Hansen 2006; Masel and Siegal 2009).

I must also note that a similar pattern was found in chapter (V): only pond fish responded to predation cues with lower lean body weight, but as the life-history traits are more labile, this result was not as unexpected as the results discussed above. In any case, plasticity re-appearing when selection for an extreme phenotype gets relaxed parallel to a general ‘decrease’ in the phenotype definitely warrants further investigation.

All in all, the main goal of my thesis was to find and understand how phenotypic plasticity varies among locally adapted populations. Some traits showed the expected pattern, i.e. local adaptation to a certain environmental factor included stronger plasticity towards variation in that environmental factor, whereas other traits showed opposite patterns and some even showed a lack of plasticity. Habitat-dependent and genetically based patterns in any quantitative trait are likely to be the result of natural selection (Endler 1986; McGuigan et al. 2005). Therefore, the habitat-dependent expression of plasticity in growth, body condition, neural architecture, lateral line development and behaviour is likely to be result of adaptive evolution.

The different sexes

Sexual dimorphism stems from divergent selective factors between sex (Shine 1980; Andersson 1994; Fairbairn 1997). Different selective pressures can influence sexes differently, which can further translate into variance in phenotypic plasticity among sexes (Fairbairn 2005). However, in many cases, studies of phenotypic plasticity do not account for sexual differences or only one sex is analysed. Recently, sex-specific plasticity has been discussed in the context of sexual size dimorphism, where it has been observed that a considerable amount of intraspecific variation in sexual size dimorphism can be due to the differences in phenotypic plasticity between sexes (Stillwell et al. 2010). In this thesis I aimed to inspect sexual dimorphism in the expression of phenotypic plasticity. I found sexual dimorphism in almost every single variable studied and interactions between sex and the treatments was found in armour (I), lateral line (III), body size (IV), energy storage (V) and behaviour (VI). The results for brain architecture showed sex specific divergence in several brain parts and a food × sex interaction in the *bulbus olfactorius* size, yet the results are not discussed in the derived paper but will be considered elsewhere.

Previous studies have shown sexual dimorphism in body size (Herczeg et al. 2010a) and morphology (Herczeg, Turtiainen & Merilä; unpublished) of nine-spined stickleback. I could confirm those results. There was considerable sexual size dimorphism in body size (IV), females being the larger sex. Also, sex specific differentiation was strong in both body shape (Fig. 8.) and armour traits and also followed those previously reported patterns from three-spined stickleback (Caldecutt and Adams 1998; Leinonen et al. 2006; Kitano et al. 2007; Aguirre and Akinpelu 2010). Females had relatively smaller heads, a more streamlined body, shorter dorsal and anal fins and longer caudal peduncles (Fig. 8). Marine fish showed sex specific differentiation in armour traits (females more armoured than males), but there was no differences in pond fish where the role of body armour is limited. In three-spined stickleback the sex specific divergence in armour and shape is thought to stem from the different life-styles that sexes have: males are more limnetic and females more benthic (Spoljaric and Reimchen 2008). While we do not know about possible sex differences in spatial preferences of the nine-spined stickleback, similar division may be the reason for the observed patterns here. However, considering how bony armour and body shape are genetically correlated in the three-spined stickleback (Leinonen et al. 2011), completely different selective factors acting on different traits might also result in similar patterns.

As energy storage traits have not been characterized in the nine-spined stickleback system before, the results showing sexual dimorphism in relative lean body, fat body and liver weight (V) were novel. Males had higher lean body weight and females had larger livers. These patterns might be explained by males often investing in muscle to succeed in territorial conflicts or mate acquisition (Bonnet et al. 1998;
Schulte-Hostedde et al., 2002; Stahlschmidt et al. 2011). Females on the other hand store energy in livers, to facilitate production of energy rich eggs through vitellogenesis (Henderson et al. 1996; Dahle et al. 2003; Guijarro et al. 2003).

I was also able to demonstrate variation in plasticity between sexes. In chapter IV, I found that pond females aimed to maximize their body size more than males; they showed the strongest response to high food treatment, and they did not sacrifice their growth under perceived predation risk (Fig. 6a). This aligns with the previous findings based on sexual size dimorphism variation across different nine-spined stickleback populations suggesting that females drive the body size divergence in the species (Herczeg et al. 2010a). Females also showed higher drive to feed in the high food treatment than males (VI) further strengthening the inference. Interestingly, females also had higher fatbody weight in the high food treatment than males (V). Divergence in plasticity patterns between sexes suggests that plasticity might play important role in the maintenance of sexual size dimorphism.

Plasticity differences between males and females were often ambiguous. For instance while marine males had a larger number of neuromasts in the anterior trunk neuromast group as a response to predation, in females only pond individuals responded and their response was dependent on food treatment (III). Similarly, the drive to feed was stronger in males in the low food treatment, but in females in the high food treatment (VI). These differences are difficult to explain and would need a considerable amount of research to understand the phenomenon in full detail (assuming the complex patterns are not artefacts). Here, it should be pointed out that photoperiod and temperature mimicked the non-reproductive season in my experimental setup, and thus the observed sex-specific differences are not likely to result from the differences that the different sexes exhibit during reproductive season (Wootton 1984).

Local adaptation

Even though the primary goal of my thesis was to explore habitat- and treatment-related trends in phenotypic plasticity, treatment-independent habitat effects also hold relevant evolutionary information because finding (genetically-based) habitat-dependent population divergence in a phenotypic trait implicates that natural selection has been the driver of the observed phenotypic divergence (Clarke 1975; Endler 1986; Foster 1999; McGuigan et al. 2005). Even though it is impossible to unequivocally separate maternal and genetic contributions to the phenotypic variation based on F, full-sib crosses (Falconer and Mackay 1996), considering the length of our experiment, most maternal effects are likely to have dissipated before the fish were measured (e.g. Green 2008). In most traits I studied, I could detect habitat-specific differences, mostly in line with previous results from this system. Body armour was reduced in pond habitats and pond individuals had generally deeper bodies and shorter caudal peduncles than the marine fish (I, Fig. 8, Herczeg et al. 2010b). Body size was larger and growth was faster (more volume per unit of time) in pond populations than in marine populations (II, Herczeg et al. 2009a, 2012). The olfactory bulbs were relatively smaller in pond compared to marine fish (IV, Gonda et al. 2009). Pond fish were more active feeders and risk-takers and showed stronger aggression and exploration than marine fish (VI, Herczeg et al. 2009b).

Variation in the number of neuromasts

![Figure 8. Variation in nine-spined stickleback body shape along the first relative warp which captured both sex- and habitat specific variation in body shape. Upper fish represents male type, whereas the lower fish represents female type.](image)
in the lateral line system (V) was also habitat dependent – marine fish had more neuromasts than pond fish in the opercular, anterior trunk and anterior trunk canal groups. In the anterior trunk canal group, neuromasts were almost completely lacking in pond populations. The ponds we sampled are small, isolated water bodies that lack other fish species besides nine-spined stickleback, have negligible aquatic vegetation, currents, and physical complexity. Such simple environments might demand less from a sensory system than the more complex marine environments. It is noteworthy though that in many cases there were also variation between populations within habitat type, suggesting that all variation is not explainable through pond-marine divergence.

Population divergence in energy reserves in the nine-spined stickleback system has not been addressed before. There were habitat-specific differences in all studied traits (V). Marine fish had larger relative liver and fat body size, but smaller relative lean body weight than the pond fish. Under negligible piscine predation risk the best strategy of pond stickleback is to overgrow their competitors, and as a consequence, to have larger fecundity and higher competitive ability (e.g. Herczeg et al. 2009a, 2010a, 2012). Hence, it is likely that pond stickleback invest heavily in growth before maturation. This can mean lowered allocation to energy storage given the trade-off between allocating energy to storage vs. somatic growth (Lankford et al. 2001; Brodin 2007; Mogensen and Post 2012). Higher investment on lean body weight might imply better competitive ability and thus can be important in pond environments (Casselman and Schulte-Hostede 2004; Stahlschmidt et al. 2011) or simply more fat or glycogen stored in muscle tissue. However, body composition analysis would be needed to reliably test the above opposite possibilities.

Conclusions and future directions

In this thesis I have explored the extent of variation in phenotypic plasticity across a range of populations from different habitats. The patterns were by and large in accordance with my predictions showing increasing plasticity induced by perceived predation risk and food supply in the order of: morphology < sensory organ / neural architecture < life history traits < behaviour. It is noteworthy that olfactory cues from a predator – without visual cues or any actual contact – alone triggered significant plastic responses in behaviour, growth, body condition and brain and sensory organ development. I am the first to demonstrate predator-induced plasticity in the lateral line system. Naturally, this raises the question of the function and the role of lateral line system in predation avoidance in nine-spined stickleback. Furthermore, as only certain neuromast groups responded to predation treatment, the functionality of these groups could be studied in more detail. Methods where neuromasts can individually be ablated are available and could be utilized to see which parts of the lateral line system are most important in certain conditions. The genomic architecture of the lateral line system in three-spined stickleback has recently been revealed (Wark et al. 2012) and a comparative study between three- and nine-spined stickleback would be interesting.

With respect to the main aim of my thesis - detecting habitat-dependent population divergence in phenotypic plasticity - I got mixed results. I predicted that local adaptation to certain environmental factors (e.g. predation in marine populations and competition in ponds) will include an increased ability to express phenotypic plasticity towards variation in environmental factors aimed to represent those selective factors. My results supported these predictions with respect to growth and behaviour, but brain and lateral line plasticity showed something unexpected: predation induced plastic responses only in pond fish that evolved in the absence of piscine predation. How plasticity appears under relaxed selection and parallel to a decrease (in size or number) of the given trait is an enigma that remains to be solved. I also detected sexual dimorphism in both mean trait levels and their phenotypic plasticity, shedding light into a very important determinant of within-population phenotypic plasticity variation.

Besides plasticity, my experiment was also adequate to draw some conclusions of local adaptations. While in most cases I could
only strengthen previous findings, by showing habitat-dependent population divergence in energy storage patterns, my work is pioneering in providing an example as how adaptation to different predation / competition regimes can result in different energy storage strategies. These results, suggestive of local adaptation, add to the body of recent research showing that relaxed selection (in this case by piscivorous predators) can cause strong phenotypic shifts even in short time scales (Lahti et al. 2009). Relaxed selection has long been an underestimated and understudied factor, and only recently the research community has started to understand the importance of these processes, and my model system provides an excellent opportunity to investigate this question in more detail.

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