The Importance of Egg Size for the Diversity of Salmonids

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Abstract

Salmonids display great diversity in terms of behaviour, life histories and morphology within and among populations. Such diversity results from a combination of genetic, environmental and ecological factors. Here I studied the short- and long-term effects of egg size on development, behaviour, growth and physiology in Arctic charr *Salvelinus alpinus* and steelhead trout *Oncorhynchus mykiss* (wild and domesticated).

In both species, egg size was smaller in domesticated populations. Egg size was negatively correlated with embryonic development. Larger eggs contained more energy in both aquaculture and wild populations of Arctic charr. Egg size related to behaviour of juveniles of both species, juveniles coming from larger eggs tended to feed more at the surface whereas juveniles coming from smaller eggs fed more on the bottom. After several months of rearing we found that the influence of egg size on behaviour and morphology of Arctic charr varied with female parentage, indicating strong maternal x genetic interactions. In steelhead trout, both origin of fish and egg size were related with growth of yearling fish reared under laboratory conditions.

The results of this are new and show that variation in egg size is crucial for phenotypic variability. My results support the hypothesis that females who grow relatively rapidly as juveniles produce a large number of small eggs as adults. Thus, changes in egg size occur rapidly (in only one domesticated generation). Finally, I discuss the implications of egg size for evolution and how diversity created by egg size can influence diversification and speciation of fishes.

Útdráttur

Hjá laxfiskum má finna mikinn fjölbreytileika, innan og milli stofa, í atferli, lífssögu og svipfari. Þessi fjölbreytni hefur orðið til vegna samspils erfða-, umhverfis – og vistfræðilegra þátta. Ég rannsakaði bæði skammtíma og langtíma áhrif hrognastærðar á þroska, atferli og lífeðlisfræði bleikju *Salvelinus alpinus* og regnbogasilungs *Oncorhynchus mykiss* (villtur og eldisfiskur).

Hjá báðum tegundum voru hrogn eldisfiska smærri en villtra fiska. Greinilegt neikvætt samband var á milli hrognastærðar og fósturþroska fyrir klak. Hjá bleikju þá reyndist stærri egg innihalda meiri orku og var það samband hjá bæði villtum og eldisfiski. Í báðum tegundunum mátti sjá samband milli atferlis og hrognastærðar. Seiði sem komu úr stórum hrognum átu meira af yfirborði á meðan að seiði úr smærri hrognum átu meira af botni. Þegar seiðin höfðu verið alin í nokkra mánuði kom fram að mæður höfðu áhrif á tengsl hrognastærðar á hegðun og útlit bleikju. Bendir þetta til sterkra samvirkni móður og gena. Hjá regnbogasilungi mátti sjá áhrif bæði uppruna fiska (eldi og villt) og hrognastærðar á vöxt ársgamallra seiða, alin í eldisstöð.

Niðurstöður þessara rannsókna eru nýjar og sýna að breytileiki í hrognastærð skiptir miklu máli fyrir svipfarsbreytileika. Niðurstöður mínar styðja þá tilgátu að hrygnur sem vaxa hratt sem seiði myndi mikinn fjölda smárra eggja eftir að þær ná kynþroska. Þannig geta breytingar á eggjastærð gerst hratt (ein kynslóð í eldi). Í ritgerðinni ræði ég hvernig hrognastærð getur haft áhrif á þróun og hvernig breytileiki sem til verður vegna hrognastærðar geti haft áhrif á myndun afbrigða og tegunda fiska.

Dedication

To my friends and my family for their support and encouragement throughout the years...
Thank you very much for being with me while I am turning this important page of my life.
Now it is just the start of something new ...

"Learning never exhausts the mind."

Leonardo Da Vinci

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Abbreviations

AIC	Akaike information criterion
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ATP	Adenosine triphospate
ATPase	enzyme that catalyze the decomposition of adenosine triphospate
CV	Coefficient of variation
df	degrees of freedom
dpf	day(s) post fertilization
DNA	Deoxyribonucleic acid
FL	Fork length
J	Joules
K^+	Potassium
MANCOVA	Mutlivariate analysis of variance
N	Total number of individuals
NA^+	Sodium
P	p-value
RNA	Ribonucleic acid
SD	Standard deviation
SE	Standard error
SL	Standard length
VIF	Variance inflation factor
YV	Yolk-sac volume

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I would like to thank two teachers that were very special to me. Jean Pierre Hue (my 5th grade teacher) introduced me to marine biology and sailing. His interest for sciences and his love for teaching definitely triggered my early interest in biology. I dearly thank David Benhaïm (B.Sc. teacher) for introducing me to Behavioral Sciences and for his time and dedication through the years. Your motivation in what you do is such a great inspiration for me and I am very pleased to work with you as a colleague and a friend.

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1 General Introduction

A major goal of evolutionary biology is to understand how and why animals and plants diversify. The conceptual framework used nowadays in evolutionary biology still relies mostly on the natural selection mechanism first described by Darwin (1859). However, advances in molecular biology, palaeontology, population-statistical genetics and analytical techniques gave birth to a new framework, the Modern Synthesis (MS), unifying the theory of heredity (Mendel) and the Darwinian theory of evolution (Ruse and Travis, 2009). The ultimate understanding from the MS is that alleles associated with higher fitness increase in frequency from one generation to the next (see Futuyma, 2006; Freeman and Herron, 2007). This theory was called a "synthesis" because it linked together not only Neo-Darwinism and population genetics, but also zoology, botany, and palaeontology. Indeed, it opened up new fields of research focusing on speciation mechanisms (Pigliucci, 2009). Although MS is still the accepted version of evolutionary theory (Futuyma, 2006), this theory is mostly a "theory of genes" (*Ipse dixit* Karl Popper and see also Ruse and Travis, 2009), where the mechanisms behind the transformation of form have yet to be explained. For the last 15 years, or so, most evolutionary biologists have tried to focus on such mechanisms leading to the Extended Synthesis (ES; Pigliucci, 2007; Pigliucci and Müller, 2010). In the ES a more important role is given to "internal" causes of variation such as development where genes are "followers" (West-Eberhard, 2003) rather than being the sole agent of variation and unit of inheritance (Pigliucci and Müller, 2010). This new framework indicates the importance of including epigenetic inheritance as an important connection between parental effects, development of the progeny and evolution (Miller, 2010). Epigenetic inheritance refers to the way genotypes translate into phenotypes beyond the action of changes in DNA sequences. Epigenetic factors encompass, for example, DNA methylation, histone modification and microRNA (Richards *et al.*, 2010). Epigenetic effects can include phenotypic plasticity and maternal effects if they are trans-generational phenomena.

Maternal effects, defined as the genetic and non-genetic contributions from a mother to her offspring that can modify the phenotype of her progeny (Cohen, 1979; Arnold, 1994; Bernardo, 1996a; Räsänen and Kruuk, 2007), can play an important role in the diversity of ecological and evolutionary processes (Mousseau and Fox, 1998; Badyaev, 2008). Indeed, they can affect population dynamics (Inchausti and Ginzburg, 2009), phenotypic plasticity (Lancaster *et al.*, 2010), life-history evolution and the evolutionary response to selection (reviewed by Wolf and Wade, 2009). Maternal effects have been put forward as an alternative to the Lotka–Volterra predator–prey model in explaining cycling population dynamics (Inchausti and Ginzburg, 2009). These effects are of interest in population dynamics because they characterize individual phenotypic variations (also accounting for current and previous environments encountered by the individuals) that can be important in understanding the demography of species.

Maternal gene products are stored in the egg during oocyte maturation and they are directly influenced by the genetic constitution and the external environment of the mother. In vertebrates and insects, these maternal factors or maternal gene products (Abrams and Mullins, 2009) drive the early stages of development (Gilbert, 2006; Lindeman and Pelegri, 2010). In zebrafish, *Danio rerio*, all processes occurring before the activation of the zygotic genome at the midblastula transition are carried out and controlled by maternally - provided products. These processes include animal-vegetal polarity, egg activation, cleavage development, axis formation, tissue morphogenesis, and germ cell development (Pelegri, 2003; Dosch *et al.*, 2004; Lindeman and Pelegri, 2010). Maternal factors also interact with the zygotic genome beyond the midblastula transition affecting embryonic morphogenesis and the embryonic body plan (Wagner *et al.*, 2004). It has been suggested that more maternal-effect genes and maternally controlled processes remain to be identified in zebrafish (Pelegri, 2003).

Inheritance of phenotypic variation has been suggested to be controlled by both genetic and epigenetic factors (Bossdorf *et al.*, 2008). One important feature of epigenetic effects, including maternal effects, is that they are more labile (unstable) than changes in DNA sequence (Johannes *et al.*, 2009; Richards *et al.*, 2010). If a novel phenotypic trait is triggered by a maternal effect it may appear at a non-negligible frequency (see discussions of Johannes *et al.*, 2009; Richards *et al.*, 2010). In that sense maternal effects could be a source of phenotypic diversity and thus, they can dramatically enhance rates of evolutionary response to selection in wild populations (models: Kirkpatrick and Lande, 1989; Riska, 1989; empirical data; Crean and Marshall, 2009; Harris and Uller, 2009; Wolf and Wade, 2009). The causal link between maternal genotype/phenotype and offspring phenotype is the critical component of the definition of maternal effect, providing the link between maternal effects and evolutionary and ecological processes. There is a general consensus in the recent literature that maternal effects can act on phenotypic development of the offspring and may generate rapid change in a population. Many maternal effects have been shaped by natural selection to enable adaptive responses to heterogeneous environments (Mousseau *et al.*, 2009), as demonstrated in birds (Crean and Marshall, 2009) and amphibians (Martin and Pfennig, 2010). Thus, they create new phenotypes that will be exposed to natural and sexual selection processes but at the same time they are also themselves the target of evolution. Maternal effects integrate development, ecology and evolution —a major and long-awaited step in evolutionary theory. Such effects are ubiquitous (Mousseau and Fox, 1998; Räsänen and Kruuk, 2007) and often reported as a key factor in the behaviour of progeny, especially in species with parental care (Reinhold, 2002). Thus they can be seen as a dynamic part of an evolutionary continuum (including many mechanisms originating and modifying phenotypes) describing the complexity and diversity of forms and functions (Mousseau *et al.*, 2009). Two major steps are now: 1) to understand and highlight the mechanisms of maternal effects that allow important phenotypic and life history changes, and 2) to do so across taxa.

Maternal effects and their evolutionary implications have not been much studied in fishes. Teleost fishes, which make up roughly half of vertebrate species, exhibit an amazing level of biodiversity that can be seen in their morphology, ecology and behaviour, as well as in many other aspects of their biology (Helfman *et al.*, 2009). This huge diversity makes fishes attractive for the study of many important biological questions, particularly questions related to evolution (Volff, 2005). Among fish species, different morphs or forms (e.g.

migratory and non-migratory forms) frequently coexist in the same location (sympatry), each adapted to their respective ecological niche (Wimberger, 1994; Skúlason and Smith, 1995; Robinson and Schluter, 2000). These adaptations can be seen in different behaviour, morphology, physiology and life history traits. These adaptations and their importance for life history differences have been much investigated in salmonids (e.g. Klemetsen *et al.*, 2003; Snorrason and Skúlason, 2004). It has been suggested that maternal effects can be important in promoting adaptations to novel environmental conditions resulting in a high degree of polymorphism in these species (e.g. Räsänen and Kruuk, 2007). However, the importance of such effects on salmonid evolution has seldom been investigated (Einum and Fleming, 1999; Heath *et al.*, 1999). Maternal effects frequently vary as a result of the environment experienced by the mother and their expression may also dependent heavily on the environment experienced by the offspring (e.g. Einum and Fleming, 1999; Räsänen *et al.*, 2003; Mitchell and Read, 2005; Räsänen *et al.*, 2005; Beckerman *et al.*, 2006).

In fishes, the most studied maternal trait is probably egg size, because of its relationship to both maternal fitness and offspring survival (Einum and Fleming, 2000a). Because the female has only a limited amount of energy resources available for reproduction, there is a trade-off between egg size and egg number (Smith and Fretwell, 1974; Elgar, 1990; Einum and Fleming, 2000a). Larger eggs are energetically more costly to produce than smaller eggs, i.e. with larger eggs having more yolk than smaller eggs and offspring survival tending to increase with increasing egg size e.g. (e.g. Roff, 1992; Einum and Fleming, 1999; Einum and Fleming, 2000a; 2002; Pakkasmaa and Jones, 2002). Egg size is also positively correlated with hatching time (Pakkasmaa and Jones, 2002), emergence and survival in salmonids (Einum and Fleming, 2000a). Furthermore egg size has been connected to feeding and mobility behaviours in juveniles of Arctic charr *Salvelinus alpinus* (Benhaïm *et al.*, 2003).

Behaviour has been suggested to be an initial factor in intra-specific sympatric divergence and speciation (Skúlason *et al.*, 1999). According to Futuyma and Moreno (1988), behaviour is often the mechanism by which specialization is exercised and an evolutionary change in behaviour frequently initiates a niche shift and directional selection on other traits such as morphology and physiology. Some authors have demonstrated that behavioural differentiation precedes morphological differentiation, especially in salmonids (De Kerckhove *et al.*, 2006; Klemetsen *et al.*, 2006; Sacotte and Magnan, 2006; Svanbäck and Eklöv, 2006). Furthermore, the ontogeny of early life behavioural patterns is important and may lead to different life history traits (Smith and Skúlason, 1996). Tinbergen (1963) emphasized the importance of ontogeny of behaviour, and scientists now acknowledge that behavioural processes may play a significantly larger role than previously suspected in the development and maintenance of variations among individuals (Stamps, 2003). Maternal contributions, e.g. through egg size and/ or yolk content, can have important effects on development (Valdimarsson *et al.*, 2000), including behaviour (Benhaïm *et al.*, 2003).

Many studies have focused on parental feeding behaviour and diet and their consequences for progeny of polymorphic fishes (e.g. Malmquist *et al.*, 1992b; Skúlason *et al.*, 1993; Klemetsen *et al.*, 2006; Sacotte and Magnan, 2006; Svanbäck and Eklöv, 2006). However, although the early development may be a crucial period to identify both proximal and long-term maternal effects on behaviour, only a few studies have focused on foraging behaviour of fish at early stage of development (e.g. Skúlason *et al.*, 1993; Benhaïm *et al.*,

2003; Sturlaugsdóttir, 2008). Benhaïm *et al.* (2003) showed that at first feeding large and small Arctic charr clearly display different foraging tactics, as well as differences in mobility and foraging rate. Because egg size largely determines body size of juvenile salmonids at first feeding (Einum and Fleming, 2000a), egg content might play an important role in embryonic development which could explain to a large extent these behavioural differences. If maternal effects influence the offspring phenotype, e.g. behavioural traits, they may have risen through the egg especially in species where maternal care is absent. Therefore, embryonic and early juvenile periods may be crucial for the operation of maternal effects.

Maternal status is known to affect offspring performance both in the field and in experimental studies on fresh- and salt- water fishes (Kamler, 2005). While egg size, egg composition and energy content can be affected by maternal environment these attributes are also influenced by maternal genes. Heritability of egg size in animals is in average equal to 0.25 (Mousseau and Roff, 1987) but can be quite high in salmonids (e.g. 0.78 Kinnison *et al.*, 2001). Additionally, the fact that observed mean egg size does not necessarily maximize maternal fitness suggests that evolutionary constraints can affect egg size (Hendry and Stearns, 2004). Egg size may result from adaptations to pre and post hatching environments. For instance, fishes with parental care produce larger eggs than fish without care (Sargent *et al.*, 1987). The abundance and size distribution of food items may also influence how egg size relates to the likelihood of starvation, and competition (e.g. see a complete discussion on how egg size may change as adaptations to pre and post hatching environments Hendry and Stearns, 2004 p 150). Environmental conditions (e.g. food and temperature) encountered by the mother (in additional to the mother's genetic background) are clearly important for the hormonal status of fish eggs and embryos (Brooks *et al.*, 1997). The relationship between egg content and development of embryos has been mainly investigated in terms of fish growth (Kamler, 2005) but no study has focused on behavioural modifications linked to egg content. Thus, behavioural consequences related to egg composition, and ultimately to the environment of the mother are unknown, even though they may be important for fitness at early stages of development and also later in life.

The potential long-term effects of egg size during ontogeny, e.g. on the growth pattern of juveniles, have been studied in aquaculture with the objective of optimizing growth (e.g. Metcalfe and Monaghan, 2001). The duration of maternal effects and their influence on offspring can vary in magnitude and in the timing of their expression (Bernardo, 1996b). Surprisingly few ecological and evolutionary studies (mostly on amphibians) have assessed how long maternal effects last, but those studies indicate that maternal effects can persist late into ontogeny and even into subsequent generations (e.g. Miller, 2010). A recent study by Martin and Pfennig (2010) reported that a maternal trait such as female body size of spadefoot toad tadpoles *Spea multiplicata* translated into larger eggs influenced the expression of a novel resource-use phenotype. It is thus, becoming clear that maternal effects may not only influence growth and metabolism early in life but they may also influence developmental pathways (Moran and McAlister, 2009), phenotypes and life history of the progeny.

The overall objective of my thesis is to assess the role of egg size as a proximate mechanism causing phenotypic variation and early divergence in polymorphic fishes. I will study how maternal effects, focusing on egg size, affect early life history traits of fishes, e.g. behaviour and morphology, as well as potential long-term effects on growth and decision

making such as the timing of smolting. My thesis is divided into five chapters referring to five hypotheses or five sets of hypotheses detailed below.

Chapter 2- As a first step in our understanding of the difference between a small and a large egg, I measure the energy content of individual egg in Arctic charr. First I test the simple hypothesis that the energy content (J/g) of yolk plus egg membrane is independent of egg size i.e. there is a perfect one to one relationship between egg volume and total energy content per egg (J). This assumption is based on the fact that a female is unlikely to control the specific energy content of the yolk that goes into each egg (Kamler, 2005; Quinn, 2005; Moran and McAlister, 2009). It is more likely that a female would vary the size of her eggs to vary the energy content enclosed in individual egg. For instance Kinnison *et al.* (2001) showed that the cost of migration in Pacific salmon strongly influences energy allocation in reproduction, favoring a higher ratio of egg number to egg size with greater migration distance. Thus, there is a trade-off between energy allocation going into the ovaries and energy allocation into migration and between egg size and egg number. Better understanding the relationship between energy content of individual egg and egg size as well as understanding how this relationship varies among populations or morphs are relevant and necessary steps before investigating further what are the potential consequences of egg size on early life history traits of salmonids. I use eggs from four wild Arctic charr morphs and one population of aquaculture charr to test if the relationship between egg size and energy content varies among different morphs of the species. Furthermore, I assess whether egg size affects yolk depletion rate and growth rate of embryos from hatching until first feeding. Valdimarsson *et al.* (2002) showed that embryos coming from smaller eggs developed faster than egg coming from larger eggs in Arctic charr. In addition, Eiriksson *et al.* (1999) showed the small benthic morph of Lake Thingvallavatn, that has small eggs, dedicated more energy towards bone development than planktivorous charr that develop from larger eggs. Rombough (1985) also showed that embryos of Chinook salmon *Oncorhynchus tsawytscha* coming from smaller eggs started feeding earlier than embryos coming from larger eggs. Taken together these results may indicate differences in depletion rate or in the utilization of yolk between small and large fish coming from small and large eggs, respectively. Having a limited amount of energy, an embryo developing from a small egg may focus its development on feeding structures, as they have to start feeding earlier than embryos coming from a large egg. Based on previous findings, I do not expect to see differences in survival between fish coming from large and small eggs (Jonsson and Svavarsson, 2000).

Chapter 3- I investigate how egg size may relate to early behaviour of fish. I hypothesize that egg size, beyond its immediate effects on the development of embryos and juveniles, is an important factor in terms of subsequent juvenile growth and feeding behaviour in first feeding Arctic charr. This hypothesis is based on a study by Benhaïm *et al.* (2003) that showed egg size to influence the foraging behaviour of juvenile Arctic charr. However, those results could be influenced by the interaction of egg size and social factors since Benhaïm *et al.* (2003) only looked at fish reared in groups. Therefore I set up an experiment aiming to remove the effects of social factors, where I measured the relative effect of egg size on juvenile growth and behaviour when charr are exposed to different social environments i.e. juveniles are maintained in long-term isolation, short term isolation or maintained in groups. This experiment was designed as the first step in testing the potential effect of egg size on early growth and behaviour of fish.

Chapter 4- Here, I test the hypothesis that egg size may continue to affect weight, length, activity and foraging behaviours of juveniles up to several months after first feeding. This hypothesis is based on my results from chapter 2. Although the effects of egg size on behaviour and growth are expected to decrease over time (see Heath *et al.*, 1999), they may still be detectable a few months after first feeding. This prediction relies on the fact that the positive correlation between egg size and body size was found to be significant in 1-year old Arctic charr (Skúlason and Steingrímsson, unpublished observations). Furthermore, I test the hypothesis that egg size affects morphology of Arctic charr progeny as a consequence of divergence in foraging behaviour. Kristjánsson (2008) demonstrated that differences in feed (benthic vs pelagic prey) could trigger rapid differences in morphology of small benthivorous Arctic charr, especially in head morphology. Thus, divergence in foraging habits and food (i.e. benthic or pelagic) may precede morphological divergence (Kristjánsson, 2008). I demonstrated that siblings coming from small and large eggs differ in terms of foraging behaviours (i.e. feeding locations; see chapter 2) with small fish coming from smaller eggs showing more bottom foraging and large fish coming from larger eggs showing more surface foraging. Thus, if differences in foraging behaviours between small and large siblings coming from small and large fish persist in time, I expect to see differences in morphology between these fish. Specifically, I predict that smaller juveniles coming from smaller eggs will show more benthic foraging behaviour and consequently have a bigger head and deeper body (i.e. benthic feeding morphology observed in Icelandic wild charr populations) when compared with larger juveniles coming from large eggs (Kristjánsson, 2008). On the other hand, large fish coming from large eggs will show a more streamlined body and a smaller head as observed in pelagic morph of Arctic charr (Kristjánsson, 2008). Additionally, I test the effect of genetic differences on morphology of Arctic charr comparing the reaction norms (i.e. the difference in morphology between small and large fish coming from small and large eggs in each female) among four females. I expect morphological reaction norms to show similar direction, i.e. fish coming from smaller eggs will show a more similar morphology and large fish coming from larger eggs will show a more similar morphology, independently of their parentage. However, I predict to see differences in morphology reaction norms among females as shown among populations of small benthivorous charr (Kristjánsson, 2008).

Chapter 5- I used steelhead trout, *Oncorhynchus mykiss*, to study difference in egg size between hatchery and wild fish and the consequences of egg size variation. I test the hypothesis that hatchery fish have smaller eggs than wild ones as has been observed in Arctic charr (chapter 1). I determine egg size variation between returning F-1 hatchery fish and wild fish from the Siletz River, Oregon USA. Although the difference between hatchery and wild fish was essentially the environment experienced during their first year (hatchery condition for F-1 hatchery fish and natural condition for wild fish) I predict a difference in egg size in returning adults. Smaller egg size in F-1 hatchery fish may results from high quality environment experienced by juveniles (i.e. hatchery; Hutchings, 1991; Einum and Fleming, 1999), and from the fact that females experiencing high growth rate as juveniles produce a large number of small eggs as adults (Jonsson *et al.*, 1996; Lobón-Cerviá *et al.*, 1997; Morita *et al.*, 1999; Fleming *et al.*, 2000; reviewed by Einum *et al.*, 2004). I also assess egg size variation within females. Little information is available on intra-clutch egg size variation in *O. mykiss*. Based on my preliminary observation I know that intra-clutch differences in egg diameter exist. I then test the hypothesis that egg size influences development, growth, and behaviour of steelhead trout during the first year of life. Contrary

to the situation in Arctic charr (chapter 2), I expect to see a rapid decrease in the influence of egg size on development and growth of steelhead trout linked to initial smaller intra-clutch egg size variation. In *Oncorhynchus tshawytscha* egg size influence on fish growth disappeared before emergence (Heath *et al.*, 1999). Additionally, *O. mykiss* develops territories through agonistic behaviour soon after first feeding (Quinn, 2005). I assume that such agonistic interactions will have a large effect on growth and feeding behaviour, thus swamping any egg size effect. For production hatcheries, fish are selected for faster growth coupled with inherited behavioural differences among which are aggressiveness and boldness (Huntingford and Adams, 2005). The literature suggests that domestication can sometimes affect aggressiveness but the direction of this effect depends on feeding regimes (Ruzzante, 1994) and on the environment in which fish are being held to screen for aggressiveness (Huntingford and Adams, 2005). In this experiment I expect offspring of the hatchery fish to have a faster growth, coupled with more aggressiveness than observed in offspring of the wild fish.

Finally, I address the question of whether egg size has potential long-term effects on growth and smolting decision. Using PIT tagged one-year-old hatchery and wild offspring, I test the hypothesis that juveniles coming from smaller eggs are smaller after one year and do not make the decision to migrate to salt water. I assess smolting status using a preference test between fresh and salt water. I predict that offspring of hatchery fish will grow faster and consequently show a different osmo- regualory status at smolting when compared to offspring of wild fish.

Chapter 6- In this last chapter, I discuss the implications of egg size for evolution of fishes and, especially how diversity created by egg size can influence diversification and speciation of fishes.

2 Female characteristics, egg size, energy content and early development in Arctic charr, *Salvelinus alpinus*, L.

Camille A. Leblanc, Skúli Skúlason, Sigurđur S. Snorrason and David L. G. Noakes

2.1 Introduction

Generally, egg characteristics such as size, shape and color, are highly variable among species, so variable that they may even be used for species identification. Egg size is an important fitness component since it is often positively linked with body size, growth and survival (reviewed by Roff, 1992; Wootton, 1999). Such relationships have been reported in amphibians, where offspring that develop from larger eggs are larger at hatching, first feeding, and metamorphosis (Kaplan, 1980; Martin and Pfennig, 2010). A similar trend has been observed in reptiles where after 3 years female turtles from larger eggs are larger than those from smaller eggs (West-Eberhard, 1998), in birds where egg size influences egg hatchability, juvenile body size and survival (Czapulak, 2002), and in fishes, where it is well known that larger eggs produce larger embryos / juveniles (see Roff, 1992).

Egg size varies considerably among and within fish populations, within females and accross reproductive seasons (Chambers and Leggett, 1996; Chambers and Waiwood, 1996; Brooks *et al.*, 1997; Kamler, 2005). Environmental and maternal effects explain a large proportion of the phenotypic variance in egg size, and consequently in hatching time and embryo / first feeding / juvenile size especially in salmonids (Einum and Fleming, 2000a). Commonly larger females produce larger eggs (Heath and Blouw, 1998; Kamler, 2005). Egg size is often considered to be a good predictor of egg survival (Brooks *et al.*, 1997). However, studies on salmonids have shown conflicting results. In Brown trout *Salmo trutta* juveniles from larger eggs have higher growth rates and higher survival than those from smaller eggs (Einum and Fleming, 1999). Egg size was positively correlated with hatching time and free swimming embryo length but not with survival in hatchery lake charr S*alvelinus namaycush* (Pakkasmaa and Jones, 2002). Srivastava and Brown (1991) recommended the use of egg size as an indicator of both development and survival of hatchery-reared and wild Atlantic salmon *Salmo salar*. The relationship between egg size and juvenile traits appears more complex in Arctic charr *Salvelinus alpinus*. In this species, intra- clutch egg size variation can be higher than egg size variation observed among females of the same population (C. Leblanc, unpublished data). Egg size is positively correlated to female body size for hatchery and dwarf populations of Arctic charr (Wallace

and Aasjord, 1984b). In an Icelandic hatchery population, egg size was not related to offspring survival from fertilization through first feeding (Jonsson and Svavarsson, 2000) whereas Wallace and Aasjord (1984b) reported a positive correlation between egg size and free swimming embryo survival for a Norwegian population. In chapter 3 I show that egg size is positively correlated with individual size (i.e. weight and fork length) up to 300 days post fertilization (dpf). Ultimately, egg composition, i.e. yolk composition, determines the total energy content of an egg (in Joules) and proportional energy content (in Joules per gram; Kamler, 1992; Brooks *et al.*, 1997). Although the chemical composition of Arctic charr eggs has been studied (Pickova and Brännäs, 2006), the relationship between the egg composition and egg size is still unknown. It is also unknown how this relationship may vary among hatchery and wild populations of Arctic charr.

Arctic charr is well known for its high degree of inter-population variability (Noakes, 2008; Klemetsen, 2010). This is reflected in a variety of traits: behaviour, morphology, life history and ecological affinities. Size of mature fish is a striking example of this diversity where mature fish are known to range from 3 g to 12 kg (reviewed by Klemetsen *et al.*, 2003). Another aspect of this diversity is the frequent occurrence of sympatric polymorphism where two or more distinct morphs occur within one lake. For instance, four distinct morphs have been described in the Icelandic lake Thingvallavatn: large and small benthivorous morphs, piscivorous morph and planktivorous morph (Snorrason *et al.*, 1989).

Skúlason *et al.* (1989) studied the early ontogeny of the four morphs. They found significant variation of egg size among the four morphs. For instance, eggs of planktivorous charr are much larger than the eggs from small benthivorous charr, a fact which may be the principal cause of planktivorous fish being longer at first feeding (159 dpf). Egg size is as strongly correlated with skeletal development and juvenile size (Skúlason, 1986; Eiríksson *et al.*, 1999) but the mechanism of how these effects may be transmitted through the egg is still unknown. A female is unlikely to control the specific energy content of the yolk that goes into each egg (Kamler, 2005; Quinn, 2005; Moran and McAlister, 2009). It is more likely that females would vary the size of their eggs to vary their energy content. For instance Kinnison *et al.* (2001) showed that the cost of migration in Pacific salmon strongly influences energy allocation in reproduction, favoring a higher ratio of egg number to egg size with greater migration distance. Thus, there is a trade-off between energy allocation going into the ovaries and energy allocation into migration. A better understanding of the relationship between energy content of individual egg and egg size as well as an improved understanding of how this relationship varies among populations or morphs are relevant and necessary steps before investigating further the potential consequences of egg size on early life history traits of salmonids. Comparative studies on egg size and mean energy content between wild and aquaculture populations of Arctic charr will help to better understand the importance of egg size and egg content for offspring traits. Such information is important for understanding recruitment and early divergence of salmonids.

Because female size is a primary factor contributing to egg size and total egg mass (Quinn, 2005) and because there is considerable variation of body size among Arctic charr populations and sympatric morphs (Snorrason *et al.*, 1989; Wilson *et al.*, 2004; Kristjánsson, 2008), I first examine the relationship between female size and total egg mass. I then focus on the relationship between female body size, mean egg size and energy content

among aquaculture population and four wild populations of Icelandic Arctic charr. Based on previous observations (e.g. Skúlason, 1986; Skúlason, 1990; Seppä, 1999; Skúlason *et al.*, 1999) I predict a positive relationship between female body size and egg size (mean egg diameter and egg dry weight). At the same time I expect to see differences in this relationship among populations and morphs, reflecting adaptations to different habitats. I also examine variation in egg size within one clutch in both aquaculture and wild populations. If egg size is determined by environmental factors, egg size and the range of egg size within one clutch may reflect habitat differences during egg maturation. It is likely that selection pressures acting on wild females and their eggs differ from those acting in aquaculture environment, resulting in differences in egg size and egg size variation within one clutch between aquaculture and wild populations. Using both aquaculture and wild populations of Arctic charr, I tested the technical assumption that the energy content (J/g) of the egg (yolk plus egg membrane) is independent of egg size i.e. there is a one to one relationship between egg volume and total energy content.

Looking at one aquaculture population of Icelandic Arctic charr, I further evaluate the significance of egg size (i.e. egg weight) for early embryonic development. Valdimarsson *et al.* (2002) showed that embryos coming from smaller eggs developed faster than embryos coming from larger eggs in Arctic charr. Besides, Eiríksson *et al.* (1999) showed that the small benthic morph, that has smaller eggs, dedicated more energy towards bone development than planktivorous charr coming from larger eggs. Rombough (1985) also showed that embryos of Chinook salmon *Oncorhynchus tshawytscha* coming from smaller eggs started feeding earlier than embryos coming from larger eggs. Taken together these results may indicate differences in depletion rate or in yolk utilization between fish coming from small and large eggs. If an individual has a limited amount of energy, as would an embryo coming from a small egg, it may have to focus its development on feeding structures, as they have to start feeding earlier than individuals coming from large eggs. Based on previous findings, I do not expect to see differences in survival between fish coming from large and small eggs (Jonsson and Svavarsson, 2000).

2.2 Methods

2.2.1 Study animals

In the summer and fall of 2002, I sampled sexually mature Arctic charr by gill nets at Ólafsdráttur (large benthivorous charr, LB) and Mjóanes (small benthivorous morph, SB, and planktivorous morph, PL) in Thingvallavatn, S-W Iceland. Similarly, mature adults of the silver morph (SM) were collected in the inflow river at Vatnshlíðarvatn, in the north of Iceland. The fish were transported to Verid, the experimental station of Hólar University College at Sauðarkrókur, Iceland, and stripped 1 day after arrival. Eggs were stripped from four ovulating LB females, 23 PL females, 20 SB females, and 11 SM females. Total wet mass of eggs per female was weighted to the nearest gram. Fork length (FL) and body weight were recorded before stripping. For wild fish, body weight after stripping was also recorded in order to calculate mass of the total eggs per female (Sibthorpe *et al.*, 2006). The eggs of each female were fertilized *in vitro* with sperm from one male of the same morph creating full sibling families. The progeny of these crosses were used by Sibthorpe *et al.* (2006) to characterize Pax7 genes expression across morphs. I used a sample of dried

eggs from that experiment (Sibthorpe *et al.* 2006) to investigate the total energy content per egg across morphs of Arctic charr.

In 2007, I stripped 13 aquaculture females from the breeding programme at Hólar University College, and fertilized their eggs with sperm from one male. Fish were from a fourth aquaculture generation. Females were measured for FL and body weight but egg mass was not recorded.

2.2.2 Female body size and egg size

I placed 200 fertilized eggs per female into individual net cages for incubation at 4 - 5ºC. The individual egg diameter of 25 eggs per female was measured to the nearest 0.01mm from a digital picture (see details in Eiríksson *et al.*, 1999) using the software SigmaScan Pro 5. A sample of 10-12 1-day post fertilization (dpf) eggs was collected from each female. Individual eggs were gently wiped with a tissue removing water around the egg, weighed to the nearest 0.0001g (wet weight) and immediately frozen at -20ºC for total energy content analysis.

Since I used extra material from Sibthorpe *et al.* (2006), I have slightly different data for the aquaculture population and the four wild populations. For each wild female, I had data on total egg mass, FL and body weight as well as egg wet weight, egg dry weight and total energy content of individual eggs. Those data were collected by Sibthorpe *et al.* (2006) but they did not include egg diameter measurements. For each aquaculture female, I collected data on female FL and body weight as well as egg diameter, egg wet weight, egg dry weight and total energy content of individual eggs. Total egg mass per female could not be calculated for aquaculture females because the females were not stripped of all remaining eggs.

2.2.3 Egg size and egg quality

To assess the relationship between egg size and energy content, I measured total water content and the total energy content of individual eggs. The diameter of individual eggs was measured to the nearest 1 mm using a caliper and then weighed to the nearest 0.0001g. Subsequently the eggs were enclosed in individual foil pouches, labelled and dried in an oven for 24 hours at 80ºC after which dry weight was measured to the nearest 0.0001g. The total energy content of individual eggs was determined with a bomb calorimeter (Calorimeter system, C 200, IKA-Werke) and expressed in terms of energy content (J/g) and total energy content per egg (J). I examined 10 eggs per female for energy content. I examined egg energy content of all females from each population.

I used the coefficient of variation (CV) of egg dry weight within females as a measure of variation in egg size (see also Einum and Fleming, 2002). Egg dry weight CV was equal to the standard deviation of mean egg dry weight for each female expressed as a percentage of the mean (Sokal and Rohlf, 1981). I use CV to compare the relative amounts of variation because populations had different mean egg dry weight.

2.2.4 Embryo development and growth rate

I followed 10 individual embryos from each of the 13 aquaculture families from the eyed-stage until first feeding. Individual eggs were placed into individual mesh cells and all 130 cells were placed into a single flow through tank (temperature 5.1 ± 0.1°C). Before assigning an egg to a cell, individual eggs were weighed (to the nearest 0.0001g) and photographed to estimate egg diameter. Data on mortality and hatching time were derived from daily inspections. I tested for correlation between egg size and mortality from fertilization until hatching and from hatching to first feeding. All embryos alive at the time of hatching were photographed on the hatching day. All free swimming embryos were individually photographed again at 138 dpf, i.e. approximately 2 weeks before first feeding. Individual embryos were placed in a petri dish filled with cold water and set on a light table with millimeter paper. The camera was mounted on a camera stand 30 cm above the petri dish.

From the digital pictures, egg diameter was estimated as the mean of the 8 longest distances accross the egg, while standard length of embryos (SL) was measured as the distance from the tip of the snout to the end of the notochord. Yolk- sac volume (YV) was estimated based on a spheroid shape: $V = LH^2 (\pi/6)$ where H is the heigth and L the length of the yolk- sac (Blaxter and Hempel, 1963). SL and YV were measured to the nearest 0.001 mm at hatching and 138 dpf using the image analysis SigmaScan Pro 5. After hatching, yolk depletion rate was calculated as the YV depleted per day and growth rate as SL increment per day.

Approximately 1 week before hatching, I examined visible melanophores . Melanophores are distinct developmental features used to characterize developmental rate of live embryos (Metcalfe *et al.*, 1990). Live embryos in eggs were examined under a dissecting microscope with lighting from beneath and the melanophores on the head were counted (for more details see Valdimarsson *et al.*, 2002).

2.2.5 Data analysis

Simple linear regressions were used to evaluate the relationships between female FL and egg size and between egg size and total energy content of the eggs. Total egg mass and the mean egg dry weight per female were log transformed to reduce variation among data points and to meet the normality assumption. Female body length and female body weight were highly correlated (Pearson's correlation across all populations: $r = 0.94$ $n = 67$ $p < 0.001$; see Figure 2.1 for correlations within population).

Differences in total egg mass per female, mean egg diameter and mean egg dry weight (per female) among Arctic charr populations were examined using analysis of covariance (ANCOVA), with female FL as covariate and fish population as main factor. Differences in mean energy content across populations were examined using first an ANOVA with 1 factor (population) and then with an ANCOVA with egg dry weight as a covariate. Scheffé post hoc tests were used to determine if any of the variables differed between wild and aquaculture populations.

Simple linear regressions were used to evaluate the relationships between female FL and CV of egg dry weight per female across all populations. CV values were log

transformed to meet a normal distribution (Shapiro- Wilk = 0.958 df = 46, p = 0.094). Differences in CV among across populations were examined with ANOVA with 1 factor (population) and then with an ANCOVA with FL as a covariate. The SB population was removed from these analyses because I had only two eggs measurements per female.

Egg diameter and mortality were not normally distributed so the correlation between mortality and egg diameter at hatching and first feeding was checked using Spearman's correlation. ANOVAs were used to investigate the influence of egg size, female origin, female FL and hatching time (random factors), on egg development (number of melanophores) and growth rate (yolk depletion rate and SL increment per day). Hatching time was included in the analysis because eggs did not hatch on the same day. Egg weight was highly correlated with egg diameter (Pearson's correlation: df = 113, p-value < 0.001, $r^2 = 0.89$) so I used egg weight as an indicator of egg size. For each dependent variable (number of melanophores, yolk depletion rate, and SL increment per day) I started with the full model, i.e. including the four factors and all their interactions, then the best model explaining the response was selected using a stepwise selection. The best-fitting model had the lowest Akaike information criterion (AIC) and differed from the other models by at least 2 units. Independent variables were checked for multicollinearity using the variance inflation factors (VIF).

2.3 Results

2.3.1 Female body size and egg size

As expected total egg mass was affected by female body length across the wild populations of Arctic charr (ANCOVA: female fork length FL as a covariate: $F_{1, 59} = 59.02$; p < 0.001, $r^2 = 0.84$). After accounting for FL, total egg mass per females differed across wild populations (ANCOVA: $F_{3, 59} = 3.22$; p = 0.029) and within all population except the PL morph from Thingvallavatn (Table 2.1).

Egg dry weight decreased with female FL across all populations, (y = -0.003 x log (egg dry weight) + 1.30; $r^2 = 0.16$ $F_{1, 59} = 10.95$; p = 0.002; Figure 2.2). Within populations / morphs this relationship was only significant for the SM from Vatnshlíðavatn and the aquaculture populations (Figure 2). PL and SB morphs had weak r^2 values ranging from 0.02 to 0.09 whereas the LB morph had a $r^2 = 0.3$ but only data from 4 females were collected (Figure 2.2). After accounting for female body length, mean egg dry weight differed between wild and aquaculture charr (ANCOVA covariate: df = 1, $F_{1, 602} = 66.14$, p < 0.001; populations: df = 4, $F_{1, 608} = 240.11$, p < 0.001); mean egg dry weight was significantly greater in wild populations (X = 0.019 ± 0.0025g; Table 2.2) than in the aquaculture population (X= 0.012 ± 0.0025 g; $F_{1, 601} = 533.28$, p = 0.001). LB and PL morphs had higher egg dry weight than SM and SB morphs (Scheffé post hoc test: p < 0.001 for all comparisons; Table 2.2).

2.3.2 Egg size and energy content

Egg dry weight was correlated with egg diameter in the aquaculture population (Pearson's correlation: $r = 0.661$ $n = 214$ $p < 0.001$; no data are available on egg diameter for wild populations).

The coefficient of variation (CV) of egg dry weight increased with female FL across all populations ($y = 0.012$ x log (CV) + 0.424; $r^2 = 0.50$ $F_{1, 45} = 43.50$; $p < 0.001$; Figure 2.3). However there was no significant relationship between CV and female FL within each population (Figure 2.3). There was no difference of slopes between populations (ANCOVA: population x FL, $F_{1, 45} = 0.634$, $p = 0.598$ and FL as a covariate: $F_{1, 45} = 1.941$, $p = 0.172$). The CV of egg dry weight was higher (12.6 %) in aquaculture population than in wild populations (LB = 5.1 %; SM = 6.2 %; and PL = 4.9 %; ANOVA: $F_{1, 45} = 15.373$, $p < 0.001$; Figure 2.3). However, after accounting for female FL there was no difference in CV among populations (ANCOVA: $F_{1, 45} = 0.932$, $p = 0.434$; and FL as a covariate $F_{1, 45} = 0.510$, $p = 0.479$).

The correlation between egg dry weight and egg wet weight was stronger in the SB and the PL morphs ($r = 0.94$ $n = 40$ $p < 0.001$ and $r = 0.85$ $n = 222$ $p < 0.001$, respectively) than in the SM and LB morphs ($r = 0.60$ $n = 94$ $p < 0.001$ and $r = -0.1$ $n = 40$ $p = 0.559$).

As would be expected for such a wide range of egg sizes a strong correlation between egg dry weight and egg energy content was recorded across all populations, ($y = 27082$ x egg dry weight $- 35.07$; $r^2 = 0.86$; ANOVA: $F_{1, 222} = 1393.06$; $p < 0.001$; $n = 224$; Figure 2.4). Energy content ranged from 18 to 33 kJ/g (3 outliers were excluded; Figure 2.4). On average, aquaculture eggs had lower energy content per egg (mean ± SD: 320.78 ± 73.26 J) than all four wild populations (477.41 ± 110.40 J; Scheffé tests: $p < 0.001$ for all pairwise comparisons). Additionally, eggs from LB and PL morphs had significantly higher energy content than eggs from SM and SB morphs (Figure 2.4). Controlling for egg size by introducing egg dry weight as a covariate in ANCOVA still returns a significant population effect ($F_{4, 218} = 5.81$; $p < 0.001$; covariate egg dry weight effect $F_{1, 218} = 337.28$; $p < 0.001$). LB eggs had significantly higher energy content than those of the SM, PL morphs and aquaculture population (Scheffe tests: $p = 0.003$, 0.001 and 0.022 respectively; Figure 2.5). Other pairwise comparisons were not significant (i.e. Scheffé tests with $p > 0.05$).

2.3.3 Egg size and early development

Mean egg diameter was not correlated with mortality from fertilization to hatching (Spearman's correlations: rho = 0.06 $n = 13$ $p = 0.859$) or from hatching to first feeding (rho = -0.33 $n = 13$ $p = 0.274$).

Embryos hatched mainly at night and hatching extended over 16 days. Most embryos (81%) hatched over a period of 5 days and the mean hatching time was 113.5 dpf. While hatching time differed significantly among females (ANOVA: $F_{1, 107} = 8.68$; $p = 0.004$) the effects of female size and egg size were not significant ($F_{1, 107} = 0.00$; $p = 0.991$ and $F_{1, 107} = 0.00$; $p = 0.965$) as were the interaction terms; female x egg size, female FL x egg size ($F_{1, 107} = 0.02$; $p = 0.882$ and $F_{1, 107} = 0.01$; $p = 0.912$) indicating that some female characteristic other than FL or egg size affected hatching time.

The additive model (standard length ~ egg weight + female + hatching time+ female FL) explained most of the variation in SL at hatching and none of the factors showed colinearity (variance inflation factors < 10). SL at hatching was affected by female ($F_{1,110}$= 42.19; p < 0.001, Table 2.3), female FL ($F_{1,110}$= 14.19; p < 0.001; Table 2.3), hatching time (with longer embryos hatching later; $F_{1,110}$= 18.53; p < 0.001) and egg weight (with heavier and larger eggs giving larger embryos; $F_{1,110}$= 62.86; p < 0.001; Figure 2.6A). SL at first feeding was affected by egg weight ($F_{1,85}$= 20.07; p < 0.001), female ($F_{1,85}$= 14.50; p < 0.001), female FL ($F_{1,85}$= 9.43; p < 0.001) and egg weight x hatching time ($F_{1,85}$ = 18.49; p < 0.001); longer embryos at first feeding came from larger eggs that hatched later (Figure 2.6A).

The additive model Yolk-sac volume (YV) at hatching ~ egg weight + female FL + hatching time + egg weight x hatching time) explained most of the variation of the YV remaining at hatching and at first feeding. YV at hatching was greater in embryos coming from larger eggs ($F_{1,86}$ = 185.73; p < 0.001; Figure 2.6B), in embryos that came from larger female ($F_{1,86}$ = 5.23; p = 0.025; Table 2.3) and in eggs that hatched later ($F_{1,86}$= 11.44; p = 0.001). At first feeding, YV was affected by the interaction between the effects of females x female FL x hatching time ($F_{1,78}$ = 4.13; p = 0.045) and by egg size ($F_{1,78}$ = 67.13; p < 0.001; Table 2.3).

Yolk sac depletion rate between hatching and first feeding was best explained by egg weight (yolk depletion per day~ egg weight), with embryos from larger eggs using up more yolk per day than embryos coming from smaller eggs ($F_{1,89}$ = 52.97; p < 0.001; Figure 2.7 and Table 2.3). The growth rate, estimated as the SL increment per day, was best described by the model (growth SL ~ egg weight + hatching time +female size + egg weight x hatching time) but only the interaction egg weight x hatching time was significant ($F_{1,85}$ = 6.64; p = 0.012). Thus, larger embryos that hatched later had a higher growth rate.

Before hatching, embryos in larger eggs had a greater number of melanophores, however only the interaction egg weight x female had a significant effect on the number of melanophores on the forehead ($F_{1,126}$ = 6.22; p = 0.014).

2.4 Discussion

This study is the first to measure the total energy content of the eggs of Arctic charr. The energy content of Arctic charr eggs (ranging from 18 to 33 kJ/g) was similar to what has been seen in other fishes producing large eggs (Kato and Kamler, 1983; Kamler, 2005). Energy content (J/g) and total energy content per egg (J) were reflected in egg size in both wild and aquaculture populations: i.e. there was a higher total energy content in larger eggs. Aquaculture eggs within one clutch were more variable than those of wild population but this effect was related to the larger size of aquaculture females. As I predicted, large wild Arctic charr females had greater total egg mass than smaller ones. Female body size influenced egg size of Arctic charr in both aquaculture and wild populations, where egg dry weight increased with female body length. This relationship was however only significant for the Silver and the Aquaculture populations. Similar results have been seen in Brown trout, *Salmo trutta* (Einum and Fleming, 1999), and in Saimaa aquaculture Arctic charr (Seppä, 1999). In my study the strength of the correlation varied among the populations of

Arctic charr. This may reflect habitat differences during egg maturation and thus selection pressures acting on females and egg size.

Variation between populations was also visible in terms of total energy content per egg. After accounting for egg size, only large benthic eggs had significantly higher energy content than the other populations. Their energy content was most similar to those of small benthic morph females that did not differ significantly in a pairwise comparison. These two morphs inhabit similar shoreline lava habitats within Thingvallavatn (Snorrason *et al.*, 1994) and feed on similar prey, mainly the freshwater snail *Lymnea sp.* (Malmquist *et al.*, 1992a). These results suggests that differences in diet among the five populations may explain the different total energy content deposited in the eggs. These energy differences may then further reinforce the observed divergence of Arctic charr by providing different amount of energy available to the embryos, and subsequently reflect differences in early development (Skúlason, 1990; Eiríksson *et al.*, 1999; Parsons *et al.*, 2010), behaviour and morphology of juvenile Arctic charr (chapters 2 and 3). These observed differences show different maternal investment across the populations, which may be direct results from different life history adaptations (Hendry and Stearns, 2004; Quinn, 2005). Similar adaptations can be seen in the variation of egg size between anadromous and non-anadromous fish within the same species, where anadromous fish are usually larger and produce larger eggs than non-anadromous fish (e.g. Heath *et al.*, 2003).

To study further the importance of egg size for early development, I looked at how egg size correlated with numerous life history traits in aquaculture charr. Larger embryos at hatching and at first feeding originated from larger eggs with larger yolk sacs and more energy. Free swimming embryo growth rate (calculated as the standard length increment per day) and yolk depletion rate were faster in larger embryos indicating that embryos from large eggs use relatively more yolk than embryos from small eggs and convert this energy into somatic growth. These results are in accordance with previous literature on salmonids where egg size is usually positively correlated with offspring length at first feeding (e.g. Einum and Fleming, 1999; 2000a; 2002; Pakkasmaa and Jones, 2002). However survival of eggs, embryos and first feeding fish were not connected to egg size, supporting the findings of Jónsson and Svavarsson (2000) for Arctic charr.

Variation in egg size and then body size of the embryo at first feeding may affect the growth and survival of young-of-the-year fish with important life history consequences (e.g. Metcalfe and Thorpe, 1992). These parameters can be highly variable in cultured fish as well as in wild stocks and may be limiting factors to the success of hatchery juvenile production and to fish recruitment in the wild. A few studies (Srivastava and Brown, 1991; Einum and Fleming, 2000a) have investigated differences in egg size between farmed fish and wild fish indicating that egg size may have influences at emergence and in turn, survival and size of juveniles at later life stages. Our results illustrate such differences in egg size (and related energy content) between aquaculture and wild populations of Arctic charr: aquaculture eggs were smaller than wild eggs. Similar results have been seen in other species where smaller egg size was observed in farmed Atlantic salmon, *Salmo salar,* compared to wild ones (Einum and Fleming, 2000b). However those authors suggested that smaller egg size may be the cause of lower survival of embryos in farmed Atlantic salmon whereas here we have shown that Arctic charr embryos coming from smaller eggs do not show higher mortality (see also Jonsson and Svavarsson, 2000). In addition to smaller egg

size and lower energy content , aquaculture egg were also more variable in terms of egg dry weight. These results may account for a lower survival of aquaculture embryos under natural conditions, and of salmonids other than Arctic charr under hatchery conditions (e.g. in steelhead trout *O. Mykiss,* C. Leblanc unpublished data). Increased variance in aquaculture egg content may be due to the suppression of selective pressures acting on both female and eggs and / or result from the action of artificial selection in hatchery condition. These changes in selection may partly explain why farmed fish are of lower fitness than wild fish in the natural environment (reviewed by Metcalfe *et al.*, 1992).

The role of egg size in early life history traits of salmonids has been widely reported (e.g. Beacham and Murray, 1990; Heath and Blouw, 1998; Heath *et al.*, 1999; Einum and Fleming, 2000a; Pakkasmaa and Jones, 2002), where changes in body size and egg size likely represent life history adaptations within and among populations (e.g. Metcalfe and Thorpe, 1992). Thus the observed differences in the egg size and energy content of the Icelandic morphs likely reflect differences in life history (Figure 2 and Skúlason, 1986), that is then reflected in phenotypic differences. These differences have been connected with differences in developmental pathways. The small benthic morph, that has smaller eggs, dedicated more energy towards bone development than planktivorous charr coming from larger eggs (Eiríksson *et al.* 1999). Because small embryos are more dependent on environmental factors (e.g. food availability) than large embryos at first feeding (Kristjánsson and Vøllestad, 1996), smaller embryos may need to develop feeding structures faster and/or earlier. Larger embryos may in turn allocate energy to somatic growth rather than to skeletal development. Besides potential differences in development connected to egg size, mobility and feeding behaviour are also related to egg size in both aquaculture and wild fish (Benhaïm *et al.*, 2003; Sturlaugsdóttir, 2008). These differences in developmental pathways observed among different morphs may be related to the evolution of direct and inderect development (Balon 1991) where the embyos coming from smaller eggs are closer to having indirect development. In amphibians, egg size influences the resource-use phenotypes of the offspring (Martin and Pfennig, 2010) with direct effects on intra- specific diversity. Therefore the connection between egg content and early life history traits of polymorphic fishes needs to be further explored. In particular maternal effects through egg size and egg content may facilitate early divergence of such species. Icelandic polymorphic Arctic charr populations inhabit relatively stable environments and display large variations in egg size between morphs. Such properties make this system very appealing to investigate the role of maternal and egg size effects in the evolutionary biology of fishes.

There is a real need to go beyond the description of correlations between egg size and offspring traits in order to better understand the role of egg size and especially energy content in the diversity of polymorphic species. Such an avenue of research may reveal that egg size is finely tuned with developmental pathways of fishes, as shown in aquatic invertebrates and amphibians (Wake and Hanken, 1996; Moran and McAlister, 2009).

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Acknowledgements

Table 2.1 Relationship between female body length (cm) and total egg mass (g) among morphs of Arctic charr. *Results from simple linear regression of female body length on log transformed total egg mass are presented. "Overall" refers to the pooling of data from all four wild populations. Degree of freedom was 1. Significant results are highlighted in bold.*

	n	r^2	slope	intercept	F	p-value
Large benthic	4	0.38	0.03	0.81	22.14	**< 0.001**
Silver morph	10	0.86	0.07	-0.24	507.44	**< 0.001**
Small benthic	20	0.62	0.12	-1.10	60.99	**< 0.001**
Pelagic morph	20	0.01	-0.01	1.25	10.99	0.131
Overall	54	0.80	0.06	-0.12	1498.31	**< 0.001**

Table 2.2: Mean egg dry weight (g) and mean female fork length (cm) for five populations of Arctic charr, Salvelinus alpinus. *N (female) refers to the number of females that were measured for each population. Mean egg dry weight (g) was calculated as the mean for each population ± SD as the standard deviation and n corresponding to the total egg number measured.*

Population	n (female)	Mean female fork length (cm)	Mean egg dry weight (g)	n (egg)
Large benthic	4	33.25 ± 2.64	0.022 ± 0.001[a]	40
Silver morph	10	23.24 ± 3.14	0.015 ± 0.003[b]	93
Small benthic	20	12.71 ± 1.90	0.016 ± 0.003[b]	40
Pelagic	20	20.53 ± 1.24	0.021 ± 0.003[a]	221
Overall wild populations	54	22.43 ± 2.23	0.019 ± 0.003	394
Aquaculture	13	54.62 ± 3.35	0.012 ± 0.003[c]	214

Results of Scheffe post hoc tests are indicated by letters in super script. When letters differ it indicates that the two populations differ significantly in mean egg dry weight with $p < 0.001$.

Table 2.3: Fork length (FL) and means (± SE) of various characteristics of Arctic charr eggs, embryos and first feeding embryos from 13 aquaculture females. All females were 4 years old and were fertilized by the same male.

Female	Female FL (cm)	Egg wet weight (mg)		Egg diameter (mm)		Standard length at hatching (mm)		Standard length at 138 dpf (mm)		Yolk sac volume at hatching (mm³)		Yolk sac volume at 138 dpf (mm³)		Yolk depletion rate (mm³/ day)		Standard length increment (mm/ day)	
1	56.20	44.67	± 2.15	4.75	± 0.13	13.77	± 0.32	17.53	± 0.39	24.45	± 3.03	13.35	± 2.70	0.36	± 0.13	0.13	± 0.02
2	58.40	39.38	± 2.04	4.60	± 0.06	12.29	± 0.35	17.19	± 0.47	23.90	± 3.25	10.23	± 1.21	0.48	± 0.13	0.16	± 0.03
3	56.70	35.63	± 1.40	4.48	± 0.09	13.23	± 0.20	16.93	± 0.42	15.51	± 1.76	9.82	± 1.50	0.17	± 0.05	0.14	± 0.03
4	55.00	39.24	± 2.72	4.55	± 0.13	13.61	± 0.33	17.21	± 0.21	18.69	± 2.12	8.84	± 1.80	0.30	± 0.09	0.11	± 0.03
5	54.70	34.91	± 1.45	4.47	± 0.09	14.00	± 0.18	18.60	± 0.61	12.37	± 1.58	5.04	± 0.52	0.31	± 0.06	0.20	± 0.03
6	54.20	36.95	± 1.55	4.58	± 0.06	14.12	± 0.23	18.22	± 0.27	16.80	± 1.85	7.25	± 0.32	0.40	± 0.07	0.17	± 0.01
7	53.50	39.02	± 1.32	4.59	± 0.07	13.25	± 0.40	17.70	± 0.59	19.31	± 1.99	9.00	± 0.48	0.34	± 0.10	0.15	± 0.02
8	56.50	41.20	± 1.15	4.66	± 0.07	14.72	± 0.27	19.13	± 0.17	17.28	± 1.15	5.31	± 0.54	0.37	± 0.09	0.13	± 0.03
9	54.60	38.37	± 0.86	4.46	± 0.04	14.39	± 0.22	18.27	± 0.45	15.30	± 1.43	10.03	± 1.45	0.19	± 0.09	0.13	± 0.03
10	52.20	34.52	± 1.18	4.41	± 0.08	12.93	± 0.36	16.73	± 0.49	15.99	± 1.32	11.94	± 1.79	0.11	± 0.08	0.13	± 0.02
11	58.60	41.83	± 1.45	4.75	± 0.06	14.09	± 0.19	18.19	± 0.32	23.87	± 1.79	12.26	± 1.07	0.44	± 0.09	0.14	± 0.02
12	55.10	35.24	± 1.59	4.41	± 0.08	13.96	± 0.34	18.08	± 0.42	13.61	± 1.67	4.96	± 0.99	0.24	± 0.09	0.14	± 0.03
13	55.20	28.86	± 1.55	4.02	± 0.11	13.00	± 0.37	17.33	± 0.29	10.04	± 1.47	2.33	± 0.43	0.28	± 0.05	0.16	± 0.01
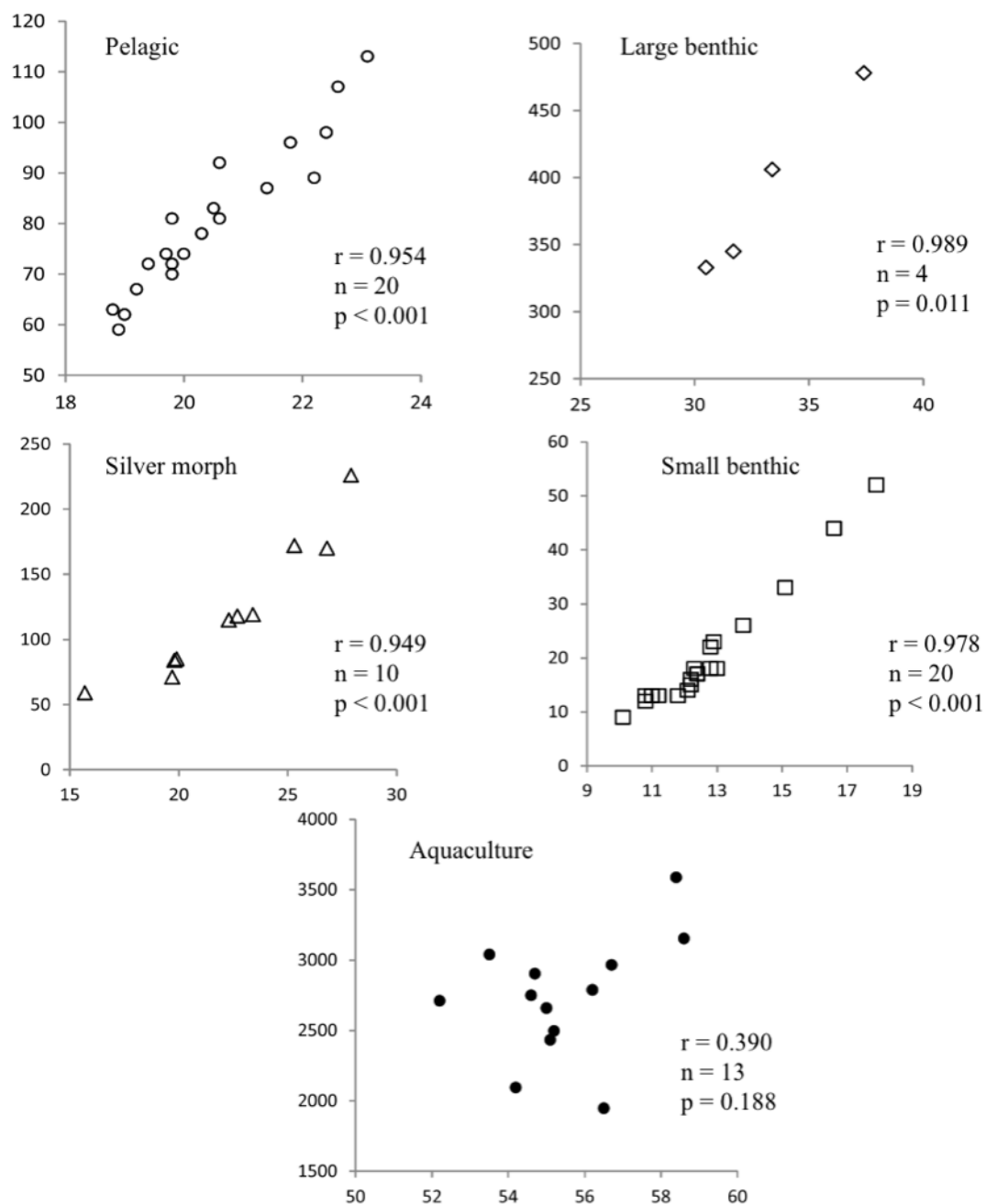
***Figure 2.1: Relationship between female fork length (cm) and female body weight (g)
for five populations of Arctic charr.*** *X-axis is female fork length (cm) and Y-axis is female
body weight (wet weight in g). Pearson's correlation coefficients (r), sample sizes (n), and
probabilities (p) are given for each population.*
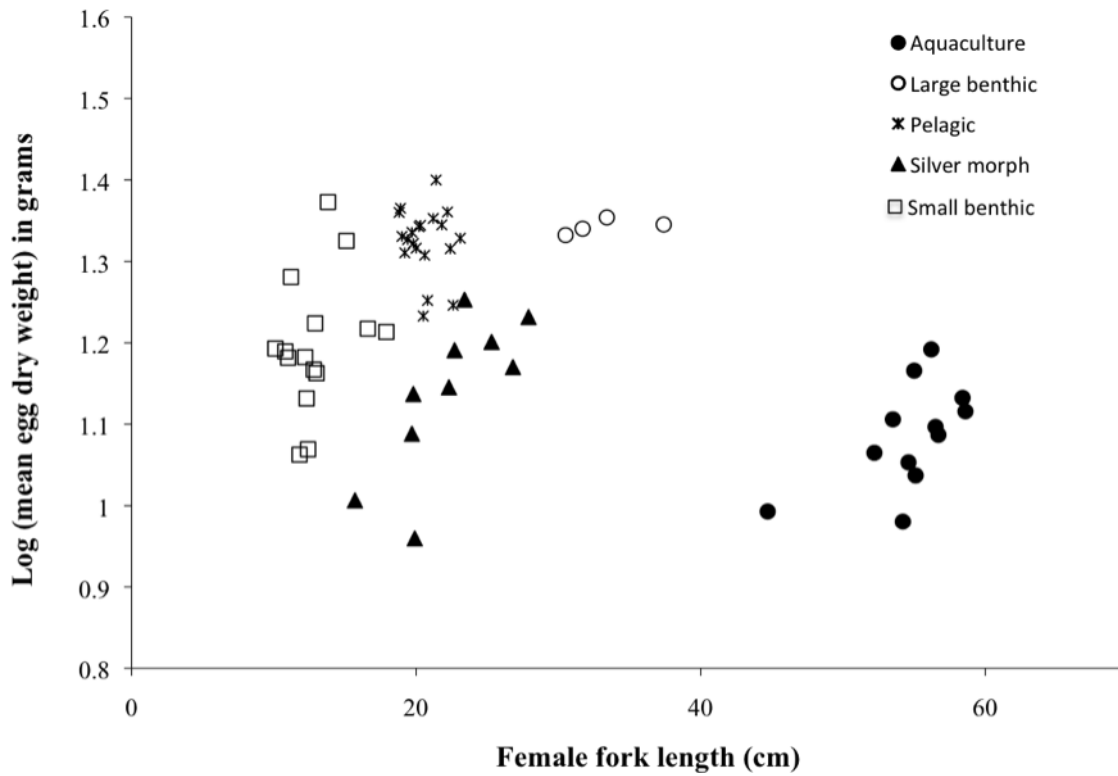
Figure 2.2: Relationship between female fork length (cm) and mean egg dry weight (g) in Arctic charr. *Each point is the log (mean egg dry weight) in grams for each female. Simple linear regressions of log (mean egg dry weight) on female FL for each population:*

Large benthic: y = 0.002 x log (egg dry weight) + 1.29 r^2 = 0.30; $F_{1, 3}$= 0.848; p = 0.454
Silver morph: y= 0.02 x log (egg dry weight) + 0.70; r^2= 0.58; $F_{1, 9}$= 11.271; p = 0.010
Small benthic: y = 0.01 x log (egg dry weight) + 1.05 r^2= 0.09; $F_{1, 14}$= 1.344; p = 0.267
Pelagic: y = -0.01 x log (egg dry weight) + 1.42; r^2 = 0.02; $F_{1, 19}$= 0.397; p = 0.536
Aquaculture: y = 0.01 x log (egg dry weight) + 0.53 r^2= 0.34; $F_{1, 11}$= 5.069; p = 0.048
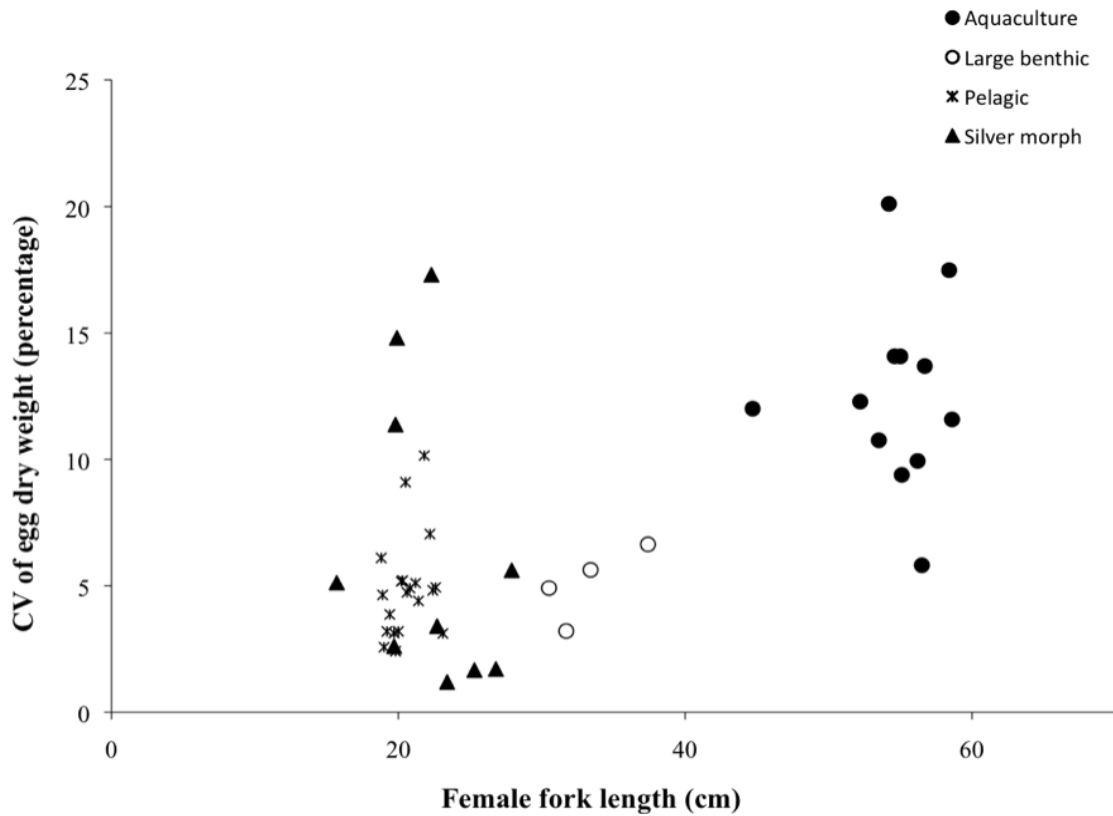
Figure 2.3: Relationship between female fork length (cm) and the coefficient of variation (CV) of mean egg dry weight in four populations of Arctic charr.
Simple linear regressions of log (CV) on female FL for each population:

Large benthic: $y = 0.03 \times \log (CV) - 0.33$; $r^2 = 0.47$; $F_{1, 3} = 1.791$; $p = 0.313$
Silver morph: $y = 0.00 \times \log (CV) + 0.671$; $r^2 = 0.03$; $F_{1, 9} = 0.012$; $p = 0.914$
Small benthic: $y = 0.01 \times \log (CV) + 1.05$ $r^2 = 0.09$; $F_{1, 14} = 1.344$; $p = 0.267$
Pelagic: $y = 0.04 \times \log (CV) - 0.16$; $r^2 = 0.10$; $F_{1, 19} = 1.962$; $p = 0.178$
Aquaculture: $y = -0.00 \times \log (CV) + 1.134$ $r^2 = 0.00$; $F_{1, 11} = 0.007$; $p = 0.937$
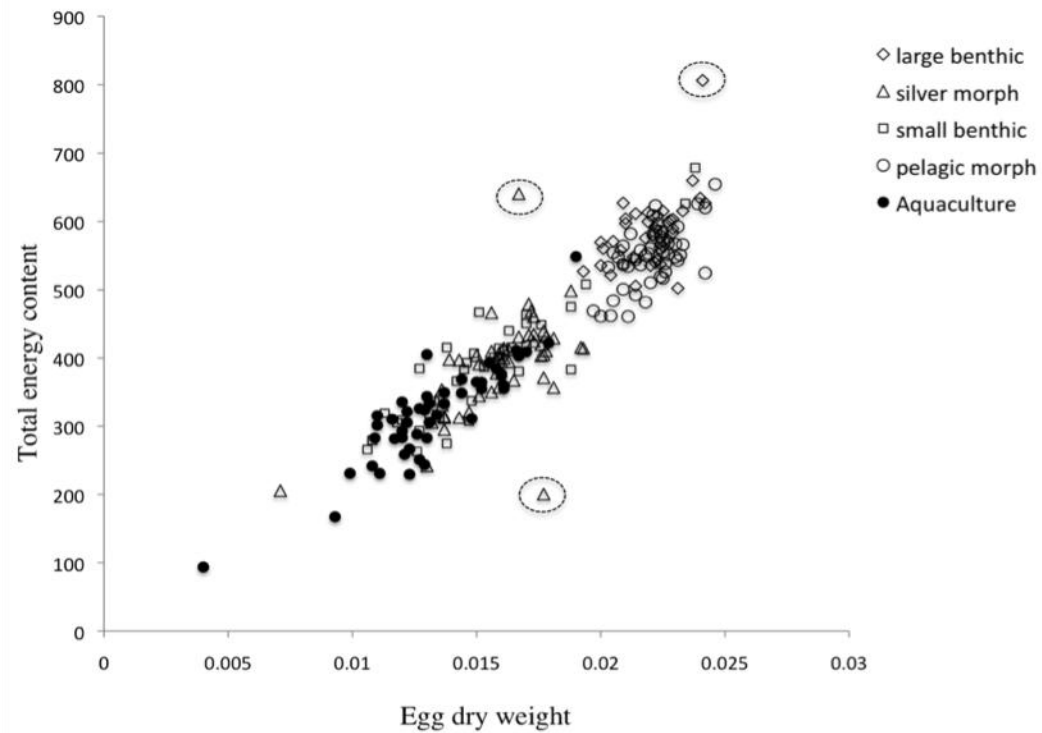
***Figure 2.4: Relationship between egg weight (g) and total energy content (J per egg) in individual egg of Arctic charr at 1- day post fertilization**. Each point represents the data for one individual egg. 3 outliers, circled data point, were removed from the analyses:*

Large benthic: y= 22140.17 x egg weight + 96.94; r^2= 0.26; $F_{1, 37}$= 13.07; p = 0.001
Silver morph: y= 20843.87 x egg weight + 55.34; r^2= 0.35; $F_{1, 47}$= 25.50; p < 0.001
Small benthic: y= 27195.76 x egg weight – 26.20; r^2= 0.85; $F_{1, 37}$= 206.47; p < 0.001
Pelagic: y= 24700.10 x egg weight + 4.34; r^2= 0.39; $F_{1, 46}$= 29.04; p < 0.001
Aquaculture: y= 25573.01 x egg weight – 20.14; r^2= 0.79; $F_{1, 46}$= 176.30; p < 0.001
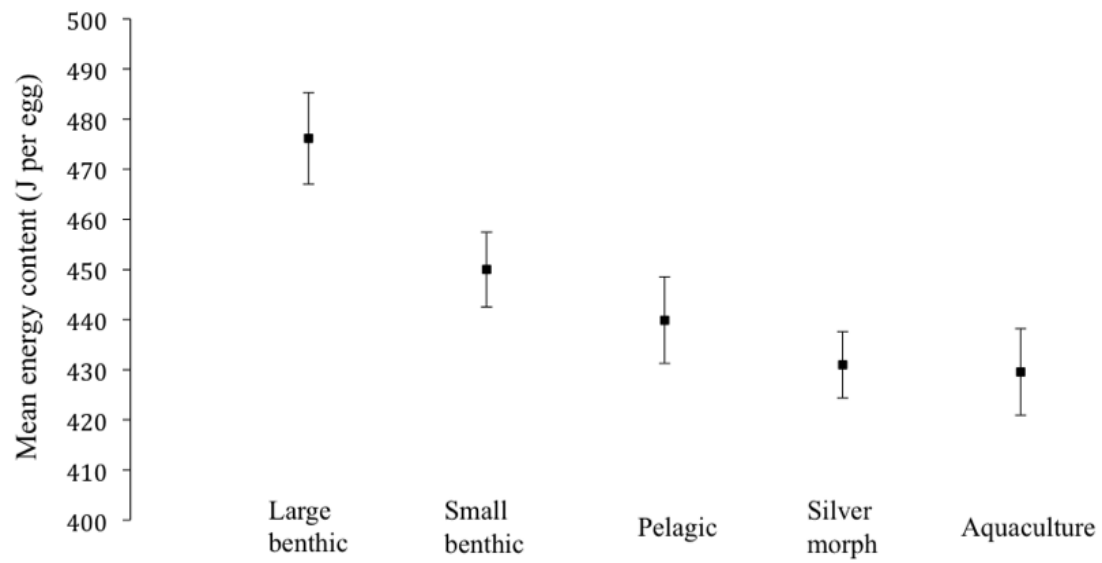
***Figure 2.5: Mean energy content (J per egg) of individual eggs of five populations of
Arctic charr after accounting for egg size****. Each point is the adjusted mean energy content
of individual egg at 1- day post fertilization, for each population. Adjusted means come
from the ANCOVA model: mean energy content ~ populations with egg dry weight as a
covariant. Error bars are standard errors.*
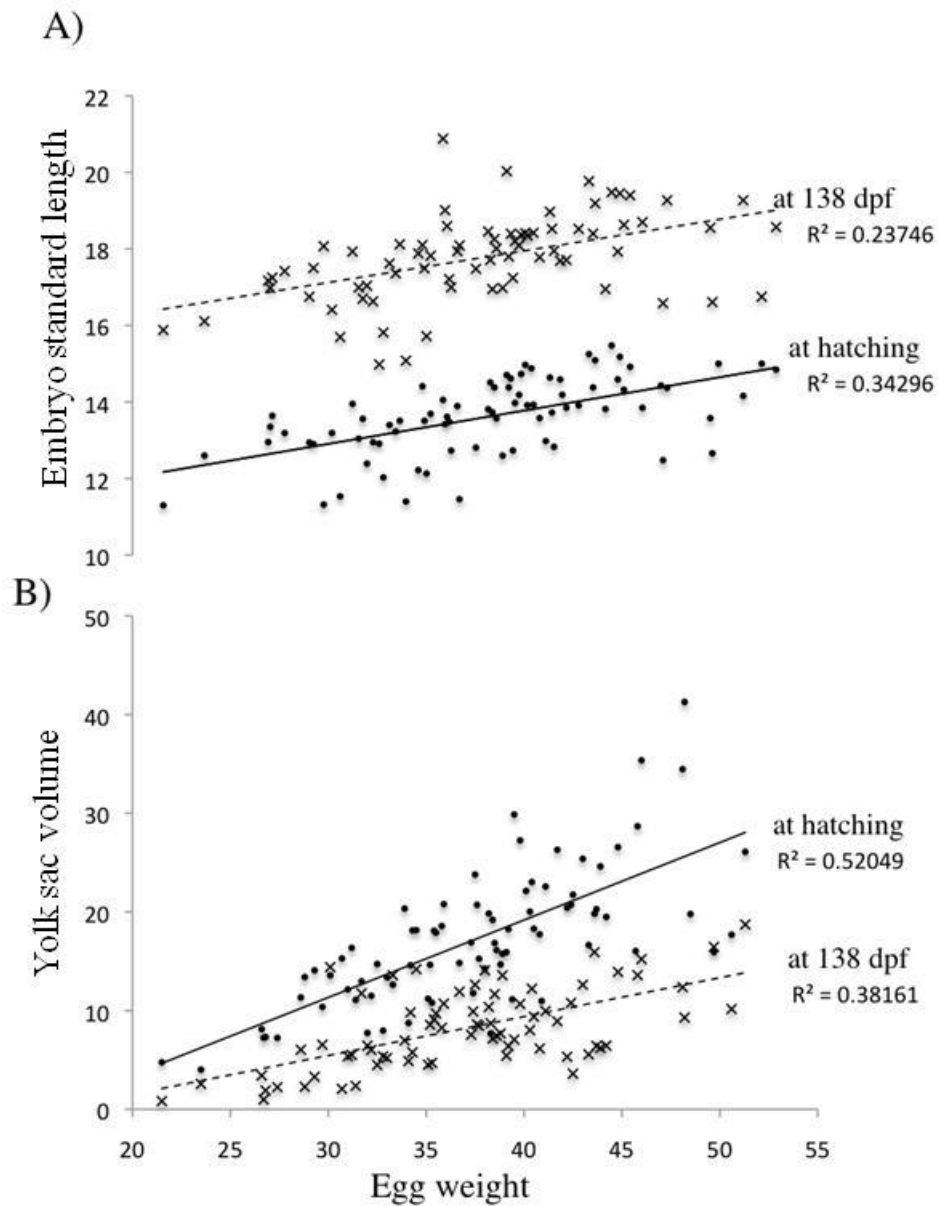
Figure 2.6: Relationship between egg wet weight (mg) and A. standard length and B. remaining yolk- sac volume (mm^3; calculated as V= LH^2 (n/6) of juvenile (free-swimming embryo) Arctic charr at hatching and 138- days post fertilization (dpf).
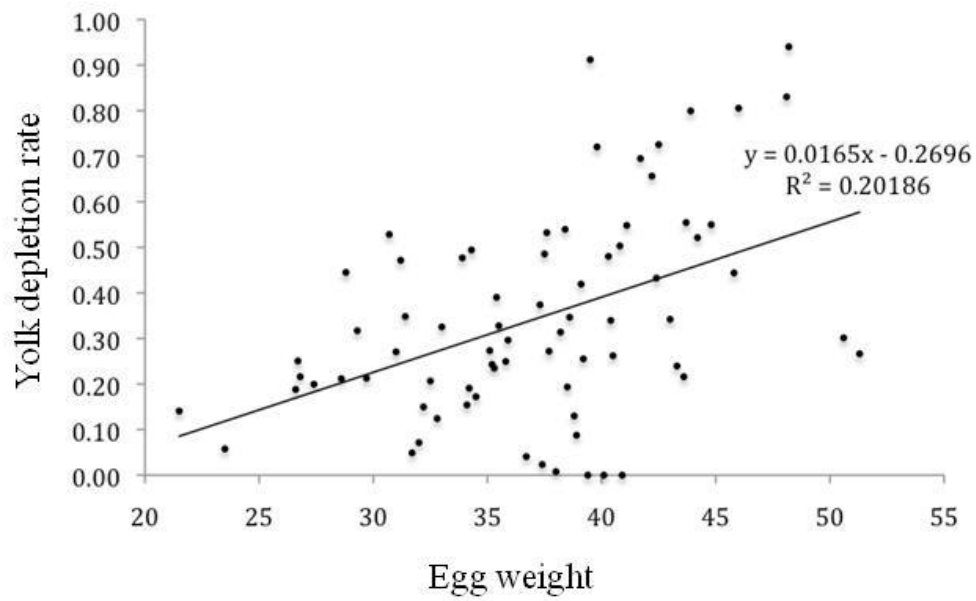
Figure 2.7: Relationship between egg weight (mg) and yolk- sac depletion rate (mm³ per day) from hatching until 138 days post fertilization.

3 The importance of egg size and social effects for behaviour of Arctic charr juveniles

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3.1 Introduction

Egg size varies considerably in fishes from microscopic eggs (e.g. 0.75 mm for the greasy grouper *Epinephelus tauvina*) up to some very large eggs in sharks and coelacanths (8 cm egg diameter). Usually a trade off exists between egg number and egg size because ovarian space and available energy for egg development are limited. Several models have attempted to explain this trade-off between quality and quantity. Lack's (1947) hypothesis and Smith and Fretwell (1974)'s model predicted that each population should have a single optimal egg size to produce the highest number of surviving offspring. Two assumptions were made in this model: (i) there is a trade-off between size and number of offspring and (ii) larger offspring have a better chance of surviving i.e. "bigger is better". Empirical data support this model in reptiles and fishes (Einum and Fleming, 2002; Heath *et al.*, 2003), but not in birds where optimal egg size was consistently smaller than the optimal egg size predicted by the model (Roff, 1992). Additionally, between-female variations within-population are in disagreement with the model predictions. Such variation is commonly associated with female phenotype (e.g. body size / age, see Roff, 1992) and parental care (Sargent *et al.*, 1987).

Extensions of the single-optimum egg size model (Smith and Fretwell, 1974) were developed to explain intra-population variation in egg size (e.g. Sargent *et al.*, 1987; Hendry *et al.*, 2001). These authors made the basic assumption that the egg-size offspring-fitness function varies with the phenotype of the mother**.** For instance, larger female Coho salmon *Oncorhynchus kisutch* produce larger eggs and provide better maternal care by guarding the redd (Quinn, 2005) resulting in higher survival of young from large eggs. Such maternal effects may explain the discrepancies with the Smith and Fretwell's model. Recent work has also explored the idea that within clutch variation of egg size may be a bet-hedging tactic as an adaptation to fluctuating environments or that it may results from other constraints (Marshall *et al.*, 2008).

In fishes, the correlation between egg size and female body size has been of interest for decades (Thorpe *et al.*, 1984; Chambers and Leggett, 1996). Much of this work has been conducted on salmonids because of their relatively large eggs (3 to 8 mm in diameter) and their important commercial value (Hendry and Stearns, 2004). They have, as well, been the focus of theoretical and empirical studies on evolutionary and ecological significance of egg size (Hendry *et al.*, 2001; Einum and Fleming, 2002; Hendry and Stearns, 2004) but the importance of egg size for population divergence has seldom been studied.

Arctic charr *Salvelinus alpinus* females show considerable variability in egg size and yolk quality resulting in a wide size distribution of juveniles at first feeding (Balon, 1980; Wallace and Aasjord, 1984a; Beacham *et al.*, 1985; Kamler, 1992; Seppä, 1999; Jonsson and Svavarsson, 2000). The correlation between egg size and Arctic charr juvenile size persists for up to 1 year after first feeding (Wallace and Aasjord, 1984a). Embryos from smaller eggs develop faster than those from larger eggs, suggesting that different timing of development is connected to egg size (Valdimarsson *et al.*, 2002). Because Arctic charr is a species lacking parental care, egg size and thus embryo size can be considered as a direct measure of maternal investment in individual offspring.

Previous studies have emphasized the high degree of polymorphism in Arctic charr (Skúlason *et al.*, 1993; Skúlason *et al.*, 1999; Snorrason and Skúlason, 2004; Klemetsen, 2010). Sympatric forms have been found to use different resources (habitat and food) and to differ in phenotypes: growth, age and size at maturity, body coloration, behaviour and body shape (summarized in Skúlason *et al.*, 1999; Klemetsen, 2010). It has been suggested that differences in early behaviour may be important for the observed diversification (Skúlason *et al.*, 1999; McLaughlin and Grant, 2001), where early behaviour is likely to influence individual behaviour later in life (Metcalfe, 1993; Salvanes and Braithwaite, 2006)**.** Thus small size differences at first feeding stemming from differences in egg size may promote differences in mobility patterns with important consequences for subsequent differences in habitat and food selection. Such differences in resource use could lead to variable life histories and promote the evolution of resource polymorphism (McLaughlin and Grant, 2001).

A common pattern proposed in the literature is that the effect of egg size on body size of progeny declines rapidly throughout development, especially when the fish starts feeding (reviewed by Mousseau and Fox, 1998; e.g. Heath *et al.*, 1999). A reduction in maternal effects e.g. egg size, through ontogeny could arise because the relative importance of the environment and genetic factors increases later in development (Lindholm *et al.*, 2006). Little is known about the interplay between the role of egg size and the environment during early developmental stages. Any effect of egg size on progeny fitness might even disappear faster in salmonid juveniles because they develop territoriality soon after exogenous feeding (Quinn, 2005). For example the effects of egg size on growth disappear at emergence in Chinook salmon *Oncorhynchus tshawytscha* (Heath *et al.*, 1999). A few days after first feeding juveniles develop a feeding territory involving agonistic interactions with conspecifics (Quinn, 2005). Such social interactions may erase the effect of egg size on growth and behaviour of salmonids early in life

The aim of this study was to investigate the mechanisms of the behavioural differences between small and large Arctic charr juveniles at the onset of first feeding as described by Benhaïm *et al.* (2003). These authors showed that large and small Arctic charr coming from large and small eggs differed in terms of mobility and foraging tactics. Those observations were conducted on fish raised in homogenous size groups but they did not account for potential agonistic behaviour that could occur in heterogeneous ones. Based on the literature, we predicted that social effects would explain most of the differences between large and small fish in fish groups. We assessed both egg size and social effects in experiments based on isolation of fish versus raising them in groups. We addressed several questions: 1) How can egg size affect early behaviour of individual offspring? and 2) How do egg size, the social environment and their interaction affect behaviour of Arctic charr? We predicted that social interactions will affect the behaviour of both size classes of fish, minimizing egg size effects on foraging and mobility: feeding behaviour and mobility will be higher in fish held in groups because of interactions with conspecifics. 3) Finally, we assessed the importance of agonistic behaviour in behavioural differences between small and large fish maintained in a group i.e. can agonistic interactions between different size fish explain the behavioural differences previously observed by Benhaïm *et al.* (2003)?

3.2 Methods

3.2.1 Eggs, Fish and Experimental Setup

We used Arctic charr from the breeding program of Hólar University College. Hatchery broodstock originated mainly from Ölvesvatn in N-W Iceland. Intra-clutch variation in egg size has been previously reported in Icelandic Arctic charr (Benhaïm *et al.*, 2003) although not studied in detail. All eggs and juveniles used in our study came from the fertilization of one female (age: 4+) with the sperm from one male (4+). We decided to use only one family as our study is the first step towards understanding how egg size and social environment affect the behaviour of salmonid juveniles. Fertilized eggs were incubated in EWOS hatching trays with flowing water (mean $\pm$ SD = 5.2 $\pm$ 0.3°C) and maintained in darkness using an opaque black plastic cover. At the eyed-stage, 50 embryos were sampled to estimate size variation. Eggs were visually sorted creating two size classes, with as much size difference as possible (paired t-test, $t_{(48)}$ = 15.8, p < 0.0001), small eggs (mean $\pm$ SD = 36.6 $\pm$ 3.1 mg, n = 25) and large eggs (mean $\pm$ SD= 51.2 $\pm$ 3.5 mg, n = 25). We placed 100 from each size class in net cages (10.5 x 10.5 x 6 cm, mesh size 0.5mm) and six from each size class were individually isolated in net cages. After the eggs had reached eyed-stage, dead embryos or unfertilized eggs were removed daily. Incubation took on average 465 degree-days and hatching date was 21 February 2005 (98 dpf) i.e. 50% of the embryos had hatched. In our experiment small fish came from small eggs and large fish came from large eggs (e.g. at 159dpf: mean $\pm$ SD small fish = 65.2 $\pm$ 6.6 mg versus large fish 98.2 $\pm$ 8.3 mg). From hatching, one group of small fish and one group of large fish were raised separately in incubating trays until being assigned to the treatment. Long-term isolated embryos were isolated at the eyed-stage and reared in the compartment for observation.

Water temperature was maintained at 4.9 $\pm$ 0.5 °C throughout the observation period and water level was held at 12 cm in each compartment. A flow velocity of 0.2 cm/s was maintained in every tray. The 12 trays were placed randomly in two tanks (250 L) and moved each week to reduce the impact of small differences in environmental variables such as temperature, light, or oxygen availability. Light intensity was about 50 lux and a 12:12 LD photoperiod was applied. The entire system was isolated from any disturbance by black opaque plastic curtains.

Juveniles were fed commercial food (EWOS micro 013C, 0.1 - 0.2 mm). Food rations were established during pre - observation periods. Fish in groups were fed a ration of 30 mg while fish kept isolated were fed 10 mg regardless of body size. Such rations were selected to allow observations of foraging by fish according to the unit volume of the trays. The food was hand-delivered once during each observation, and both amounts of food were sufficient to sustain regular growth. Daily food leftover and faeces were removed after each observation. Between observations, fish were fed three times a day accordingly to aquaculture ration for Arctic charr juveniles.

3.2.2 Experiments and behavioural observations

Three social environments were tested: no isolation i.e. group of 6 fish, short isolation, and long isolation. Short isolation refers to fish that were maintained in a group and were then isolated 24h before observation. Long isolation refers to embryos that were isolated since

eyed-stage. We had 6 small and 6 large fish in long isolation. Mobility, foraging behaviour and space use were estimated by comparing behaviour of large and small fish maintained in the three different social contexts. Behaviour was observed using focal animal sampling (Altmann, 1974) before and after food delivery. Behaviour before food delivery was recorded as a base line of activity before feeding.

The first experiment aimed to compare during development the behaviour between small and large fish coming respectively from small and large eggs, isolated since eyed embryo stage (cf. first question in the Introduction). The same 12 fish (6 large and 6 small) were individually observed 5 times during development (Table 3.1). In a second experiment we compared small and large fish in different social environments i.e. group of 6 fish versus 6 shortly isolated fish (cf. question 2 in the Introduction). We used three replicates of 6 small fish in groups and 6 large fish in homogenous group and six replicates of individual small and large fish in short isolation (Table 1). In a third experiment, we compared agonistic behaviour of juveniles from different size classes maintained in a group (cf. question 3 in the Introduction). We compared 3 replicates of small and large fish maintained in homogenous groups and 3 replicates of mixed groups i.e. three small and three large fish in a group (Table 3.1). The behavioural sampling method (Altmann, 1974) i.e. counts of behaviour occurrences before and after food presentation was used to compare agonistic behaviour between homogenous and mixed groups.

In these 3 experiments, every trial lasted for 3 minutes i.e. 1 minute before food delivery and 2 minutes after (Benhaïm *et al.*, 2003). The behaviour of fish was voice recorded to collect both the occurrence and the duration of behavioural items. The target fish was selected randomly as the first individual crossing a randomly selected area. Food pellets were supplied by hand above the left side of the unit where the food tended to drift out of the compartment. Therefore, the mobility of fish was maximized towards the feeding area. Observations were carried out daily between 09:00 hrs and 13:00 hrs. At each time fish were observed 4 days in a row in each treatment.

Experiments started 6 days after the onset of first feeding and observations were repeated five times at 159, 173, 180, 187 and 194 days post fertilization (dpf). Different fish were observed at each observation date except for the fish in long isolation. Fish were not fed for 2 days before observations, providing a similar level of appetite without causing discomfort from food deprivation. A 2 -day fasting period has been used in Arctic charr (Lahti and Lower, 2000) and other fish species without causing starvation of juveniles (e.g. Enders *et al.*, 2005). One day before observations juveniles were anesthetized and measured for length and weight (to the nearest 0.1mm and 0.001g). Then the juveniles were assigned to one of the two social environments: group or short isolation.

3.2.3 Behavioural Variables

After hatching, juveniles were kept in 12 EWOS hatching trays (39.5 x 42.5 x 17.2 cm). Each tray was longitudinally divided into six compartments, each compartment being a unit of observation for a single fish or a group of fish. In order to collect data on fish movement we visually divided each compartment into five equal viewing areas (8 x 7 cm) in length and three areas in depth: areas were marked with a waterproof marker. Using these visual landmarks we were able to describe the position of the fish horizontally and vertically, and

to record mobility. The depth of the compartment was divided in three equal parts: the surface, the water column and the bottom. Each snap by a fish at a particle in these locations was respectively called surface foraging, foraging in the water column and bottom foraging. Reaction time was also recorded and defined as the latency (in seconds) before the first bite at a food particle.

Immobility and mobility were recorded in a similar way to that described by Benhaïm *et al.* (2003): horizontal and vertical stationary movements, slow and regular swimming, jerky swimming and speed swimming (see Benhaïm *et al.*, 2003 for ethogram). We recorded both the occurrence and the duration of each activity. The total number of items corresponded to the sum of all behavioural occurrences in one observation. Additionally, space use was assessed for each fish recording the number of zones (horizontal dimension) and levels (vertical dimension) visited. We also calculated the total number of crossed areas i.e. the sum of all visited zones and levels.

Aggression level was characterized by two relevant agonistic behavioural items previously described in juvenile fish. Chase was defined as pursuit of one individual by another for at least one body length (Kim *et al.*, 2004). Escape behaviour referred to a burst and fast swimming by one individual to move away from a conspecific (Noakes, 1980).

3.2.4 Data Analysis

We used SPSS 14.0 Windows Student Version (SPSS, Inc., Chicago, Illinois, U.S.A.) for statistical analyses. Differences of weight between fish coming from large and small eggs were analyzed with a paired t-test. Data before food delivery provided a baseline of behaviour / activity shown by the fish before feeding. Data after food delivery were analyzed to assess mobility, foraging behaviour and space use. Data were obtained by averaging the behaviour from 4 days of observations for each treatment, each replicate and each time. Data from the focal animal sampling method were behaviour durations in seconds while data from behaviour sampling method were behaviour occurrences. Data were analyzed for normality with a Shapiro-Wilk test and for homoscedasticity with a Bartlett's test.

In the first experiment, differences in behaviour, mobility, and space use between small and large fish, reared in isolation, were assessed using a repeated measures analysis of variance (ANOVA) because the same fish were followed over time (Table 3.1). Egg size (small and large) was the between-subjects factor and time was the within-subjects factor. In the second experiment we used an ANOVA where egg size, social environments and time were defined as fixed factors (Table 3.1). The model included three fixed factors, 2- and 3-way interactions. To analyse the origin of the significant differences we conducted post hoc Newman-Keuls tests in both ANOVAs.

3.3 Results

Over the course of the experiment, fish coming from large eggs were on average 32.5 ± 8.5 % larger than fish coming from small eggs. At the end of the experiment the large fish weighted 194.9 ± 24.3 mg and small fish 131.1 ± 13.3 mg (t = 14.89 df = 82 p < 0.001).

Before food presentation all fish held alone, independently of their previous social context, were immobile at least 90% of the time.

3.3.1 Experiment 1: Egg size effect on behavioural development (long term isolation)

Differences between small and large fish were detected in foraging activity, mobility and space use. Large fish foraged significantly more (i.e. total foraging) and faster (i.e. reaction time) than smaller ones (Table 3.2). On average, larger fish foraged 4.6 ± 3.3 times more than smaller ones (Figure 3.1A) and reacted to food delivery 1.4 ± 0.6 times faster than smaller ones. They were also more mobile (Figure 3.1 B) and had more active behaviour than smaller ones (Table 3.2). For instance, larger fish spent $72.8 \pm 21.3\%$ of the time immobile whereas smaller fish spent $88.0 \pm 24.0 \%$ (Table 3.I). Additionally larger fish crossed in averaged 2.4 ± 1.9 more areas than smaller ones.

Interestingly such differences became significant through development with the exception of the last observation (e.g. Figure 3.1). Reaction time to food delivery illustrates this trend where differences between large and small fish increased over time (with the exception of 194 dpf): large fish foraged on average 27.5 s earlier than small fish. This relationship became significant at 180 dpf ($p = 0.024$), 187 dpf ($p = 0.007$), 194 dpf ($p = 0.043$). Same trend was observed in the number of visited zones (173 dpf, $p = 0.035$; 180 dpf, $p = 0.020$; 187 dpf, $p = 0.050$) and the total foraging activity (180 dpf, $p = 0.055$; 187 dpf, $p = 0.093$; and 194 dpf, $p = 0.110$).

3.3.2 Experiment 2: Interaction between egg size and social effects on behavioural development (short term isolation versus group)

Egg size, social environment and time affected foraging, mobility and space use of young Arctic charr. Egg size significantly affected bottom and total foraging, but not mobility or space use (Table 3.3). However, most variables characterizing foraging and mobility and all variables characterizing space use showed a social effect (Table 3.3). For instance, fish in groups reacted faster (36.4 ± 17.5 seconds) to food delivery than fish in short isolation (93.9 ± 15.5 s). Fish became more mobile over the course of the experiment: stationary, rapid swimming, total number of visited areas and number of displayed items increased (time factor in Table 3.3). Additionally, the reaction time to food delivery significantly decreased and foraging activities increased (significantly for bottom foraging, and marginally significant for foraging in water column and surface; factor time in Table 3.3) resulting in weight gain: small fish gained in average 66.1 ± 8.9 mg and large fish gained in average 100.7 ± 14.0 mg over the experimental period of 45 days.

Bottom and total foraging activities were affected by a two-way interaction between egg size and social effect (Table 3.3). Large fish in groups foraged more than large fish in isolation, small fish in-group and small fish in isolation (post hoc tests: all $p < 0.001$; Figure 3.2A). Only rapid swimming activity, a rather rare and brief behaviour, showed the same interaction with large fish in groups displaying more rapid swimming than other groups (post hoc tests: all $p < 0.001$). The interaction between egg size and time was not found in any variables but the interaction between social effect and time was found mainly in foraging

activities and rapid swimming (Table 3.3). Fish in group at 180 and 187 dpf displayed more bottom foraging and total foraging than fish shortly isolated at all times (post hoc tests: all p < 0.001; Figure 3.2A). Similarly at 187 dpf, fish in groups displayed more rapid swimming than other groups at all times (post hoc tests: all p < 0.001).

Additionally, foraging, mobility activities and the total number of crossed areas were affected by a three-way interaction of factors (egg size, social factor and time; Table 3.3 and Figure 3.2). Overall, this interaction illustrates a gradient of activity (foraging, mobility and space use) with large fish in-group being more active than small fish in- group being more active than large fish in isolation being more active than small fish in isolation. A 3-way interaction may indicate that the interaction between egg size and social effect changed over time. For example, the average total number of foraging (Figure 3.2A): large fish in-group at 180 and 187 dpf foraged more than small fish in group and small and large isolated fish at all time (post hoc tests: all p < 0.001). Another type of 3-way interaction was observed in the number of items (Figure 3.2) and in the total number of crossed area (Table 3.3) where similar results were observed: both variables were higher in large fish in groups at 187 dpf compared to all other categories (post hoc tests: all p < 0.001) except for small fish in groups at 173 and 180 dpf, large fish in short isolation at 159 and 194 dpf, large fish in groups at 194 dpf.

3.3.3 Experiment 3: Agonistic behaviour (mixed versus homogeneous size groups)

In groups, agonistic behaviour (chase or escape) was rarely observed and no significant differences were detected between heterogeneous and homogenous size groups.

3.4 Discussion

Our results show how social environment and body size may affect behaviour at early stages of development and indicate as well how behavioural patterns may change over time. They highlight the relative importance of both egg size and social effects for small and large fish in foraging, mobility and the use of space. In long isolation, egg size affects both mobility and foraging activities. We also demonstrated that social interactions, other than agonistic behaviour, play an important role in mobility and foraging of first feeding fish. Overall, a social effect was observed in almost all behavioural items we looked at. Fish in groups were more mobile over time and space and foraged more than fish placed in short isolation. Egg size clearly affected foraging activities (larger fish foraging more than smaller fish) but did not affect mobility or space use. However, we observed 2-way interactions (egg size x social environment and social environment x time) and 3-way interaction (egg size x social environment x time) in foraging and mobility indicating that social effects alone did not explained the observed behavioural differences. The interaction egg size x social environment affected foraging behaviour and one mobility variable (i.e. rapid swimming) revealing that the combination of factors egg size and social environment do not influence much mobility or space use of the fish (Table 3.3). The influence of time was difficult to interpret: overall mobility and foraging activities increased over time up to 187 dpf. However, activities were overall lower at 194 dpf. This could reflect plasticity or an artefact of measures.

Our study supports the hypothesis that variation in feeding behaviour may not be primarily the result of social hierarchies but rather the result of a strong genetic component and / or parental effects (Ferguson and Noakes, 1982; Ferguson and Noakes, 1983; Kamler, 2005; Martins *et al.*, 2005a; b). There is substantial genetic basis for many observed differences in early history and behaviour (Noakes, 1989; Boake, 1994). When behavioural differences are observed between two populations, the assumption is often made that those differences stem from inherited i.e. genetic differences rather than maternal effects (Huntingford, 2004). However, our study is one of few showing the importance of egg size on behaviour of juvenile salmonids. These behavioural differences may have their roots in differences in egg chemical composition provided by the mother. Differences in egg size may reflect differences in egg content with potential consequences for later development of embryos. Preliminary results on total energy content of individual eggs of Arctic charr indicate that larger eggs have more energy content than smaller eggs (C. Leblanc, unpublished data). In charr, non- genetic maternal effects i.e. all materials transferred from mother to egg beyond genes, may play an important role in early stages of fish development including the development of behaviour. Behavioural differences may also be the result of interaction between genetic and maternal effects but our experiments were not designed to measure such effects.

Our study showed that early behaviour of fish can be influenced by egg size with direct consequences for growth. Such results may be important in terms of evolution of fishes and dynamics of populations (Green, 2008). In fact egg size maybe a tool used by the mother to adapt to fluctuating environments to increase her fitness. Our results and those of Benhaïm *et al.* (2003) indicate that each egg size may correspond to a different behavioural tactic, especially in terms of mobility and foraging behaviour. Different phenotypes may arise from different egg size as seen in spadefoot tadpoles *Spea multiplicata*. Martin and Pfennig (2010) showed that larger females invested in larger eggs, which in turn produce larger tadpoles better able to capture shrimp that induce carnivore morphology. Egg size may indeed be a source of novel resource-use phenotype. More work is needed regarding the considerable scope for egg size and egg quality for fish behaviour and morphology. Experimental designs including several females will help to better understand the importance of egg size and maternal investment on behaviour of fishes and its potential role in evolution of fish phenotypes.

From our study it is possible to conclude that social environment plays an important role in mobility and foraging of first feeding fish, where fish in groups were more active than fish maintained in isolation. These results are consistent with previous studies examining isolated fish (Koebele, 1985; Jobling and Baardvik, 1994; Martins *et al.*, 2005a; b) where isolation generally induces fewer foraging attempts, longer food biting latency (Gómez-Laplaza and Morgan, 1991), decreases in mobility (Gómez-Laplaza and Morgan, 2003) and less flexibility in behaviour (Salvanes *et al.*, 2007) by reducing competition pressure, predation risk and the absence of social facilitation. The greater feeding latency that we observed in isolated fish is most likely due to the absence of social interactions (see also Gómez-Laplaza and Morgan, 1991) and the lack of visual contact with conspecific providing increased feed intake and growth rate in a group of fish (Sundstrom and Johnsson, 2001; Martins *et al.*, 2006). Additionally, it has been hypothesized that aggressive interactions are higher in heterogeneous size groups especially in salmonids (Abbott *et al.*, 1985). Unlike other studies, we observed almost no differences in aggressive interaction

between mixed and homogenous groups. These results are similar to low levels of aggression previously found in similar-sized Arctic charr (Benhaïm *et al.*, 2003).

We have shown that differences in behaviour between small and large Arctic charr juveniles were triggered by egg size, social environment, time and the interaction of those factors. Additionally our results show that egg size effects were not cancelled out by the effect of social environment but rather interact with the social environment to affect early behaviour. This is surprising for salmonid juveniles where the importance of social interactions has been widely reported in both laboratory and field studies (e.g. Glova, 1986). Heath *et al.* (1999) reported that the effect of maternal size on offspring size disappeared shortly after emergence in Chinook salmon *Oncorhynchus tshawytscha,* with offspring tending to resemble their fathers more than their mothers. We showed that egg size affects behaviour early in development and may still affect mobility and foraging of fish later in life.

In a polymorphic system like Arctic charr, differences in feeding tactics between small and large fish could be linked to evolutionary processes. Indeed variation in behaviour, stemming from small size differences at first feeding, may influence habitat and food selection that can lead to divergence of fish populations, especially if there are clear interaction between maternal and genetic effects (Leblanc *et al.* unpublished observations). Sturlaugsdóttir (2008) showed important genetically fixed differences in mobility among wild Icelandic Arctic charr morphs (pelagic/benthic). Evidence for genetic differences in the behaviour of offspring of a "profundal" and a "littoral" morph have previously been suggested in a Norwegian population of Arctic charr (Klemetsen *et al.* 2002). Those differences may be related to habitat and diet specialization of the morphs. Considering the importance of egg size may greatly improve our understanding in many areas of evolutionary biology (Räsänen and Kruuk, 2007) especially our understanding of maintenance of diversity within a species. Such a maternal effect may for example be an important contribution to the large intraspecific diversity seen in Icelandic populations of Arctic charr (Skúlason *et al.*, 1999). The importance of egg size and more generally the importance of maternal effects for resource polymorphism and evolution of diversity of fishes is a new field that needs to be further studied.

Table 3.1: Experimental design table. *This table shows for each environment the group composition, the number of replicates, and the nature of the fish used in the three experiments. Experiment one compared small and large fish isolated at eyed stage. Experiment 2 compared small and large fish maintained in groups of 6 fish vs small and large fish placed in short isolation. Experiment 3 compared 3 groups of small or large fish vs 3 groups of mixed small and large fish. Observations were made at 5 ages: 159, 173, 180, 187 and 194 days post fertilization. The column replicates refers to the number of replicates for each treatment at each age.*

	Ages	Group composition	Replicates	Nature of fish	Environment	Statistics
Experiment 1	5	1 small fish vs 1 large fish	6	same individuals followed over time	long term isolation	repeated measures ANOVA
Experiment 2	5	group of 6 small or 6 large fish	3	different individuals/groups at each age	homogenous group vs short term isolation	ANOVA
		vs 1 small or 1 large fish	6			
Experiment 3	5	group of 6 small or 6 large fish	3	different individuals/groups at each age	homogenous vs heterogeneous group	ANOVA
		vs group of 3 small and 3 large fish	3			

Table 3.2: Summary of repeated measures ANOVA results for fish placed in long- term isolation. *We compared the effect of egg size on each behavioural variable for 6 small fish and 6 large fish placed in isolation since eyed-stage. Fish were observed at 5 different times.*

		Egg size df=1	
		F	P
Foraging			
	Reaction time to food	3.1	0.098
	Foraging in water column and surface	2.3	0.160
	Bottom foraging	2.5	0.140
	Total foraging	4.7	0.036
Mobility			
	Immobility	3.6	0.074
	Stationary	0.6	0.680
	Vertical stationary	0.7	0.630
	Slow and regular swimming	0.4	0.802
	Jerky swimming	1.2	0.394
	Rapid swimming	1.5	0.311
	Number of items	8.6	0.010
Space use			
	Total number of crossed areas	2.0	0.212
	Visited zones	4.6	0.046
	Visited level	3.3	0.095

Table 3.3: Summary of ANOVA results for fish in groups vs fish in short isolation. *F value, degrees of freedom and the probability p are displayed for each dependent variable. The factor "Size" refers to the effect of egg size (large versus small). "Social" effects refer to the two different social treatments tested: group of 6 fish versus short isolation. Short isolation refers to fish that were maintained in-group and were isolated 24h before observation. The symbol "*" is used to characterize the interaction between factors. The factor time refers to the 5 different ages at which fish were observed.*

	Size df=1		Social effect df=1		Time df=4		Size*Social df=1		Size*Time df=4		Social*Time df=4		Size*Social*Time df=4	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Foraging														
Reaction time to food	1.4	0.250	90.9	0.000	3.4	0.010	0.9	0.340	1.2	0.310	0.4	0.830	1.2	0.300
Foraging in water column and surface	0.1	0.780	0.6	0.450	2.1	0.091	0.0	0.940	0.0	0.890	1.1	0.350	0.2	0.910
Bottom foraging	6.1	0.016	15.5	0.000	3.1	0.020	7.8	0.007	1.1	0.370	5.7	0.001	3.4	0.010
Total foraging	5.4	0.020	14.8	0.000	2.0	0.100	6.3	0.014	1.0	0.440	3.9	0.007	3.5	0.010
Mobility														
Immobility	0.6	0.430	10.2	0.002	1.9	0.130	0.0	0.950	0.6	0.650	0.4	0.840	3.0	0.025
Stationary	2.8	0.097	4.4	0.040	2.4	0.060	0.0	0.910	1.6	0.180	2.3	0.070	2.6	0.045
Vertical stationary	3.5	0.067	0.1	0.730	1.6	0.186	4.7	0.540	1.3	0.280	0.4	0.780	0.7	0.680
Slow and regular swimming	0.6	0.450	1.2	0.270	1.4	0.240	0.3	0.610	0.2	0.950	0.2	0.910	1.4	0.250
Jerky swimming	0.1	0.820	2.3	0.130	2.9	0.003	0.0	0.870	0.7	0.560	1.6	0.180	0.6	0.610
Rapid swimming	2.5	0.110	8.0	0.006	2.7	0.039	7.5	0.008	1.4	0.260	2.6	0.046	1.1	0.360
Number of items	2.1	0.150	14.7	0.000	5.4	0.001	0.0	0.930	0.8	0.510	1.1	0.340	3.8	0.018
Space use														
Total number of crossed areas	1.1	0.290	7.5	0.008	7.2	0.001	0.0	0.860	0.8	0.560	0.6	0.650	2.5	0.053
Visited zones	0.2	0.660	10.6	0.002	3.3	0.015	0.0	0.890	1.2	0.300	1.5	0.230	2.1	0.090
Visited level	2.5	0.120	2.7	0.100	3.2	0.018	0.8	0.360	1.1	0.370	0.6	0.670	1.1	0.390
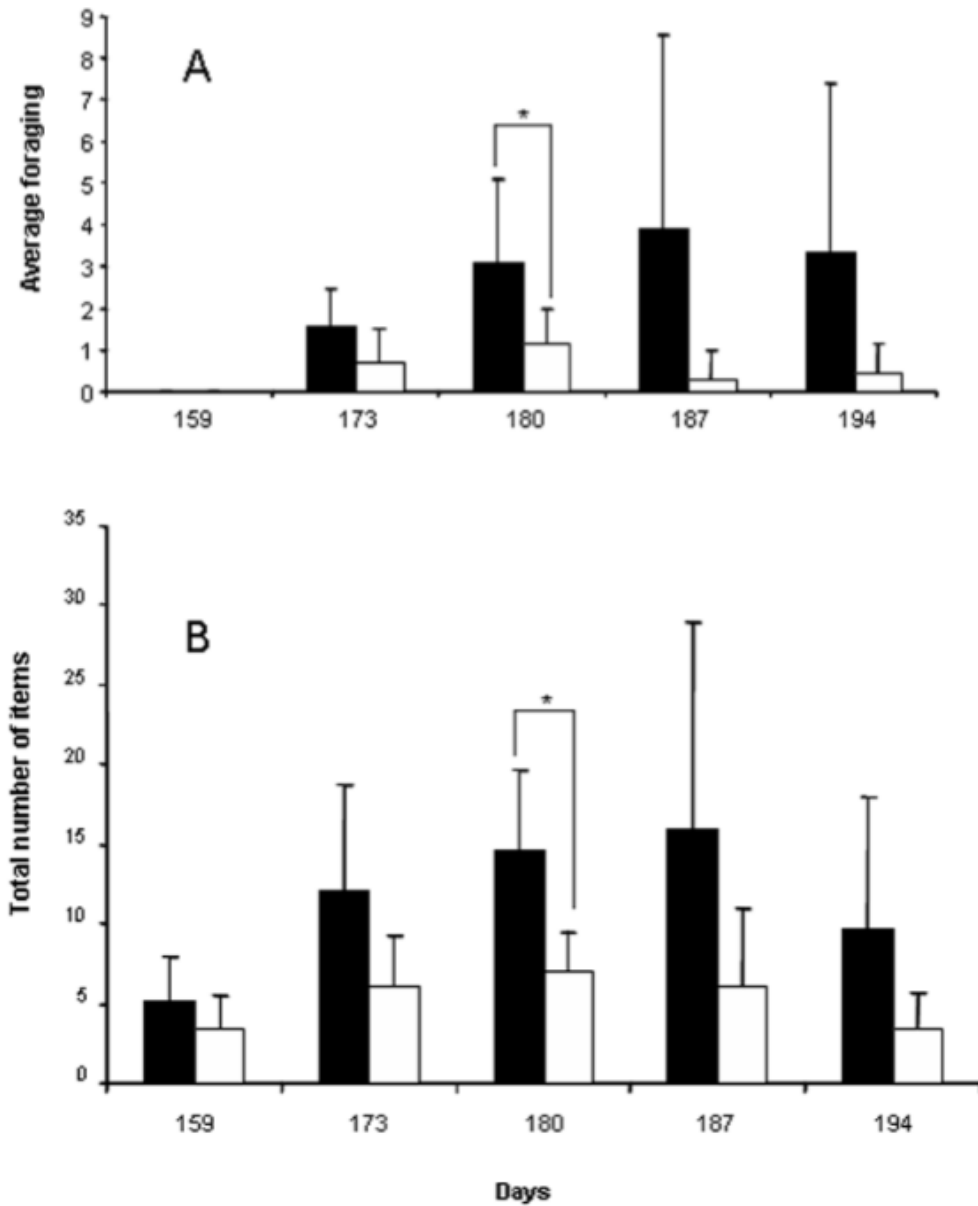
Figure 3.1: Foraging and mobility of large and small juveniles of Arctic charr isolated since hatching. *Foraging (A) and total number of behavioural items performed (B) during the two minutes of observation after food presentation are shown. Means + S.D. values are given. Large fish are in black and small fish in white. Differences between small and large fish (Newman-Keuls post-hoc tests) are shown: * p <0.05.*
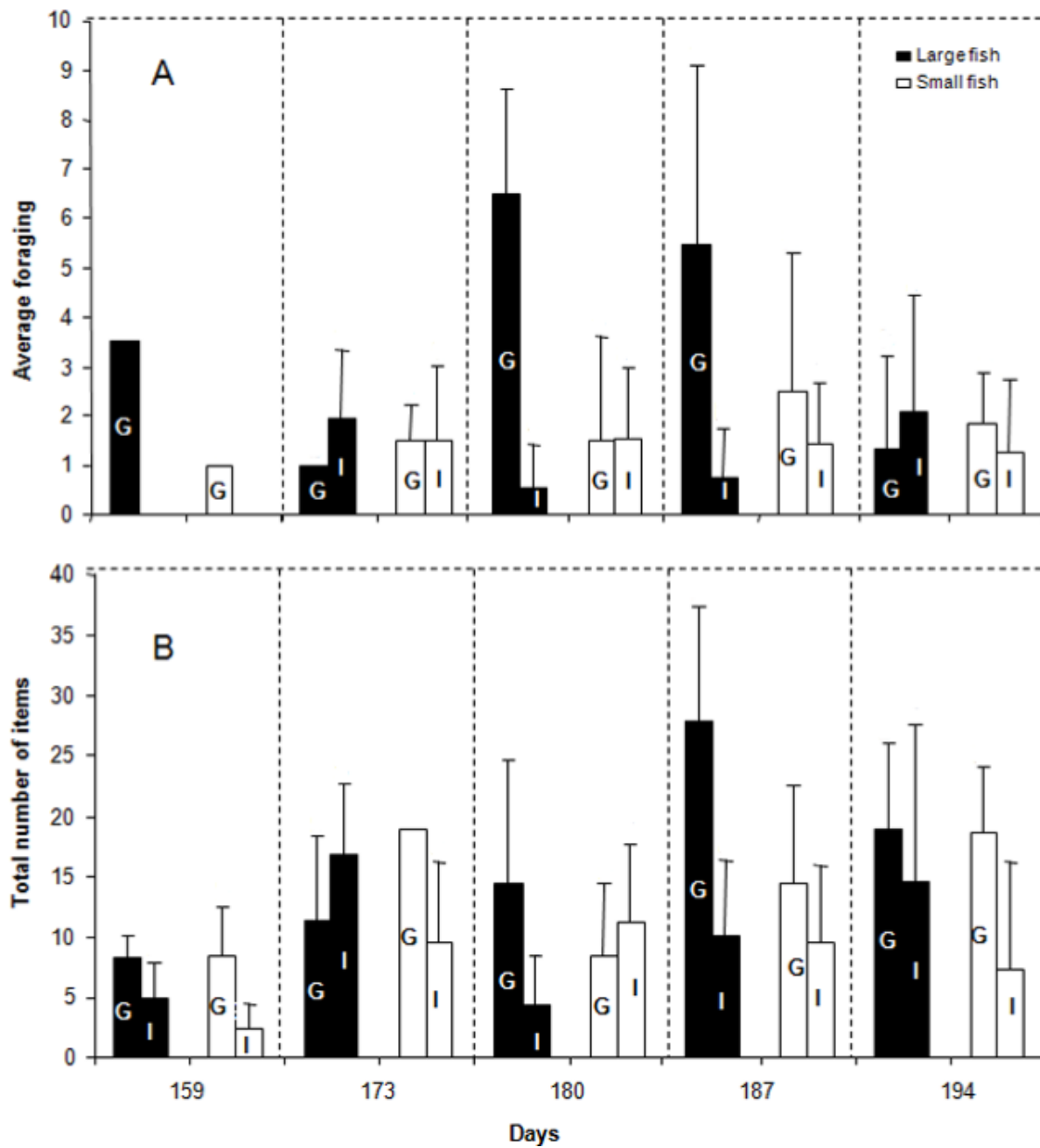
Figure 3.2: Foraging and mobility of large and small juveniles of Arctic charr in different social environments. *Foraging (A) and total number of behavioural items performed (B) during the two minutes of observation after food presentation are shown. Means + S.D. values are given. G: fish observed in group condition; I: fish shortly isolated. Large fish are in black and small fish in white.*

4 Potential role of egg size for divergence in fishes

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4.1 Introduction

The modern synthesis in evolutionary biology has mostly focused on genetic differentiation to explain observed phenotypic differences among individuals and populations. Pigliucci (2007) suggests that important additions will have to be made to the modern synthesis for a better understanding of evolutionary processes. One of the additions is to focus more on the importance of phenotypic plasticity as a source of variation. The ability of animals to be plastic for a given trait is now known to be inheritable, and phenotypic plasticity is known to be an evolvable trait (Pigliucci, 2005; 2007). Furthermore, it is acknowledged that the non-genetic influence of parents on the phenotype of their offspring represents a form of plasticity (Mousseau and Fox, 1998; Uller, 2008).

Maternal effects have for long time been recognized as a cause of phenotypic variation (Mousseau and Fox, 1998). They are often adaptive and they may play a significant role in adaptive evolution of organisms e.g. in rapid population differentiation and speciation (Roff, 1992; Rossiter, 1996; Mousseau and Fox, 1998; Reinhold, 2002). Maternal effects can be caused either by the genes inherited from the mother i.e. genetic maternal effects (Räsänen and Kruuk, 2007), or they can be caused by various factors such as energy, hormones, mRNA, mitochondria, that the mother provides to the offspring i.e. non-genetic maternal effects (Cohen, 1979; Arnold, 1994; Bernardo, 1996a; Marshall and Uller, 2007). Non-genetic maternal effects can be mediated by the mother through provisioning of the eggs and/or through any form of maternal care provided to the offspring (see examples for different taxa and at different life stages in Mousseau and Fox, 1998). They were originally considered as a bias or noise in quantitative genetic studies and then poorly understood. But recently they have been the focus of an increasing number of studies (e.g. McAdam *et al.*, 2002; Pakkasmaa and Jones, 2002). Non-genetic maternal effects, also called inherited environmental effects (Rossiter, 1996) or epigenetic inheritance (Richards *et al.*, 2010), frequently vary as a result of the environment experienced by the mother and, as

it is the case with genetic effects, their expression may also be heavily dependent on the environment experienced by the offspring (Einum and Fleming, 1999; Räsänen *et al.*, 2005). Therefore, the expression of maternal effects must be seen as a dynamic process (Mousseau and Fox, 1998) and their adaptive significance is being increasingly recognized in a wide range of taxa (reviewed by Räsänen and Kruuk, 2007).

The growing literature on maternal effects in fishes (Reznick *et al.*, 1996; Einum and Fleming, 1999; Heath *et al.*, 1999; Einum and Fleming, 2000a; Heath *et al.*, 2003; Gagliano and McCormick, 2007) indicates that maternal effects are more common than first believed. In species with no parental care egg size, i.e. the amount of yolk, can be directly linked to maternal effects and may affect important traits in the offspring (chapter 1). In addition to fishes, this has been the focus of a number of studies in birds, especially the maternal transfer of antibodies reviewed by Boulinier and Staszewski (2008). In fishes, egg size is an important fitness determinant especially for early life history. For instance, egg size has in size at hatching (Heath *et al.*, 1999), size at emergence and sometimes survival (Einum and Fleming, 2000a). There is an increasing literature on how egg size may influence individual early life history traits. However, some of these studies consider egg size as a continuous variable (across females) and do not account for potential confounding genetic effects. It appears crucial to disentangle the effect of genetic and non-genetic variation on early life history traits to understand how egg size relates to evolutionary dynamics (Sinervo and Doughty, 1996; Einum and Fleming, 1999). Although maternal effects are thought to play a role in evolution, the importance of maternal effect or egg size for evolution and sympatric divergence of fishes has been little studied.

Northern freshwater fishes offer a good system to study the potential importance of maternal effects in facilitating divergence processes. These fish often show extensive phenotypic variability among populations and sympatric morphs or species are common (Wimberger, 1994; Skúlason and Smith, 1995; Smith and Skúlason, 1996). Such morphs are commonly adapted to harvest specific resources in their environment, termed resource polymorphism (Skúlason and Smith, 1995; Smith and Skúlason, 1996). Differences between sympatric morphs can be seen in morphology, behaviour, physiology and life history characters. Inter - and intra-specific competitions as well as predation are thought to be important factors in driving sympatric resource polymorphism (Snorrason and Skúlason, 2004; Svanbäck *et al.*, 2008), although other ecological factors may also influence this process. It is believed that phenotypic plasticity may be an important factor in the early divergence of sympatric morphs. In particular, plasticity in behaviour and to some extent plasticity in morphology are thought to be crucial to allow fish to colonize and adapt to a new environment or to a new resource. Behavioural plasticity may facilitate colonization of a new habitat. In fact plasticity might even "jump start" the morph separation by facilitating early divergence in a stable environment (Skúlason *et al.*, 1999; Snorrason and Skúlason, 2004).

The importance of egg size variation for the evolution of sympatric morphs has not been studied. A good candidate for such studies is Arctic charr (*Salvelinus alpinus*). The high degree of polymorphism and the considerable, but variable, egg size makes Arctic charr a good model for studying maternal effects and their importance for behavioural and morphological differences. The polymorphism seen in Arctic charr is clearly reflected in resource - related characters such as body size, diet, and trophic morphology (Skúlason *et*

al., 1999; Klemetsen *et al.*, 2003; Snorrason and Skúlason, 2004; Klemetsen, 2010). Different morphs show clear segregation in behaviour especially in foraging behaviour (Skúlason *et al.*, 1993; Snorrason and Skúlason, 2004; Klemetsen, 2010). Behavioural differences between morphs can be related to morphological differences and resource use (e.g. Hindar and Jonsson, 1982; Adams *et al.*, 2003). Such differences are in part genetic (Sturlaugsdóttir 2008) and can commonly be seen at the onset of first feeding (Skúlason *et al.*, 1993; Parsons *et al.*, 2010). In Sturlaugsdóttir's (2008) study behavioural differences were partly genetic, as the fish had been reared in a common garden environment, and so behaviour could be related to the habitat and diet specialization of the parent morphs. She also reported important behavioural differences between large and small fish within some of the morphs. When looking at morph pairs among lakes it is clear, however, that the morphological differences among them are variable and those differences are in some cases reflected in genetic differentiation and reproductive isolation (Gíslason *et al.*, 1999; Kapralova *et al.*, 2011).

It has been suggested that behavioural differences may appear before morphological differentiation in the divergence of sympatric morphs (Futuyma and Moreno, 1988). For example differences in mobility and feeding behaviour at the onset of feeding in juveniles may play an important role in this respect through differentially shaping their life histories. More specifically we put forward the hypothesis that egg size, a non-genetic maternal effect, may play an important role in creating behavioural and morphological variation at an early age, which in turn could be important for the first steps of morph formation. To test this hypothesis we examined the variance in behaviour and morphology among Arctic charr juveniles coming from small and large eggs. We predicted that juveniles coming from large eggs will be more mobile and forage more towards the surface than those coming from small eggs. This prediction stems from the fact that we already know that at first feeding large and small siblings, coming from large and small eggs, differ in mobility and foraging behaviour (chapter 2; Benhaïm *et al.*, 2003). Although such observations were made on the progeny of one female we expect to see similar results for large and small siblings within and across females. We would expect that these differences will persist up to few months after first feeding and will further be reflected in morphology since behaviour may precede morphological changes in polymorphic species (Futuyma and Moreno, 1988). Differences in behaviour and morphology between large and small eggs among and within families will be a clear indication of interaction between egg size and the genetic of the mother. We may expect that egg size effect or reaction norm differs from one female to another indicating that non-genetic maternal effect, such as egg size, may be a trait on which selection can act upon.

4.2 Methods

Eggs were obtained from the breeding program of Hólar University College. Eggs came from nine females (age 4+) all fertilized with the sperm from one single male (age 4+). The females belonged to three different families with three sisters from each family. An additional group was created and composed of pooled eggs from virgin females (3+) that were fertilized with the sperm of the same male.

The fertilized embryos were placed in net cages (105 x 105 x 55 mm) made of 2 mm mesh screen. The individual chambers were placed in an EWOS incubator with a constant flow of water (Sauðarkrókur tap water, originating in bore holes (temperature 4.1°C +/- 0.57(SD)), and held in total darkness. The embryos were observed daily and regularly treated with malachite green (1:500.000) to prevent fungal infection. When the embryos had reached the eyed stage, dead embryos were manually removed daily. As the embryos were placed in the net cages, large and small embryos from each female were visually sorted (intermediate-sized eggs were not used). Thus a total of 20 experimental groups (9 females + 1 virgin females pool) x 2 size classes) were formed with 50 eggs per group. A sample of 25 eggs was taken from each crossing. Eggs were individually weighed (to the nearest 0.01 mg), then placed in a petri – dish and photographed to assess egg size. The egg size difference between large and small eggs was estimated by measuring egg diameter (SigmaScan Pro 5) according to Eiriksson (1999). Within a female, large eggs were on average 40% heavier than small eggs (mean ± s.d.; small = 30.65 ± 4.1 mg, large = 42.58 ± 4.2 mg; $t = 20.29$, $p < 0.000$, paired samples t-test). The embryos were allowed to hatch in the incubation net cage (average hatching time = 105 days post fertilization). Shortly before first feeding (155 dpf), free-swimming embryos were transferred to rearing tanks (30 L) with continuous water flow ($8.7 \pm 0.8°C$). Juveniles were fed twice a day by hand until satiation for the first 2 months after which automatic feeders were used.

Behavioural observations took place 300 days after fertilization. A video camera (Sony Handycam DCR HC 32E) was placed 50 cm above each tank to allow for the observation of the whole arena. Observation started approximately 20 minutes after setting up the recording system. All observations were performed between 08:00 and 13:00 hours in a randomized order. The fish were deprived of food for 24 hours to provide similar level of appetite prior to observation. The behaviour of all fish in a tank was recorded 1 minute before and 1 minute after food delivery. From the video and the audio recordings, bottom foraging and surface foraging attempts were counted for each individual in each tank. The observer was both recording the video and commenting on foraging occurrences documenting the locations of foraging attempts (bottom or surface foraging). The reaction time to food delivery (i.e. the latency to first foraging attempt in a tank; Benhaim et al. 2003) was estimated from the video recordings. Behavioural data were analyzed using SPSS 14.0 for Windows, Student Version (2006, SPSS Inc., USA). A chi - square test was used to estimate differences in the total number of foraging attempts at the surface and at the bottom, comparing family and body size (categorical variable: large or small). Reaction time data were checked for normality and analyzed using an analysis of variance to determine whether reaction time differed between families, females and large *versus* small and to test for the interactions of this three factors.

One day after behavioural observations, all fish were killed by an overdose of phenoxyethanol. A high-resolution digital photograph (Nikon Coolpix 4500) was taken of the left side of each fish (Figure 4.1). We used relative warp analysis in tps-relw to analyze for differences in morphology, while controlling for geometric body size. This analysis scales the landmarks from each fish (Figure 4.1) to a centroid configuration (mean shape), position and rotation. The program then defines principal warps from the centroid configuration, which are axes along which shape variation away from the centroid configuration can occur. Partial warps and two uniform components are then calculated (weight matrix) to contain a score for each fish that describes the realized amount of

bending and stretching necessary for the configuration of an individual to fit the centroid configuration. The partial warps and uniform components describing how individuals differ from the mean along a certain axis of shape variation were used in further analyses. To visualize the observed differences we used tps-splin to create thin plate spline images. The tps program package was developed by F. James Rohlf and can be obtained as a freeware at the homepage http://life.bio.sunysb.edu/morph.

To assess differences between families, between "large"/"small" fish and the interaction of families and size we used a MANCOVA on partial warp scores. The centroid size, which is the square root of the summed squared distances of all landmarks from their centroid, was used as a measure of fish size and was accounted for by using centroid size as a covariant. Thus the MANCOVA model was Body shape = Constant + Centroid size + family + egg size + family x egg size. To estimate the magnitude of differences between egg size groups within families the MANCOVA was conducted between "large" and "small" within each family. F-values were proportional to the differences between large and small within each family. We used a discriminant function analysis (DFA) of shape data to look for the correct assign of individuals to their family and size class based on their morphology. DFA helps to visualize differences in morphology across and within families for each egg size. The percentage of correctly classified individuals gave a measure of the morphological differences between groups. The weight matrix was used as the dependent variable and family, egg size and family x egg size as independent variables (categorical variables). First, we did this looking only at family, next looking only at egg size and thirdly by looking at both family and egg size.

4.3 Results

Overall fish derived from large eggs were 6% heavier (mean ± SD: 2.95 ± 0.64 g) and 3% longer (6.91 ± 0.40 cm) than fish derived small eggs (2.8 ± 0.79 g and 6.73 ± 0.56 cm; ANOVAs respectively df =1 $F_{1, 368}$=5.76 p=0.017 and df =1 $F_{1, 365}$=15.16 p < 0.001) after 300 dpf. Within each female, fish coming from large eggs were larger and heavier than fish coming from smaller eggs (nested ANOVAs: df =1, $F_{1, 367}$ = 3.33 p= 0.069 and df =1, $F_{1, 364}$ = 6.39 p = 0.012).

Fish from larger eggs foraged more near the surface than fish from smaller eggs (df = 20, χ^2 = 31.06, p = 0.044; Figure 4.2). There were no differences among families in this respect (df =20, χ^2 = 79.29, p > 0.05; Figure 2. Fish from smaller and larger eggs did not differ in their foraging near the bottom (df = 20, χ^2 = 18.22, P =0.573 Figure 4.2). Reaction time to food delivery did not differ between large and small fish (df =1 $F_{1, 23}$=3.47 p = 0.544), between females (df =9 $F_{9, 23}$=13.30 p = 0.324) nor when egg size was nested within female (df =8 $F_{8, 23}$=0.94 p = 0.566).

We tested for differences in morphology using MANCOVA with centroid size (a measure of relative size) as covariant. The effect of centroid size was significant ($F_{48, 291}$ = 15.9, p < 0.001). There were differences in morphology between families ($F_{144, 879}$ = 5.4, p < 0.001), between egg size groups ($F_{48, 291}$ = 3.8, p < 0.001) and the interaction of those was significant ($F_{144, 879}$ = 3.2, p < 0.001; Figure 4.3). There were clear differences in body shape

between juveniles coming from larger and smaller eggs: fish coming from smaller eggs were thinner, had larger anal fins, larger heads and their eyes were lower on the head than fish coming from larger eggs.

The morphological differences were further explored using a DFA. We could correctly classify 79% of the fish to family (Wilks λ = 0.16, $\chi^2_{(138)}$ = 594.3, p < 0.01, table 1), 76% to larger eggs and 84% to smaller eggs (Wilks λ = 0.62, $\chi^2_{(46)}$ = 154.5, p < 0.01), and 75% to both family and egg size class (Wilks λ = 0.03, $\chi^2_{(322)}$ = 1121.9, p < 0.01 table 1). The discriminant analyses clearly show differences across families in the degree of morphological differences between egg size groups within families (Figure 4.4). Fish from larger eggs were deeper bodied in all families except among the virgin females. There were also commonly differences in the caudal region and in the head shape of the fish, usually with larger fish having smaller heads (Figure 4.4). F-values from the MANCOVA are indicative of the magnitude of the morphological differences between large and small fish within each female. They range from 2.3 for family B to 5.5 in family C (Figure 4.4) indicating that more differences between large and small fish were observed in family C than in family B.

4.4 Discussion

Our study has two main findings. First, siblings of Arctic charr juveniles derived from eggs of different size differ in morphology and behaviour at 300 dpf. Second, the relationship between egg size and morphology differs among families. There are clear differences in the effects of egg size among families, indicating strong maternal x genetic interactions. In other words, the body shape changes between small and large siblings were not identical among the families. This is the first time to our knowledge that geometric morphometrics have been used to assess the effect of egg size (beyond genetic inheritance) in fishes.

Behaviour of offspring may be influenced by parents and in turn the offspring will themselves influence the expression of phenotypes in subsequent generations. However, behaviour is a high flexible trait that often reflects the adaptation of a phenotype to his environment, but it is also constrained by body size (Travis, 1994). In Arctic charr where juvenile size is highly influenced by egg size it is likely that behavioural consequences may derive from size variation originating from maternal effects. Heath (1999) demonstrated that the female size - egg size - offspring body size correlations are a true maternal effect that rapidly dropped to zero soon after first feeding in Chinook salmon *Oncorhynchus tshawytscha*. For this reason most of the studies investigating egg size effect on early life history traits of fishes have been terminated shortly before or after first feeding. In this study we observed a subtle but significant relationship between egg size, progeny size and foraging behaviour at 300 dpf. In Arctic charr effects of egg size on growth, foraging behaviour and morphology persist longer than in other salmonids and could in turn have significant fitness consequences.

Our findings show that independently of their genetic origin large and small juveniles differ in their body shape. These differences could most clearly be seen in the head, and body shape i.e. larger fish were overall slimmer than smaller fish. These results are very interesting when taking into account the ecological context of Arctic charr as a species. Salmonids are known to emerge more or less at the same time in the close proximity of the redd (Quinn, 2005). Differences in behaviour and mobility at first feeding may reflect

different resource use early in life (Benhaïm *et al.*, 2003) that can later translate into different body shapes. Egg size may thus be a mechanism influencing phenotypic plasticity of Arctic charr triggering or facilitating early divergence in resource use.

Furthermore, we have shown that relative egg size (within a female) influences behaviour and morphology of juveniles (Figures 4.3 and 4.4). The magnitude of the effect is influenced by female parentage, which suggests that the differences may be due to the interplay between maternal factors, egg size and genetic factors. Assuming that the phenotypic differences observed can have different fitness consequences, selection could act on traits connected to this interplay.

Phenotypic plasticity has been suggested as an important factor for the evolution of biological diversity (e.g. Pfennig *et al.*, 2010; Pigliucci and Müller, 2010). We identified egg size as a potential source of phenotypic plasticity in several months old Arctic charr. At the same time our results suggest that initial individual differences in behaviour and morphology could have a more complex and important role in facilitating divergence and resource polymorphism. The importance of egg size as a mechanism of plasticity in natural condition will need to be specifically tested. The next step would be to explore variation in maternal effects among Arctic charr morphs to highlight the ongoing processes of natural selection and better understand the origin of divergence in polymorphic species. Different maternal effects between two morphs may confirm an important role such effects have in evolution of polymorphic species.

Acknowledgements

Table 4.1: Differences in morphology between offspring coming from larger and smaller eggs, within four families of Arctic charr Salvelinus alpinus. The table shows F values from MANCOVA with centroid size as covariant. The proportion of fish from different families correctly classified, using a discriminant function analysis on families, and the proportion of fish coming from larger and smaller eggs within families correctly classified.

Family	F	Correct class (%)	Large (%)	Small (%)
A	4.4	82	79	95
B	2.3	76	73	55
C	5.5	74	62	84
Virgin	2.4	90	85	96

Figure 4.1: Landmarks used to capture the morphology of Arctic charr juveniles. *A total of 26 landmarks were digitized and 5 of those were sliding landmarks.*
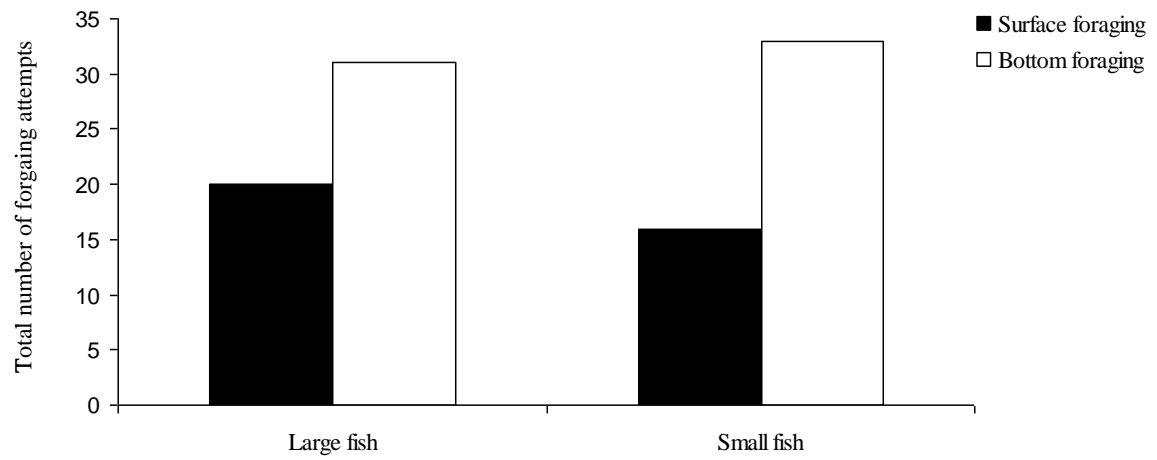
Figure 4.2: Location of foraging attempts of Artic charr juveniles originating from smaller or larger eggs. Surface foraging is in dark and bottom foraging in white. The total number of foraging attempts corresponds to the total number of observed foraging for large and small fish during one-minute observation after food delivery.
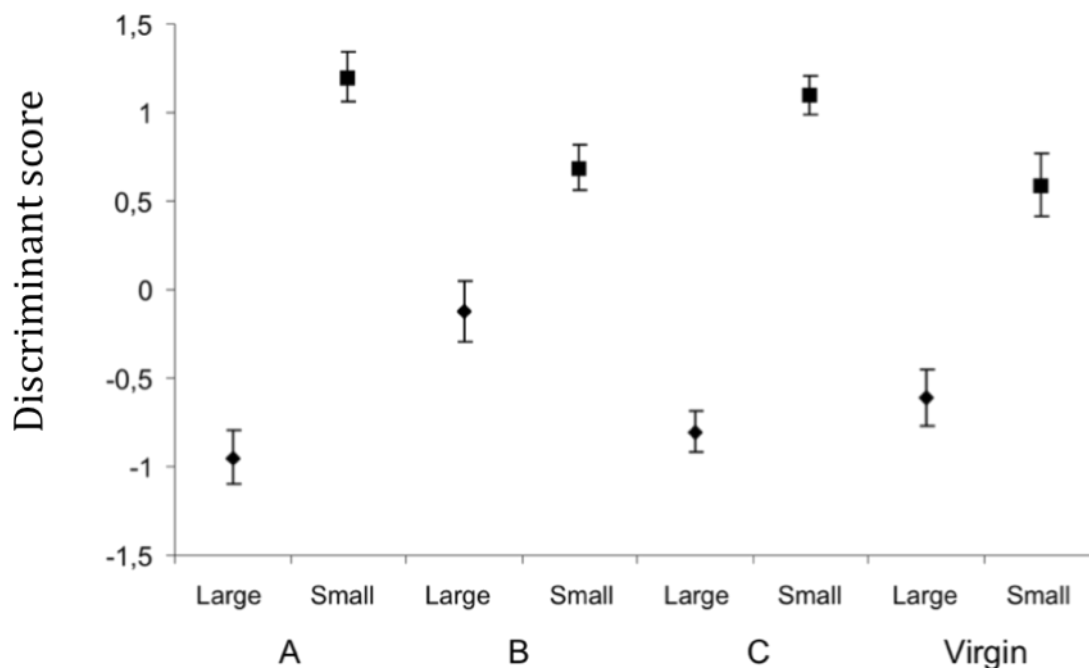
Figure 4.3: Results of a discriminant analysis for fish derived from larger and smaller eggs. *The figure shows the morphological distribution of Arctic charr juveniles derived from large and small eggs within four different families (families A, B, C and virgin). "Virgin" family refers to females that were spawn for the first time. The y-axis represents the discriminant score from the discriminant analysis separating fish coming from larger and smaller eggs.*
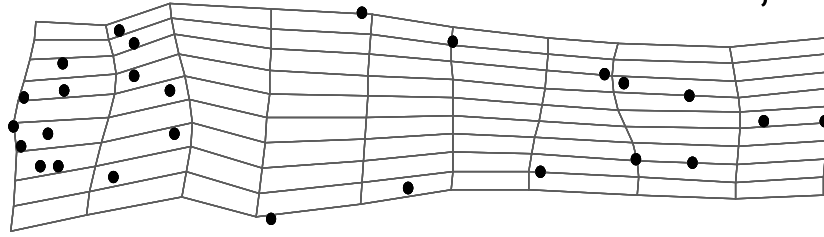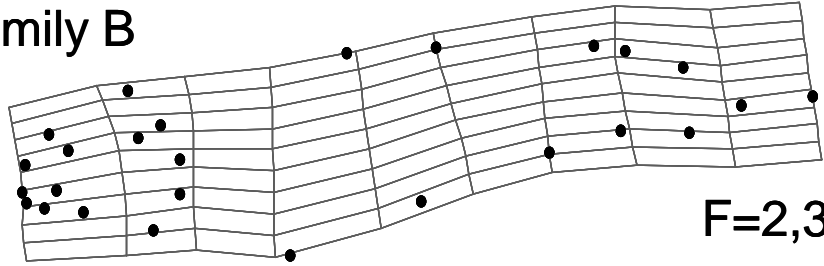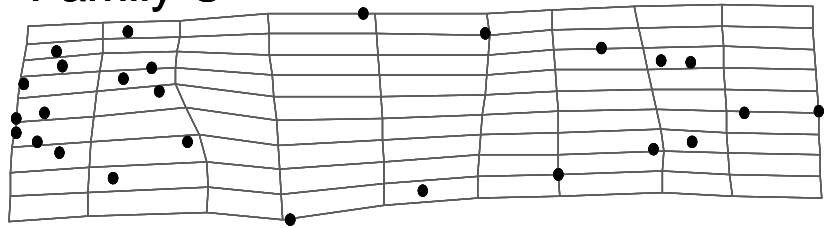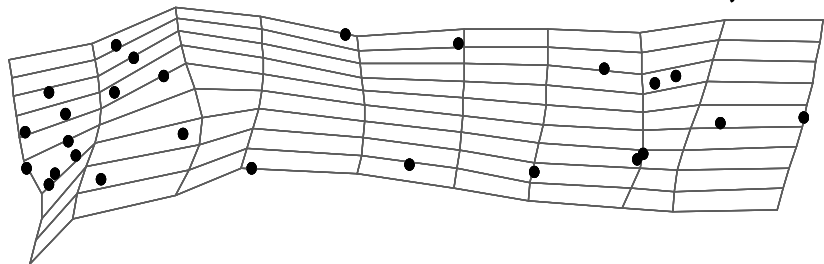
Figure 4.4: Body shape differences between families of Arctic charr. *The deformation grids show morphology of fish going from large to small fish in different families, with a 3x magnification. The F-values, obtained from the MANCOVA are indicative of the magnitude of body shape difference between fish coming from smaller versus larger eggs within each family.*

5 Influence of egg Size on offsprings of Hatchery and Wild Steelhead Trout, *Oncorhynchus mykiss*, W.

Camille A. Leblanc, Sigurður S. Snorrason, Carl Schreck and David L. G. Noakes

5.1 Introduction

For decades hatchery programs for enhancing threatened Pacific salmon and steelhead trout *Oncorhynchus* spp. populations have been developed all around the North Pacific Rim. Such implementation programs were first intended to produce fish for harvest and then used to restore declining natural populations (e.g. Augerot, 2005; Williams, 2006). However, such hatchery programs have become more contentious as wild stocks continue to decline (Augerot, 2005; Hill *et al.*, 2006). Evidence suggests that hatchery fish have lower fitness than wild fish when they breed in the wild (Berejikian and Ford, 2004; Araki *et al.*, 2007; 2008; Theriault *et al.*, 2010). Araki *et al.* (2007) showed that domestication reduced subsequent reproductive capabilities by ~ 40% per captive-reared generation when fish are released to natural environment. These results indicate that there are important genetic and fitness differences between hatchery and wild fish, and that such heritable differences can arise in only a few generations.

An important aspect of research on salmonids has been the comparison of performances of hatchery versus wild fish at different life stages. In addition to genetic differences, studies have revealed differences in life history traits of returning hatchery and wild fish (e.g. Knudsen *et al.*, 2006). Behaviour and habitat use have been the focus of numerous studies on juveniles of salmonids in streams and hatchery conditions (Weber and Fausch, 2003; Hill *et al.*, 2006). For instance, Negus *et al.* (1999) reported differences in fright response (wariness) between progeny of resident and migratory *O. mykiss*. Such differences may have important impact on survival of juveniles when expose to natural conditions. These studies were all aimed to highlight differences between hatchery and wild fish (sometimes coming from the same genetic background) in order to minimize the genetic and ecological impacts of hatchery fish have on wild populations. Additionally, hatchery fish

differ from wild fish in terms of physiology, morphology and behaviour when they are released as smolts (reviewed by Weber and Fausch, 2003; Hill *et al.*, 2006). Smolt development is controlled by abiotic and biotic factors such as photoperiod, temperature, and growth pattern (Thorpe *et al.*, 1998; McCormick *et al.*, 2000; Beckman *et al.*, 2003) which differ greatly between hatchery and natural environments, and in turn may explain the differences between hatchery and wild smolts. Hill *et al.* (2006) reported some differences in morphology, behaviour and physiology (Na+, K+ -ATPase activity) of first generation hatchery fish when compared to wild steelhead trout reared respectively under hatchery and natural conditions. Despite the vast literature on differences between hatchery and wild fish (e.g. Berejikian and Ford, 2004), little is known about the mechanisms that may trigger such differences and how early in life these differences arise.

Hatchery fish commonly have smaller eggs than wild fish when captive-reared for a few generations (chapter 1; Einum and Fleming, 2000b). These changes can occur rapidly as in Chinook salmon *Oncorhynchus tshawytscha*, where egg size of hatchery fish and wild fish placed in hatchery conditions decreased significanlty and rapidly (Heath *et al.*, 2003). Several explanations have been put forward to explain smaller egg size in hatchery fish. Egg size should be smaller when the environment experienced by juveniles is of high quality such as in a hatchery (Hutchings, 1991; Einum and Fleming, 1999), females experiencing high growth rate as juveniles typically produce a relatively high number of small eggs as adults (Jonsson *et al.*, 1996; Lobón-Cerviá *et al.*, 1997; Morita *et al.*, 1999; Fleming *et al.*, 2000; Olsen and Vøllestad, 2003; reviewed by Einum *et al.*, 2004). In addition the relationship between egg size and survival is weaker under hatchery conditions than in the wild (Heath *et al.*, 2003). In salmonids, egg size is known to influence early life history traits (e.g. Pakkasmaa and Jones, 2002; Einum *et al.*, 2004) and in some cases such effect may last until after emergence and first feeding (chapters 2, 3 and 4). Generally juveniles coming from larger eggs are larger than juveniles coming from small eggs (e.g. Hutchings, 1991; Einum and Fleming, 1999; Heath *et al.*, 1999). In *O. mykiss* larger eggs produce larger first feeding juveniles up to fours weeks after first feeding (Springate and Bromage, 1985). It has been suggested that egg size has no direct implications for overall egg quality and early offspring survival (but see also Kato and Kamler, 1983), as survival for the first three months after first feeding was not affected by egg size (Springate and Bromage, 1985). A more recent study showed that the relationship of egg size and absolute growth of *O. mykiss* juveniles persisted longer within family than across families, i.e. a positive relationship between egg size and juvenile weight was found for 9 weeks after hatching across families but up to 15 weeks within each family (Blanc, 2002). Despite the interest in aquaculture and in fisheries management of *O. mykiss*, no study has examined the potential relationships of egg size with embryo developmental features and first feeding juveniles phenotypic traits that may vary between hatchery and wild *O. mykiss*. Additionally, it is unclear 1) how egg size varies between females of either hatchery and wild origin 2) and how egg size may relate to early and later development of juveniles. Yet, no study has investigated the effect of egg size as a potential mechanism in divergence of wild and hatchery fish.

Steelhead trout is the anadromous form of resident rainbow trout *Oncorhynchus mykiss*. Some North American populations of *O. mykiss* have been listed as "threatened

populations" according to ESA (NOAA Fisheries, http://www.nwr.noaa.gov/ESA-Salmon-Listings). This species is the most "complex salmonids" species in terms of life- history with up to 32 different life-history types (Thorpe, 1998). It has been extensively studied for farming purposes, with studies focusing on fecundity, embryo survival (used to define egg quality) and growth (e.g. Kato and Kamler, 1983; Springate and Bromage, 1985; Bromage *et al.*, 1992; Kristjánsson and Vøllestad, 1996). Steelhead trout reared in the Pacific Northwest are generally released as smolts correspoding in most cases to one year growth under hatchery conditions before release in natural environment (Kostow, 2009). Despite genetic similarity, hatchery and wild smolts differ in size, body shape and physiology (McCormick and Bjornsson, 1994; McCormick *et al.*, 2003; Hill *et al.*, 2006). Smolting development has been examined using morphology (e.g. silvering and condition factor) as well as size and physiology. An array of physiological and endocrine indicators of parr-smolt transformation has been extensively used to assess smolt quality or readiness, such as gill Na+, K+ -ATPase activity (e.g. McCormick, 1993) and thyroxine hormones (T3 and T4; Hoar, 1988). Additionally, first year growth pattern appears to be crucial in the expression of life history strategy e.g. migration to salt-water versus residualism (Sharpe *et al.*, 2007).

The main objective of this study was to test the hypothesis that egg size influences steelhead embryonic developmental features, growth and behaviour. Based on earlier findings (Arctic charr chapters 1 and 3; Atlantic salmon Einum, 2003; Moffett *et al.*, 2006), I expect that juveniles coming from larger eggs will be larger than juveniles coming from smaller eggs. Similar to first feeding Arctic charr, I expect large fish coming from large eggs to develop slower, to be more mobile and feed more than small fish resulting from small eggs. Especially I expect juveniles coming from larger eggs to feed more at the surface, with juveniles coming from smaller eggs feeding more of the bottom (see previous results in Arctic charr chapters 2 and 3). Additionally, I examine the long- term relationship of egg size with growth and smolting development (physiology and salt- water preference test) from fish of both hatchery and wild origin. In contrast to my predictions for early growth, I do not expect that egg size positively relates to growth of 1- year- old steelhead trout neither with physiological characteristics and salt- or fresh- water preference at smolting (e.g. Heath *et al.*, 1999).

In order to better understand the differences between hatchery and wild fish, these above predictions were tested on wild and hatchery steelhead trout (with the same genetic background). I expected that hatchery eggs would be smaller than eggs from wild parents (e.g. chapter 1, and previous information) and that progeny of hatchery fish would grow faster than progeny of wild fish like demonstrated in Atlantic salmon *Salmo salar* (Einum and Fleming, 1997; Fleming *et al.*, 2002) and in brook trout *Salvelinus fontinalis* (Vincent, 1960) under laboratory conditions. However I predict that hatchery steelhead trout will be larger than wild ones (Hill *et al.*, 2006) after one year raised in laboratory conditions, based on the fact that hatchery fish are usually selected to grow and mature faster (reviewed by Vincent, 1960; Fleming *et al.*, 2002; Weber and Fausch, 2003). Based on the study of Hill *et al.* (2006) I expect hatchery fish to show lower osmoregulatory status when compared to wild fish and hatchery fish to choose less consistently salt- water rather than fresh- water. If the origin of the fish and/or egg size affect early life events and physiological charasteristics and behaviour of yearling fish, it suggests that important changes in early life history of

salmonids may occur in only one generation. In other words, the effect of rearing fish in hatchery conditions for only one year (before being released) may have impact on egg size and on the growth and behaviour of the following generation.

5.2 Methods

5.2.1 Study animals

Returning steelhead trout of hatchery and wild origin from the Siletz River (Oregon, USA) were caught in spring 2009 and 2010 as part of the Oregon Department Fisheries and Wildlife (ODFW) monitoring and broodstock programs. Here, hatchery fish refers to first-generation (hereafter F- 1) hatchery fish i.e. eggs were collected from wild parents and their progeny were raised for one year until they were released as smolts in the river. For later identification, the adipose fin of hatchery fish were clipped off before they were released as smolts. Hatchery F-1 fish matured in the ocean similarly to wild fish and were caught when returning to the Siletz River. Wild fish were adult steelhead with adipose fins intact, captured at the same times and locations in the ODFW trap on the Siletz River. Their intact adipose fins identified them as wild fish, i.e., they had not spent any portion of their life in a hatchery.

In 2009, 5 females and 5 males with ripe gonads from each origin were selected and, in 2010 ten females and ten males were selected. In 2009, all females were fertilized with the milt from all males. However, only the progeny of one male per origin (crossed with the five females) was selected to assess the long- term influence of egg size on behaviour and growth. One male from each origin was selected to minimize the potential paternal effect on egg size and development. In 2010, eggs from one female were fertilized with the milt of one male. Body weight and length of parental fish were measured and 2 scales per adult fish were collected to estimate age (Bagliniere *et al.*, 1985). 60 ml of eggs from each female were fertilized mixing with a few millilitres of milt from each male. Viability of the eggs was checked a few days after fertilization and I retained families with more than 90% fertilization rate to conduct the experiments. In 2009, the progeny of two females was excluded because of poor survival. Viable eggs from other crosses were returned to the ODFW North Fork Alsea River Hatchery at the eyed embryo stage.

Fertilized eggs were incubated at the Oregon Hatchery Research Center in hatchery trays supplied with freshwater from Fall Creek (mean ± SD, 8.2 ± 0.5 °C) and kept in darkness up to emergence (first external feeding). One day after fertilization, a sample of 25 eggs was taken from each crossing, placed in a Petri dish and photographed to assess egg size. Measurements of egg diameter were performed according to the method of Eiríksson (1999) and the mean egg diameter for each family was estimated using the software SigmaScan Pro 5 (chapters 1 and 3). Mortality was assessed weekly and dead embryos removed. When embryos had pigmentation in the eyes, they were visually sorted for smaller and larger eggs within each family. Mean egg diameter for each size class was assessed as described above.

5.2.2 Egg size from hatchery versus wild steelhead

For each female, twenty- five eggs were measured to calculate mean egg diameter and its variance. I compared the mean egg diameter per female and its variance for both hatchery and wild steelhead trout. Both variables were normally distributed. Female fork length (FL) and female age were positively correlated as well as female FL and mean egg size per female (Pearson's correlations across both hatchery and wild females: $r = 0.89$ $n = 38$ $p < 0.001$ and $r = 0.66$ $n = 38$ $p < 0.001$; Figure 1). Thus, origin (hatchery vs. wild), year (2009 vs. 2010), the interaction origin x year, and female FL (covariate) were the factors in a 2- way ANCOVA used to test for differences in egg size.

5.2.3 Development and early growth

Assessing the relationship between egg size, developmental rate and early growth was achieved by following individual embryos from each crossing. When embryos had pigments in eyes, ten embryos from each cross were individually weighted (to the nearest 0.01g), measured (using digital photographs as described above) and then placed in individual rearing cell. Rearing cells were made of circular PVC pipes (10 cm diameter and 25 cm height) with a mesh bottom (mesh size 0.25 x 0.25 cm). Isolating the individual embryos in these cells allowed observations of development and growth of individual embryos in relation to egg size, female and origin (hatchery vs. wild fish). Ten cells were placed in a randomized order in 6 covered flow- through tanks (60 x 30 x 14 cm) with constant water flow (mean $\pm$ SD 11.4 $\pm$ 0.7°C).

Individually kept embryos were observed 1 month after fertilization for developmental features. The features examined were: the number of melanophores on head and trunk, the darkening (pigmentation) of the eye, the formation of the vitelline vein and the intensity of blood color (Table 1). For this inspection, each embryo was placed in a Petri dish covered with water and placed under a microscope. Hatching time was recorded and individual growth rate was estimated as the standard length increment per day between hatching and emergence. Embryos were photographed at hatching and at emergence. At emergence (first external feeding), fish were also weighted. Standard length (to the nearest 0.01mm) was measured from the digital pictures using the software SigmaScan Pro 5. After first feeding, individual fish were reassigned to their family and egg size groups.

Spearman's correlations were used to test whether egg size is related to early developmental features. Egg weight was used as an indicator of egg size. I removed the effects of female length on egg length by using the standardized residuals from a linear regression of egg weight on female FL. These residuals were used as a measurement of egg size. To test whether female and origin (hatchery vs. wild) affected early development I used mixed model of covariance (ANCOVA), with female nested in origin as a random factor, origin as a fixed factor and egg size as a covariate.

5.2.4 Early behaviour

First feeding juveniles from 3 hatchery and 3 wild families were observed to estimates differences in early behaviour related to origin and egg size. Within each family I observed

six juveniles coming from smaller eggs and six juveniles coming from larger eggs. Thus, a total of 12 experimental groups (3 hatchery + 3 wild families) x 2 egg size classes) were observed two days after first feeding.

In the morning of the observation 6 juveniles per experimental group were randomly selected and placed together in an aquarium (30 x 24.5 x 30.5 cm), isolated from adjacent aquariums with black plastic. 5 hours later three fish were randomly selected (as the first fish crossing a pre determined area of the aquarium) and observed for mobility, agonistic interactions and feeding behaviour. Behavioural sequences were video recorded with a camera (Canon Elura 100) placed 50 cm away from the aquarium side. One observation consisted of one minute before food delivery and 4 minutes post food delivery. Fish were hand fed with Silver Cup Diet (aquaculture food, size 0) on the surface. Fish were subsequently anesthetised (50 mg/L MS-222 buffered with 125 mg/L NaHCO3 to pH= 7.0) and measured for body weight and fork length (FL).

Mobility and foraging behaviour were recorded in a similar way as described by Benhaïm *et al.* (2003). Mobility included stationary display, regular swimming, and rapid swimming. Foraging behaviour included the count of foraging events at the surface, in the water column, at the bottom and the total number of foraging per fish, and the reaction time to food delivery (see chapters 2 and 3 for definitions). Agonistic behaviour represented the number of pursuit of one individual by another for at least one body length, i.e. chase sensu (Kim *et al.*, 2004). Behavioural data were collected from video-tapes using EthoLog 2.2 software (Ottoni, 2000), encoding behavioural duration for mobility and reaction time to food delivery, and behavioural occurrences for agonistic interactions and foraging behaviour.

Mobility and foraging behaviour of the hatchery and wild progeny coming from different egg size were compared using 2- way ANOVA. Mobility data met the assumptions of ANOVA but foraging behaviour and reaction time data were log-transformed (log x+1) to meet the assumption of homogeneity of variances. This transformation still violated the assumption of normality but none of the transformation satisfied both homogeneity of variances and normality. Origin (hatchery vs. wild) and size (smaller eggs vs. larger eggs) and their interaction origin x size were the factors in the ANOVA. After transformation agonistic behaviour data still violated both assumptions of normality and homogeneity of variances, thus independent Kruskal-Wallis tests were performed to assess the effect of origin and the effect of size on agonistic behaviour.

I also estimated the fright response and wariness of the same juveniles directly in their rearing tray (60 x 30 x 14 cm) (see also Negus, 1999). The number of juveniles in each tray was equal to 20. I had 5 replicates for each treatment; that is: 1) hatchery juveniles coming from smaller eggs; 2) wild juveniles coming from small eggs; 3) hatchery juveniles coming from large eggs; and 4) wild juveniles coming from large eggs. Juveniles were fed to excess four to five times a day. Differences in fright response exhibited by each egg size category and each origin were tested in 5 trials over the week after first feeding. The design of this experiment on fright response is adapted from Vincent (1960) and Negus (1999). A video camera was mounted 1.5 m above each tray, and fish were allowed to resume normal activity with a 15 minute- interval before video recording started. Video recording lasted for

four minutes starting at the fright event. To create a fright response, open hand of the observer was quickly pivoted toward the water surface in the middle of the tray. The hand was held just above the water surface for 3 s, and quickly withdrawn. When videos were analysed a 20-cm by 11-cm-rectangle outlines were drawn on each video. These outlines delimited the area where the fright event occurred. Fish located outside these outlines were counted from the video one second after the hand was removed i.e. I estimated the percentage of fish at the edge of the tray. I also recorded the elapsed time from disturbance after which the group of fish resumed a random distribution i.e. 35% of the fish were observed in the outlined rectangle corresponding to 35 % of the tray.

Percentage of fish at the edge of the tray and time to resume random distribution were compared using a 2- way ANOVA with repeated measures. Time to resume random distribution met the assumptions of ANOVA whereas percentage of fish at the edge of the tray violated the assumptions of normality and equal variances. Thus, the data were ranked within each observation time before applying the ANOVA with repeated measures. Data from repeated experiments were plotted versus time to visualize the data and determine if wariness declined with experience.

5.2.5 Body size, physiology and salt-water preference tests after one year of rearing

One week after first feeding, juveniles were moved to circular outside tanks (86 cm diameter x 60 cm). The treatments were: hatchery fish coming from small eggs (HS), hatchery fish coming from large eggs (HL), wild fish coming from small eggs (WS) and wild fish coming from large eggs (WL). Each treatment had 3 replicated tanks of 50-60 fish per tank. Fish were raised under natural photoperiod and tanks received water from Fall Creek (10.6 ± 1.3 °C). Fish were fed with hatchery rations of food Silver Cup Diet (SCD) of size #0, #1, #2, #3, #4 and Bio-Oregon 2.0 mm. In October 2009, fish were anesthetized (50 mg/L MS-222 buffered with 125 mg/L $NaHCO_3$) and individually PIT tagged (8.5 mm, BioMark) in the body cavity using a beveled edge syringe at an angle of 30 degrees. A total of 577 fish were PIT tagged (8mm PIT tags BIOmark; mean ± SD: fish body weight 11.46 ± 3.36 g and fork length (FL) 100.1 ± 10.7 mm) and carefully monitored for 24 hours after tagging procedure. Mortality of 1.7% was observed within the first 24 hours and 4.8% of the fish lost their tagg in the following days. Fish were individually measured for weight and FL (to the nearest 0.01g and 1 mm) every month from October 2009 to March 2010 (Figure 6).

During spring 2010, 60 fish from each treatment, i.e. 10 fish per replicated tanks, were euthanized by anaesthesia overdose (200 mg/L MS-222 buffered with 125 mg/L $NaHCO_3$ to pH= 7.0) at 15 days intervals starting on 15th of April until 7th of June. This period corresponds to the time when wild smolts from Fall Creek migrate downstream and go through smolting metamorphosis (Leblanc *et al.* unpublished data). Fish were classified as either unsilvered (clearly visible parr marks), partially silvered (few parr marks and some silvering), or fully silvered (no parr marks, silvering and dark caudal fin; see coloration index in Birt and Green, 1986). Gill tissue was collected. Two to four filaments from the first left arch were collected and placed in ice-cold SEI buffer (250 mM sucrose, 10 mM EDTA, 50 mM Imidazole, pH 7.3), frozen on dry ice and then stored at -80°C. Gill samples

were assayed for Na+, K+ -ATPase activity using standard methodology of McCormick (1993). Blood was collected from the caudal vein using heparinised syringes, and immediately centrifuged (10 min at 3000 G) to collect plasma. Plasma was frozen on dry ice, and stored at -80°C before being assayed for thyroxine (T_4) concentration using the radioimmunoassay from Dickhoff *et al.* (1978). A high Na+, K+ -ATPase activity and a low T_4 concentration are suggestive of smolting transformation in salmonids (Ewing *et al.*, 1984; McCormick, 1993).

Size and physiological data were compared among origin, egg size classes and sampling time using a 3-way ANOVA with origin and egg size classes as fixed factors and time as random factor. There was no difference between replicated tanks ($F_{(1,2540)}= 0.21$ p = 0.64). When interactions effects were detected, Scheffe post- Hoc tests were used to assess where the difference originated.

Salt-water preference tests were carried out on one- year old fish (i.e. 2009 crosses). Arenas for the salt- water test were four rectangular fiber- glass 800- l tanks (2.2 x 0.6 x 0.6 m), each tank being divided in two compartments of equal volume by a fiber- glass divider (Figure 2). Two header tanks (1m diameter) were used to prepare salt- water. Approximately 120 l of salt-water was prepared the night before the trial in the header tanks using Instant OceanR artificial salt. This volume of salt- water was selected accounting for size and steelhead smolts activity. On the morning of the trial, salt was entirely dissolved and salinity was measured before introduction into the experimental tanks. Header tanks were equipped with a pump sending salt- water into the bottom of two experimental tanks through PVC pipes (one tank after another to ensure constant salt- water flow). The two compartments of each experimental tank were connected to independent PVC pipes allowing switching fresh- water and salt-water sides between trials. The tanks were supplied with air- stones, and flow- through- fresh- water (4-5 l/ min) in both sides of the tank. The tanks were enclosed behind black plastic curtains to reduce disturbance. Overhead incandescent light bulbs (60 watts) were suspended above both compartments of each tank and were used to match ambient light and photoperiod. Light was also proven to encourage fish movement (see also Price and Schreck, 2003).

A few minutes prior to salt- water introduction, the fresh- water supply and aeration were turned off to prevent mixing in the expérimental tanks. Each trial started with salt-water introduction into one compartment of each experimental tank. The valves from the header tanks were then opened and salt- water began flowing into either compartment of the experimental tanks, pushing the lighter fresh- water out of the top- draining standpipe. Salt-water was introduced very slowly (2 L/min) to obtain a stable layer of salt- water i.e. bottom third of the water column (20 cm deep) became saline and the top two third was fresh. Salt- water introduction triggered fish movement; they could "sense" salt- water even when stationary in the opposite compartment. The halocline was visible allowing observers to distinguish easily whether fish were located in salt- or fresh- water. Fish were allowed to choose between salt- and fresh- water for 2 hours after which a separation partition was placed between the two compartments. Some fish would hold close to the surface of the salt- water compartment i.e. they were in the layer of fresh- water above the salt- water, but not in salt- water (Figure 2) and some fish would consistently switch between compartments. None of these fish were considered in the data analyses. Salt- water

concentration and stability of the layer was checked at the end of the trial, salinity on the top two third was always < 2 ppt. Afterwards, fish were removed from the tank for identification and the salt-water compartment flushed with fresh- water. Fresh- water was reintroduced in both compartments, and fish were put back into the same experimental tank and allowed to re- acclimate for 48 hours before next trial. A total of six consecutive trials were carried out with the same group of fish to evaluate the consistency of fresh- or salt-water choice.

On 18 April, 10 May and 6 June 2010, 10 fish from each treatment group (HS, HL, WS, WL) were introduced into the four experimental tanks. Each month, each treatment was randomly assigned to one of the four experimental tanks. Before each trial, the compartment receiving salt- water was randomly chosen. Fish were acclimated for 48 hours in freshwater (Price and Schreck, 2003). Fish were exposed to salt-water every other morning for 12 consecutive days resulting in six repeated trials per treatment (see above). Behaviour was recorded with a high-definition camera (GOPRO HD Hero) placed 2 meters above the tank. Behaviour was recorded during the one-hour salt-water introduction and two hours after salt-water was introduced. Pre-observations over 24 hours prior to salt-water introduction revealed that steelhead smolts were most active for the first one and half hour after which they choose either fresh- or salt- water compartment. At the end of each trial, I identified individual fish by their PIT tags and then I noted final fish position: fresh- or salt- water. When the same group of fish had performed 6 consecutive trials I estimated the consistency of the choice per fish: salt- water if the fish chose salt- water at least 4 times out of 6, fresh- water if the fish chose fresh- water at least four times out of six and no choice if the fish chose three times fresh- water and three times salt- water. I compared fish choice across treatments (origin and egg size category) using chi square tests. Additionally, fish movement data (direction of the switch) were collected from video clips, every five minutes for a 1- minute duration. Data were collected on first, third and sixth trial each month for each treatment. Direction of the switch was expressed as fresh or salt- water switch per fish per hour. Mann-Whitney tests were used to compare switching behaviour across origin and across egg size and Kruskal- Wallis tests were used to compare switching behaviour across time and trials.

5.3 Results

5.3.1 Egg size from hatchery versus wild steelhead

The factors origin x year and year did not significantly affect egg size (respectively $F_{(1,35)}=$ 0.08 p = 0.777 and $F_{(1,35)}=$ 0.99 p = 0.325). Wild fish had significanlty larger eggs than hatchery fish after accounting for female size (respectively 6.84 ± 0.40 and 6.56 ± 0.35 mm; $F_{(1,35)}=$ 23.19 p < 0.001; Figure 3). After accounting for female size, variance in egg size tended to be greater in hatchery fish compared to wild fish ($F_{(1,35)}=$ 2.86 p = 0.092; Figure 3). Variance in egg size increased with female size ($F_{(1,35)}=$ 18.92 p < 0.001) and also differed between years (df = 1 $F_{(1,35)}=$ 56.56 p < 0.001).

5.3.2 Development and early growth

In general, developmental features of embryos observed before hatching were negatively correlated with egg size in both wild and hatchery fish (Table 2). Hatching time was slighly correlated with egg size when both origin groups were combined but not within each origin group (Table 2). After hatching, growth rate, weight and length at emergence were positively correlated to egg size in both wild and hatchery fish (Table 2).

Before the embryos hatched, origin and female did not significantly affect most of the developmental features after accounting for egg size (Table 3). However, hatching time was longer for hatchery fish than for wild fish (respectively 44.92 ± 5.51 vs 43.69 ± 4.18 days; Table 3 and 4). Wild embryos were longer, heavier and grew faster than hatchery embryos even after accounting for egg size (Table 3 and 4). After accouting for egg size, origin of the mother and female FL, significantly affected most of the developmental features before hatching as well as hatching and growth of embryos up to emergence (Table 3 and 4).

5.3.3 Early behaviour

First feeding juveniles coming from larger eggs were larger than juveniles coming from smaller eggs (respectively 30.4 ± 0.7 versus 25.3 ± 0.7 mg; ANOVA: $F_{(1,56)} = 30.56$ p < 0.001) but fish of wild and hatchery origin did not differ in body weight (respectively 28.4 ± 0.7 versus 27.3 ± 0.7; ANOVA $F_{(1,56)} = 1.30$ p = 0.258). Wild juveniles tended to perform more bottom foraging than hatchery juveniles and, within each group, juveniles coming from smaller eggs tended to perform more bottom foraging than juveniles coming from larger eggs (Table 5). Additionally, wild juveniles coming from larger eggs performed more surface foraging than wild juveniles coming from smaller eggs, and hatchery juveniles coming from both smaller and larger eggs (Table 5). Only juveniles from smaller eggs displayed agonsitic behaviour (Table 5).

On the first fright event, hatchery fish retreated less to the edge than wild fish but over time wariness decreased more rapidly in wild fish than in hatchery fish (Figure 4). Fish coming from smaller eggs were fewest at the tray edge on the last two days of observation compared to fish coming from larger eggs (Figure 4). Additionally, wild fish took twice as long than hatchery fish to resume random distribution on the first day of observation (Figure 5). In the last trial all groups resume random distribution in about 5 seconds. Individual behaviour of wild fish was more variable than that of hatchery fish (error bars in Figure 5). Hatchery and wild fish differed neither in the percentage of fish at the edge of the tray nor in time to resume random distribution ($F_{(1,6)} = 0.02$ p = 0.890 and $F_{(1,5)} = 0.55$ p = 0.490 respectively; Figures 4 and 5). Fish coming from small and large eggs did not differ in the percentage of fish at the edge of the tray neither in time to resume random distribution ($F_{(1,6)} = 0.00$ p = 0.996 and $F_{(1,5)} = 0.036$ p = 0.857 respectively; Figures 4 and 5). At the same time the interaction origin x size was not significant for both the percentage of fish at the edge of the tray and the time to resume random distribution ($F_{(1,6)} = 0.02$ p = 0.905 and $F_{(1,5)} = 0.679$ p = 0.447 respectively).

5.3.4 Body size, physiology and salt-water preference tests after one year of rearing

After one year progeny of hatchery steelhead trout were larger (body weight and fork length) than wild steelhead trout ($F_{(1,2540)}$= 7.65 p = 0.006). Fish coming from smaller eggs were larger than fish coming from larger eggs ($F_{(1,2540)}$= 114.33 p < 0.001). Such relationship was observed at each sampling time (October $F_{(1,545)}$= 29.66 p < 0.001; November $F_{(1,505)}$= 27.56 p < 0.001; December $F_{(1,503)}$= 27.27 p < 0.001; February $F_{(1,493)}$= 29.38 p < 0.001; March $F_{(1,495)}$= 29.44 p < 0.001; Figure 6). The 3-way interaction origin x size x time was close to significance ($F_{(4,2540)}$= 2.30 p = 0.057; Figure 6) and the 2-way interaction origin x size was significant only in March ($F_{(1,495)}$= 6.29 p = 0.012). Juveniles coming from smaller eggs were larger than fish coming from larger eggs and hatchery juveniles were larger than wild juveniles (see Figure 6).

After march 2010, fish coming from smaller eggs were significantly longer and heavier than fish coming from larger eggs, and hatchery fish tended to be longer and heavier than wild fish (Table 6). Hatchery fish coming from small eggs were more silvery than wild fish from small eggs and hatchery fish from large eggs (Scheffe post hoc tests: respectively df = 135 p < 0.001; df = 146 p < 0.001; Table 6). Gill Na+, K+ -ATPase activity was significantly lower in hatchery fish at each sampling time except the sampling on mid – April (Figure 7; Table 6). At the same time Gill Na+, K+ -ATPase activity was significantly lower in larger fish at each sampling time (Figure 7; Table 6). Over time, thyroxine levels were significantly lower in wild fish than in hatchery fish and in fish coming from large eggs compared to fish coming from small eggs (Figure 7, Table 6).

Fish coming from larger and smaller eggs did not differ in their final choice of salt- or fresh- water (df = 2, χ^2 = 0.23, p = 0.892) and 28 % of fish did not choose consistently fresh- or salt- water. However 49 % of hatchery fish chose salt-water consistently and 60% wild fish chose consistently fresh-water (df = 2, χ^2 = 21.26, p < 0.001). 32% of hatchery fish were not consistent in their water choice compared to 25% of wild fish. Wild fish switched more often to fresh- water than hatchery fish (Mann-Whitney: n = 35 U = 72 p = 0.007) but there was no difference between fish coming from larger and smaller eggs (Mann-Whitney: n = 35 U= 140.5 p = 0.684). There was no significant difference in salt- water switch between hatchery and wild fish (Mann-Whitney: n = 35 U = 119.5 p = 0.273) and between fish coming from larger and smaller eggs (Mann-Whitney: n = 35 U = 138 p = 0.636). There was no differences in fresh- and salt- water switching behaviour across time (Kruskal-Wallis: $H_{(2)}$= 1.06, p = 0.590 and $H_{(2)}$= 1.20, p = 0.550). Switching behaviour did not decrease with trials (switch to fresh- water $H_{(2)}$= 0.79 p = 0.675 and switch to salt- water $H_{(2)}$= 0.82 p = 0.664).

5.4 Discussion

This study revealed important relationships between egg size, early embryonic development, juvenile behaviour and growth of both hatchery and wild steelhead trout. Furthermore, hatchery and wild steelhead trout differed in egg size, egg size variance and embryo growth. After accounting for female body size, F-1 hatchery fish had smaller and more variable egg

size when compared to wild fish. Thus, wild progeny grew faster, were larger and longer at emergence. Embryos coming from smaller eggs were developing faster than embryos coming from larger eggs. Contrary to my prediction, both origin and egg size could still be related with growth of one year old fish. Thus, hatchery fish coming from smaller eggs were larger than wild fish coming from smaller eggs and both wild and hatchery fish coming from small eggs were larger than both hatchery and wild fish coming from larger eggs. In addition, hatchery and wild steelhead trout differed in physiological characteristics and salt-water preferences. Hatchery fish showed lower levels of gill Na+, K+ -ATPase activity and higher levels of thyroxine when compared to wild fish raised under the same laboratory condition. These results are consistent with previous studies that found that hatchery fish were larger and had reduced levels of gill Na+, K+ -ATPase activity (Hill *et al.*, 2006). However, salt- water preference tests did not follow these physiological data: hatchery fish preferred salt- water whereas wild fish preferred fresh- water.

The effects of domestication on salmonids have been of interest for both hatchery practices and restoration of wild populations using hatchery fish. Large reductions in relative fitness have been observed for hatchery steehead trout compared to wild ones (Berejikian and Ford, 2004). Genetic effects of domestication considerably reduce the reproductive capabilities of hatchery fish when released to natural environments, i.e. 40% decrease in fitness per generation raised in hatchery (Araki *et al.*, 2007). Here, I showed that egg size was smaller in first generation hatchery fish and egg size was also more variable when compared to wild fish (see chapter 1; Einum and Fleming, 2000b). The originality of my study is that wild and hatchery fish shared parental genetic similarities and parents differ only in their first year of rearing, i.e. captivity for hatchery fish and in the river for wild fish. The smaller size of wild fish compared to hatchery fish has been suggested to be the result of females experiencing high growth rate as juveniles produce a relatively large number of small eggs as adults (Jonsson *et al.*, 1996; Lobón-Cerviá *et al.*, 1997; Morita *et al.*, 1999; Fleming *et al.*, 2000; Olsen and Vøllestad, 2003; reviewed by Einum *et al.*, 2004). It is clear that this can be a quick response as I have seen here significant changes in egg size after only one generation in a hatchery. In addition to that, the fish I observed had only spent the time until smolting in the hatchery, but after that they lived as wild fish.

Hatchery propagation of steelhead trout involves a full year of hatchery rearing before being released as smolts. Such practices shortens the two years in fresh- water usually observed in wild populations of coastal steelhead populations (Busby *et al.*, 1996). In the Siletz population, most parental fish spend 2 years at sea before coming back to freshwater to spawn (L. Borgerson, ODFW, unpublished data). Thus, 1 year in a hatchery environment may represent a substantial part of the life cycle (Berejikian and Ford, 2004) resulting in rapid divergence of reproductive capabilities of hatchery and wild fish.

Egg size related to development of embryos before and after hatching. Interestingly smaller embryos coming from smaller eggs developed faster than larger embryos. These observations are similar to what have been shown in Arctic charr (chapter 1 and Valdimarsson *et al.*, 2002). Showing a consistent in developmental trends across these two salmonids species. Few studies have explored how egg size affects developmental pathways of fishes (but see Balon, 1999; 2002). In invertebrates, egg size is strongly associated with developmental mode: species with small eggs (i.e. small amount of yolk) have planktonic

larvae, disperse, feed on plankton, and then undergo metamorphosis, whereas species with large eggs (i.e. large amount of yolk) tend to have short-lived, non feeding larvae or have no larvae stage at all (reviewed by Moran and McAlister, 2009). These two extreme modes of development refer respectively to indirect and direct development. Similar developmental trajectories linked to egg size (i.e. yolk amount) have been described in fishes (Balon, 1999). Embryos coming from small eggs may have to develop feeding structure faster than embryos coming from larger eggs because they have less yolk. In fact egg size variation among and within a species may reflect different way of using the energy available in individual egg and it may also reflect different patterns of development in fishes. In fact, differences in egg size between hatchery and wild fish may indicate differences in pattern of development that are later mirror by differences in growth and behaviour.

Short term effects of egg size were observed on foraging and agonistic behaviours of both hatchery and wild first feeding fish. As predicted, fish coming from smaller eggs tended to feed more on the bottom whereas fish from larger eggs tended to feed more at the surface. Effect of egg size on foraging behaviour is similar to what has been shown in Arctic charr (chapters 2 and 3; Benhaïm et al., 2003). So again there is a great consistence in early behaviour that is related to egg size in these two salmonids species. In polymorphic species like Arctic charr and steelhead trout, differences in feeding tactics between fish coming from small and large eggs can be linked to habitat use and evolutionary processes. Indeed variation in behaviour, stemming from small size differences at first feeding, may influence habitat and food selection that may lead to divergence of fish populations, especially if there are clear interaction between maternal and genetic effects (chapter 2). Such divergence in early behaviours may lead to subsequent changes in growth.

Interestingly, egg size affected absolute growth of embryos and juveniles steelhead trout up to the age of smolting. At emergence, juveniles coming from larger eggs were larger as classically reported in salmonids (Arctic charr chapters 1 and 3; Atlantic salmon Einum, 2003; Moffett et al., 2006). However this positive relationship between egg size and growth observed at emergence turned into a negative relationship from that fall up to the spring (Figure 6). Thus, egg size affected growth of juvenile steelhead trout beyond few weeks after emergence and in a way never reported before, i.e. fish coming from smaller eggs became larger than fish coming from larger eggs in both hatchery and wild fish. Environmental effects were minimized since fish were raised in similar hatchery condition with controlled density, feeding, photoperiod and temperature, from egg to 1- year old juveniles. Also, their genetic background were similar (see discussion above). A few studies have reported long term relationships of egg size and growth of salmonids (e.g. Blanc, 2002). In Arctic charr, I observed a long- term positive effect of egg size on growth (chapter 3). Two explanations as to why only a few studies have focused on egg size consequences on growth can be provided. First, many salmonids studies indicate that egg size effects on growth disappear quickly after first feeding (i.e. genetic and environmental factors become more important than the effect of egg size), but also many experiment are terminated shortly after first feeding (Srivastava and Brown, 1991; Heath et al., 1999). Hence the long term effect of egg size may be ignored because experiments terminates too early. The second explanation is the variation of egg size itself. Indeed, egg size effect have perhaps received little interest in species where egg size variation within females is not obvious and where differences in egg size did not appear to affect survival. However, recent

results on *O. mykiss* indicate that egg size influences on juveniles growth lasted longer within families rather than across families (Blanc, 2002). My results show as well that egg size varies across and within *O. mykiss* females, resulting in significant differences in growth and physiological characteristics. However the effect of egg size on fish reared in natural environment still needs to be tested.

As previously described, hatchery fish are reared for 1- year in fresh- water and released as smolts whereas wild fish reared in natural conditions usually spend 2 years in fresh- water before migrating downstream (Quinn, 2005). Preference tests clearly indicated a salt- water preference by hatchery fish and a fresh- water preference by wild fish after 1- year of rearing under hatchery conditions. Physiological results indicated a lower osmo- regulatory status for hatchery fish. Such mismatch between salinity preference and osmo- regulatory status may translate into lower survival of hatchery fish in the estuary and in the ocean environment. Reduced smolt survival has been linked to decreased osmo- regulatory status, hormone levels and migratory tendency (Muir *et al.*, 1994; Beckman *et al.*, 1999; Hill *et al.*, 2006). Some studies (Jonsson *et al.*, 2003; Chittenden *et al.*, 2008) have found hatchery salmon smolts to have higher mortality rates in marine environments as well as lower survival rates in the estuary and a longer in-river downstream migration. Thus, the tendency to prefer salt- water combined with a lower osmo- regulatory status in hatchery fish may result in lower survival when released as smolts in the river. However, other studies have failed to consistently identify survival differences between hatchery and wild fish (Welch *et al.*, 2004; Johnson *et al.*, 2010). Whether physiological differences and differences in salt- water preference observed in this study reflect differences in subsequent ocean survival between hatchery and wild fish still needs to be tested.

The short term effects of egg size on embryonic development and behaviour of first feeding fish were similar to those I have described in Arctic charr (chapters 2 and 3). These results emphasize that egg size effects are consistent across two salmonids species and that egg size is a source of diversity in these fishes. My study is the first to look at the long term effect of egg size and steelhead trout origin (hatchery vs wild). The study has revealed siginificant differences in early development, behaviour and first year growth and physiological status of fish. These differences are even more striking since they were identified in first generation hatchery fish that originated from the same genetic pool as wild fish. Moreover, both hatchery and wild fish were raised under similar hatchery conditions for one year whereas some previous studies compared performances of hatchery versus wild fish without controlling for the environment (e.g. Jonsson *et al.*, 2003; Chittenden *et al.*, 2008; Johnson *et al.*, 2010). Further studies are needed to investigate the role of egg size in the natural environment from early stages of development up to the age of smolting. First year environment and growth patterns of steelhead trout appear to be crucial with important consequences in terms of reproductive capabilities i.e. egg size and performances of the next generation. Egg size may be a mechanism from which differences in growth and behaviour between hatchery and wild fish originate.

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Acknowledgements:

Table 5.1: Description of developmental features used to characterize early development of steelhead trout embryos and their associated indexes. Modified from Valdimarsson et al. (2002).

Developmental feature	Index	Description
Intensity of blood color	0	no red color in the vein
	1	slight pink color in the vein
	2	clear red colour observed
Formation of vitelline vein	0	clear vein observed
	1	clear vein with one ramification across the yolk
	2	clear vein observed with multiple ramifications
Darkening of eyes	0	light shadow in eyes
	1	dark shadow in eyes
	2	dark coloured cells clearly visible
Number of melanophores	0	no sign of coloured cells
	1	less than 10 melanophores visible
	2	less than 100 melanophores visible
	3	more than 100 melanophores visible

Table 5.2: Spearman's rank correlation coefficients for indexes of developmental features in relation to egg weight.

Developmental features	Origin	Spearman correlation coefficient	N	p-value
Before hatching				
Melanophores on the head	Hatchery	-0.534	70	**<0.001**
	Wild	-0.397	90	**<0.001**
	Both	-0.524	160	**<0.001**
Melanophores on the trunk	Hatchery	-0.491	70	**<0.001**
	Wild	-0.292	90	**0.005**
	Both	-0.462	160	**<0.001**
Darkening of the eyes	Hatchery	-0.077	70	0.526
	Wild	-0.142	90	0.181
	Both	-0.251	160	**0.001**
Blood color intensity	Hatchery	-0.255	70	**0.033**
	Wild	-0.149	90	0.162
	Both	-0.177	160	**0.025**
Vein ramification	Hatchery	-0.263	70	**0.028**
	Wild	-0.264	90	**0.012**
	Both	-0.270	160	**0.001**
Hatching time (dpf)	Hatchery	0.105	96	0.308
	Wild	0.184	90	0.082
	Both	0.169	186	**0.021**
After hatching				
Growth rate	Hatchery	0.269	87	**0.012**
	Wild	-0.180	90	0.089
	Both	0.407	177	**<0.001**
Length at emergence	Hatchery	0.319	85	**0.003**
	Wild	0.057	90	0.596
	Both	0.597	175	**<0.001**
Weight at emergence	Hatchery	0.533	85	**<0.001**
	Wild	0.435	57	**0.001**
	Both	0.715	142	**<0.001**

The standardized residuals of egg weight from the linear regression model egg weight ~ female fork length were used to account for female effect on egg weight.

Table 5.3: Results of mixed model analyses of covariance on early development of hatchery and wild steelhead trout. Analyses on developmental features are performed with egg weight as a covariate. The term female is nested within the origin of the fish (origin): wild or hatchery. Origin was a fixed factor while female (origin) and egg weight were random factors. n df and d df refer to numerator and denominator degrees of freedom respectively.

Developmental features	Factors	n df	d df	F	p-value
Melanophores on the head	origin	1	143	9.99	**<0.001**
	female(origin)	14	143	4.14	**0.002**
	egg weight	1	143	53.36	**<0.001**
Melanophores on the trunk	origin	1	143	3.04	0.083
	female(origin)	14	143	2.73	**0.001**
	egg weight	1	143	22.49	**<0.001**
Darkening of the eyes	origin	1	143	0.24	0.624
	female(origin)	14	143	6.04	**<0.001**
	egg weight	1	143	8.34	**0.004**
Blood color intensity	origin	1	143	0.66	0.417
	female(origin)	14	143	1.35	0.185
	egg weight	1	143	3.17	0.077
Vein ramification	origin	1	143	0.06	0.815
	female(origin)	14	143	1.12	0.345
	egg weight	1	143	0.74	0.390
Hatching time (dpf)	origin	1	166	415.80	**<0.001**
	female(origin)	17	166	659.49	**<0.001**
	egg weight	1	166	3.01	0.085
Growth rate	origin	1	157	46.21	**<0.001**
	female(origin)	17	157	24.28	**<0.001**
	egg weight	1	157	14.78	**<0.001**
Length at emergence	origin	1	155	67.83	**<0.001**
	female(origin)	17	155	11.57	**<0.001**
	egg weight	1	155	15.09	**<0.001**
Weight at emergence	origin	1	125	117.62	**<0.001**
	female(origin)	14	125	22.09	**<0.001**
	egg weight	1	125	94.82	**<0.001**

The standardized residuals of egg weight from the linear regression model egg weight ~ female fork length were used to account for female effect on egg weight.

Table 5.4: Differences in developmental traits between hatchery and wild steelhead trout, Siltez OR (mean ± SD). *dpf: days post fertilization.*

	Hatchery	Wild
Female fork length (cm)	66.80 ± 3.94	70.78 ± 5.49
Egg size (mm)	6.56 ± 0.35	6.84 ± 0.40
Hatching time (dpf)	44.92 ± 5.51	43.69 ± 4.18
Length at emergence (mm)	26.89 ± 1.56	28.80 ± 2.60
Weight at emergence (mm)	19.36 ± 2.62	22.44 ± 5.16
Growth rate (mm/day)	0.34 ± 0.10	0.40 ± 0.10

***Table 5.5: Origin and egg size influences on behaviour of first feeding steelhead trout.** Origin refers to hatchery versus wild fish and egg size to fish coming smaller eggs or larger eggs. All behavioural items were analyzed with 2- way ANOVA (ndf= 1 ddf=26 for each factor and the interaction) except the agonistic behaviour data that did not meet the assumptions for ANOVA and thus independent Kruskal- Wallis tests were performed to assess the effect of origin and the effect of size.*

	Factors	F	p
Foraging behaviours			
Bottom foraging	origin	3.05	0.092
	egg size	3.05	0.092
	origin*egg size	1.46	0.238
Water column foraging	origin	0.33	0.571
	egg size	1.22	0.280
	origin*egg size	0.07	0.793
Foraging surface	origin	0.06	0.804
	egg size	9.96	**0.004**
	origin*egg size	4.04	0.055
Total foraging	origin	0.60	0.448
	egg size	1.24	0.275
	origin*egg size	0.83	0.372
reaction time to food delivery	origin	0.50	0.471
	egg size	0.97	0.333
	origin*egg size	0.24	0.626
Mobility			
Stationary display	origin	0.33	0.572
	egg size	0.35	0.560
	origin*egg size	0.69	0.414
Regular swimming	origin	0.05	0.819
	egg size	0.13	0.724
	origin*egg size	0.90	0.353
Rapid swimming	origin	3.58	0.070
	egg size	0.14	0.708
	origin*egg size	0.89	0.355
Agonistic behaviour	origin	1.26	0.261
	egg size	4.44	**0.035**

Levene's tests were performed to check the homogeneity of variances (n df=1, d df= 26; p> 0.05 for all items, except agonistic behaviour).

Table 5.6: Analyses of variance for fish body weight, fork length, condition factor, gill Na+, K+ -ATPase and thyroxine levels for hatchery and wild steelhead smolts coming from large and small eggs. *Fish were raised in hatchery conditions for their first year and 5 samplings were performed from mid april to june 2010 during smolting.*

Factors	df	Body weight F	p	Length F	p	Condition F	p	Color F	p	ATPase F	p	Thyroxine F	p
origin	1	0.39	0.530	0.04	0.840	3.31	0.070	22.22	**<0.001**	5.37	**0.021**	1.61	0.205
size	1	14.37	**<0.001**	12.61	**<0.001**	0.27	0.602	13.19	**<0.001**	4.21	**0.041**	39.51	**<0.001**
time	4	7.74	**<0.001**	5.56	**<0.001**	7.14	**<0.001**	6.54	**<0.001**	6.21	**<0.001**	8.01	**<0.001**
origin x size	1	5.88	**0.015**	3.61	0.058	2.24	0.136	20.32	**<0.001**	0.48	0.491	2.34	0.127
origin x size x time	4	1.33	0.251	1.55	0.173	0.51	0.767	0.72	0.609	1.13	0.343	3.93	**0.004**

Levene's tests were performed to check the homogeneity of variances (p> 0.05).
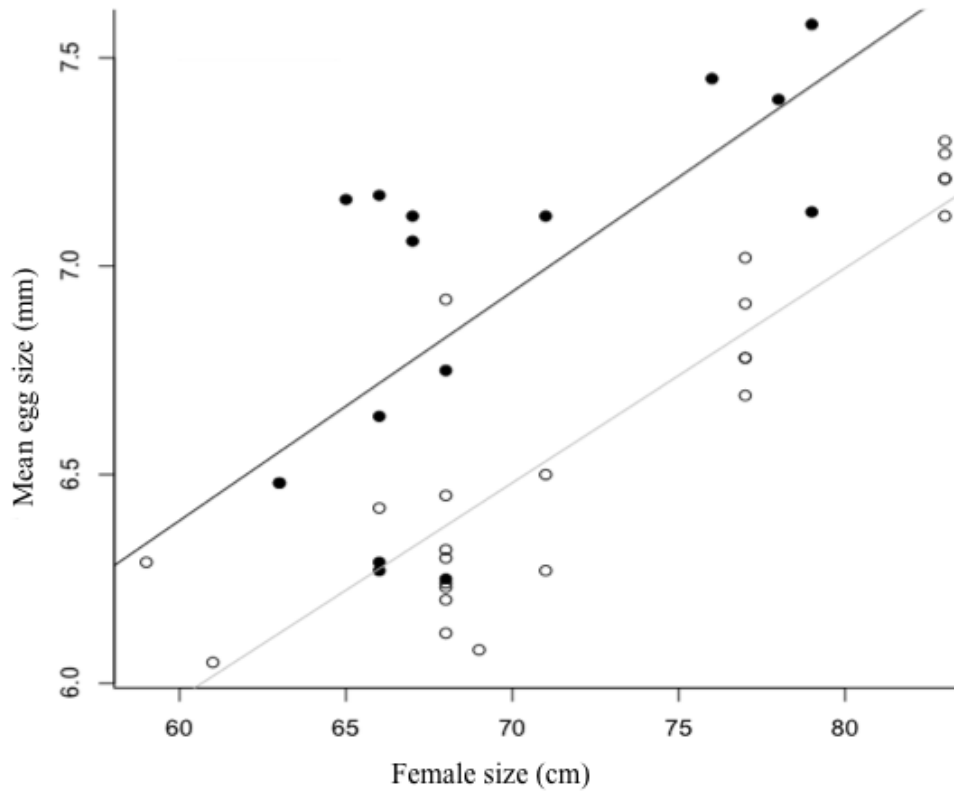
***Figure 5.1: Correlation between female fork length and mean egg size per female in hatchery versus steelhead trout, Siletz OR.*** *Results for 15 wild females (●) and 23 hatchery females (○) are presented with associated linear relationships within each origin. Scales from one hatchery and one wild female were not readable. Pearson's correlations across: hatchery: r = 0.88 df = 22 p < 0.001; wild: r = 0.69 df = 14 p = 0.003.*
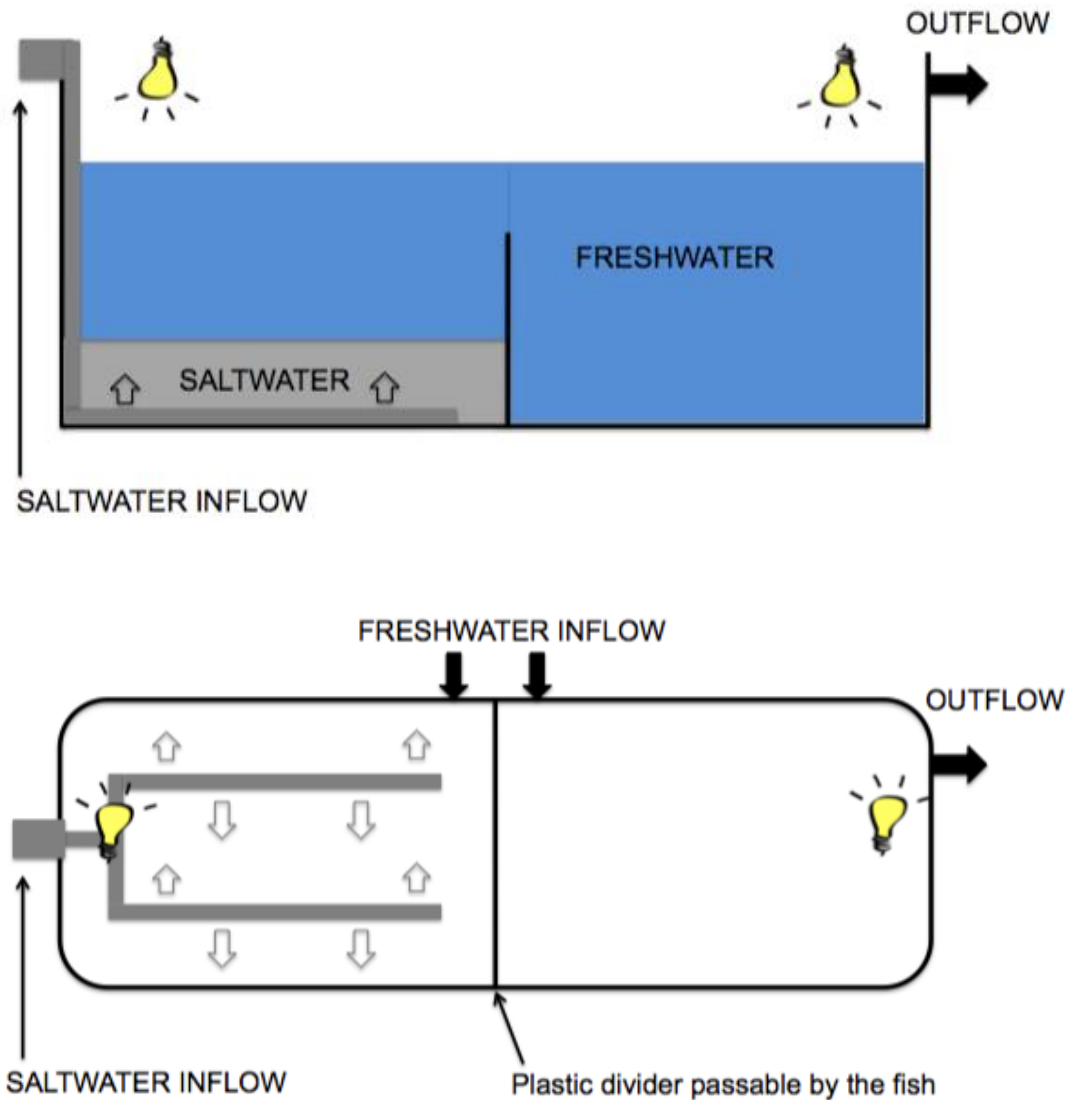
Figure 5.2: Diagram of one salt- water preference tank. *The upper diagram is a side view of the experimental tank and below is a bird view. Tank is made from plastic and measure 2.2 x 0.6 x 0.6 m. A black plastic curtain enclosed the tank. The pipe from the header tank split into two lines on the bottom of the preference tanks (bottom diagram, grey pipe). Salt- water was slowly introduced through holes drilled every 5 cm in two rows along the sides of the pipes, shown by the open arrows in the bottom view. At the end of the trial, a separator was lowered to isolate fish in side or the other. Light bulbs were suspended above each compartment of the tank. Fresh and salt- water compartment were inter- changeable with independent pipes system (adapted from Price and Schreck, 2003).*
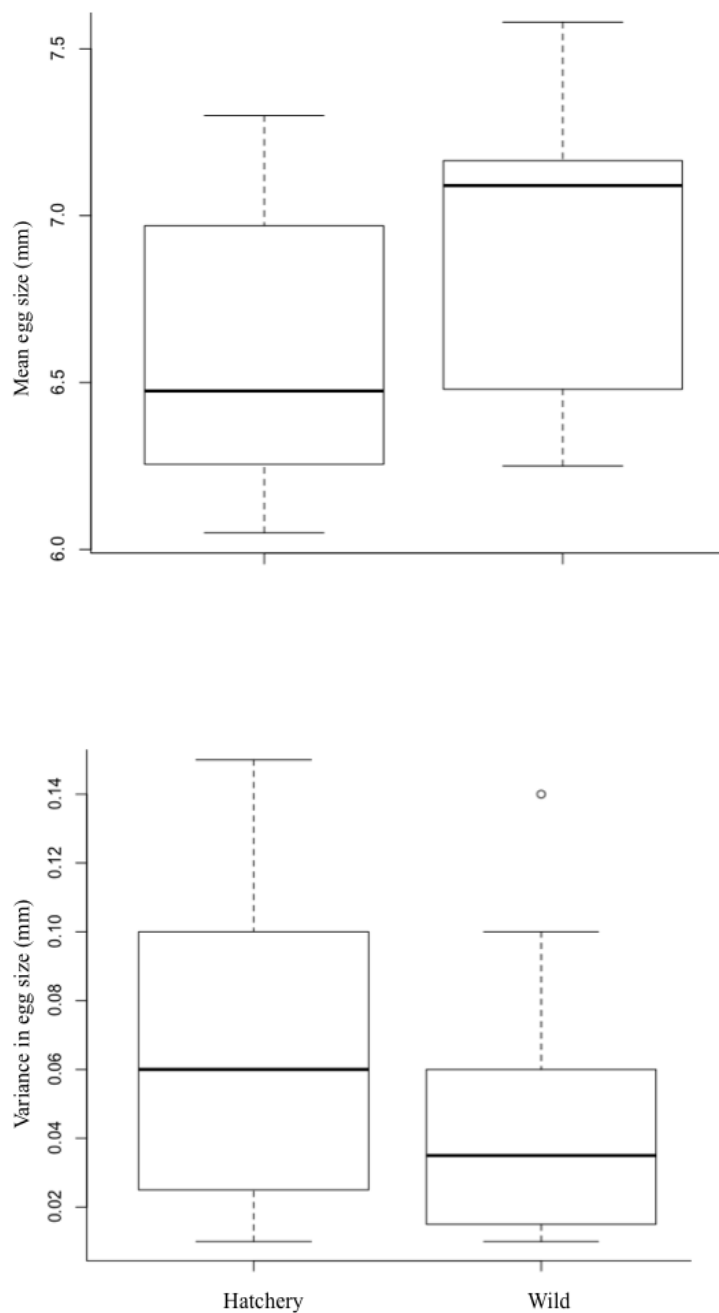
Figure 5.3: Mean egg size and variance in mean egg size per female of steelhead trout from hatchery and wild origins. Upper graph presents the mean egg diameter (mm) and the lower graph presents the variance in mean egg diameter (mm). Results from 40 females total are presented. The bold horizontal line in each box represents the median, the bottom and top edges lines represent the 25th and 75th percentiles respectively, and error bars are the 10th and 90th percentiles.
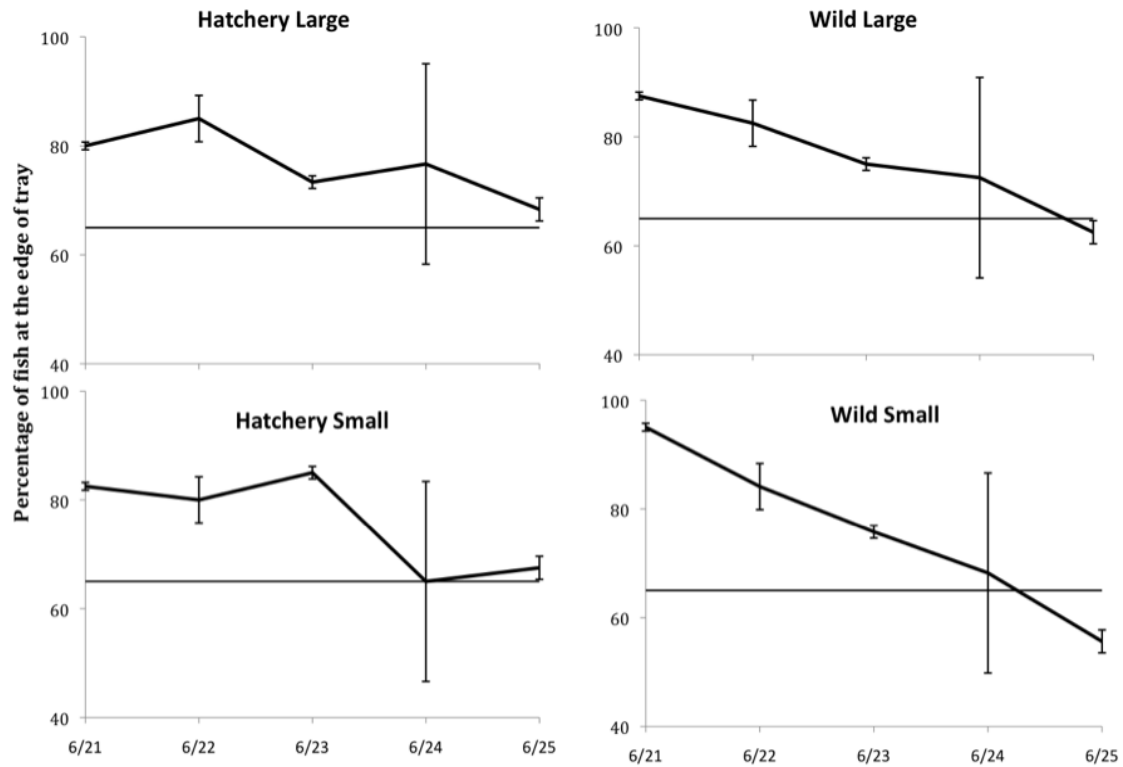
Figure 5.4: Percentage of fish located at the edge of the tray in the fright response trials. *The line drawn at 65% represents the portion of fish that would be expected in the edges of each tray if they were randomly distributed (i.e. 65% of the tray area was located outside the 20-cm by 11-cm-rectangle outlines ; inspired by Negus 1999).*
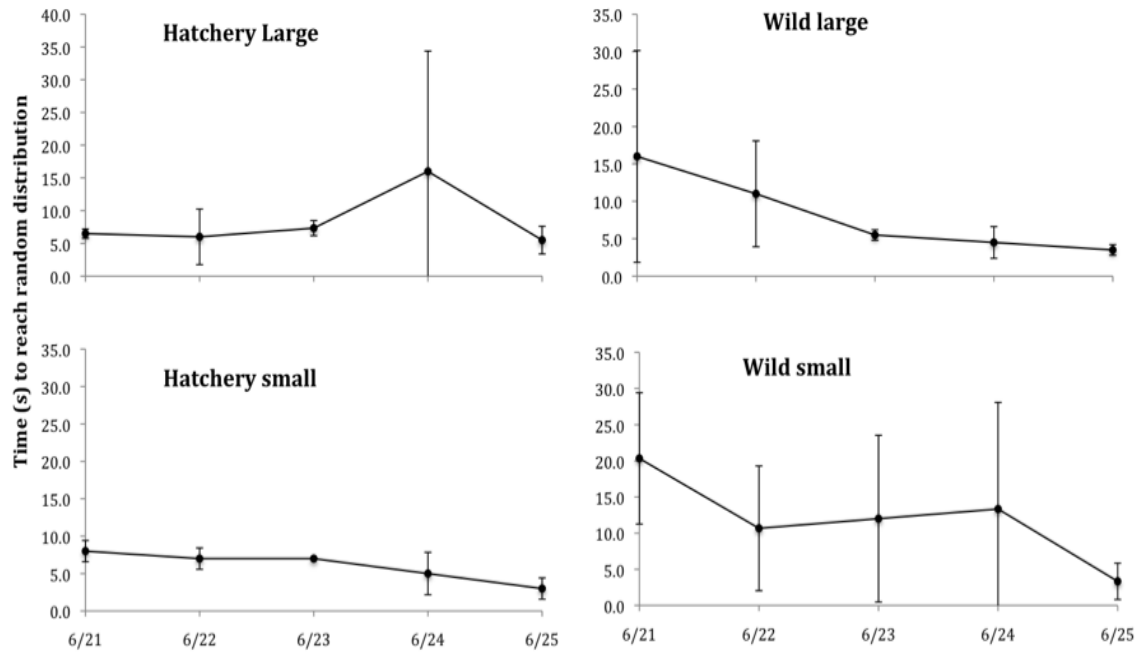
Figure 5.5: Time to reach a random distribution after a startling event in the fright trials. *Random distribution was achieved when 35% of the fish were observed inside the 20-cm by 11-cm-rectangle outlines representing 35% of the tray area. Error bars are standard deviation.*
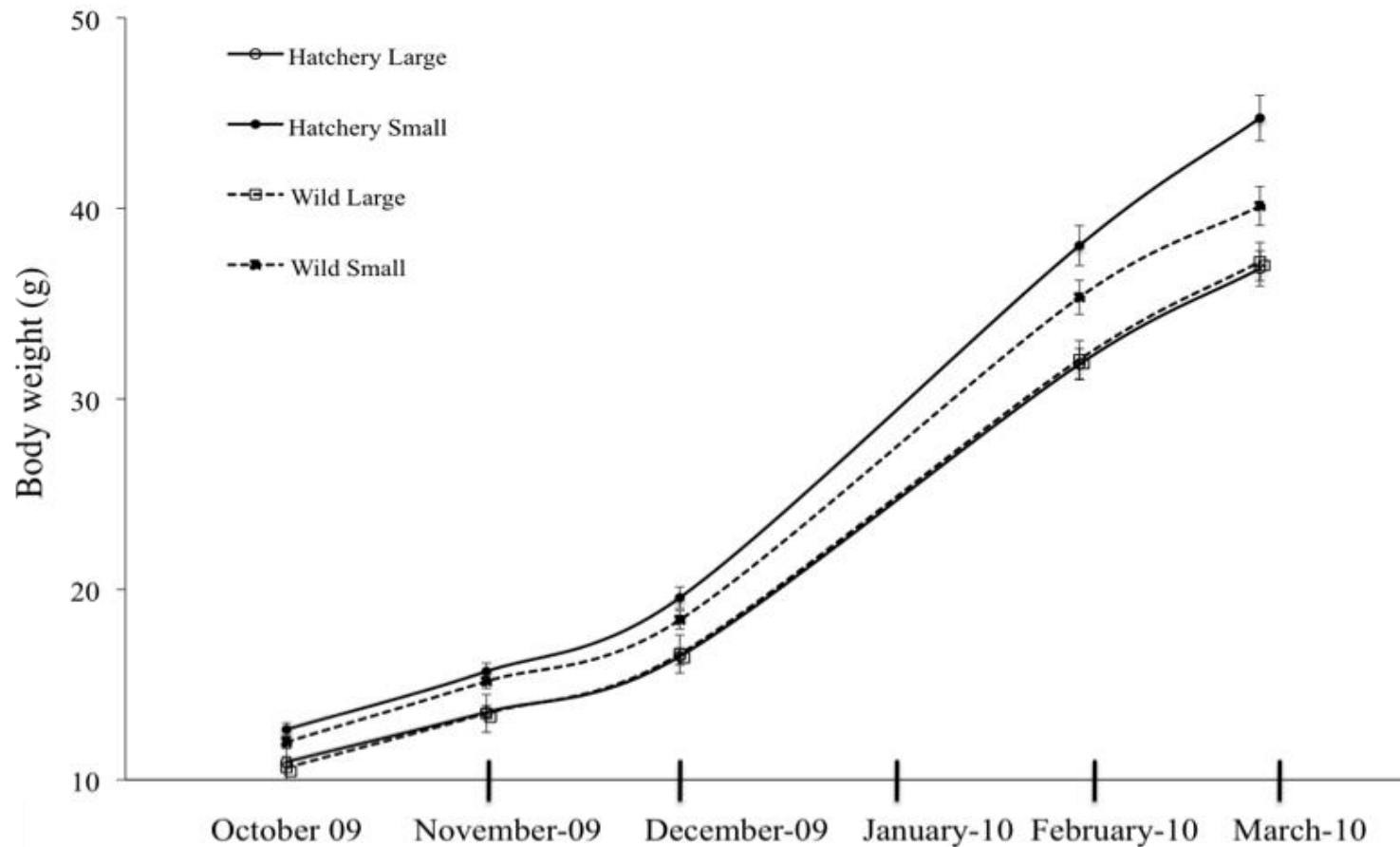
Figure 5.6: Weight (mean ± SE) of hatchery and wild steelhead trout in their first year raised under hatchery condition. *"Large" and "small" refer to egg size classes. Dashed lines refer to wild fish and solid lines refer to hatchery fish whereas (°) represents fish derived from smaller eggs and (•) represents fish derived from larger eggs. Results of the three-way ANOVA with origin and egg size classes as fixed factors and time as random factor are: origin x size x time interaction $F_{(4,2540)}= 2.30$ p = 0.057; origin x size: October $F_{(1,545)}= 0.41$ p = 0.523; November $F_{(1,505)}= 0.39$ p = 0.523; December $F_{(1,503)}= 1.82$ p = 0.166; February $F_{(1,493)}= 2.87$ p = 0.091; March $F_{(1,495)}= 6.29$ p = 0.012. Growth significantly increased accross time (Scheffe post hoc tests: p < 0.001).*
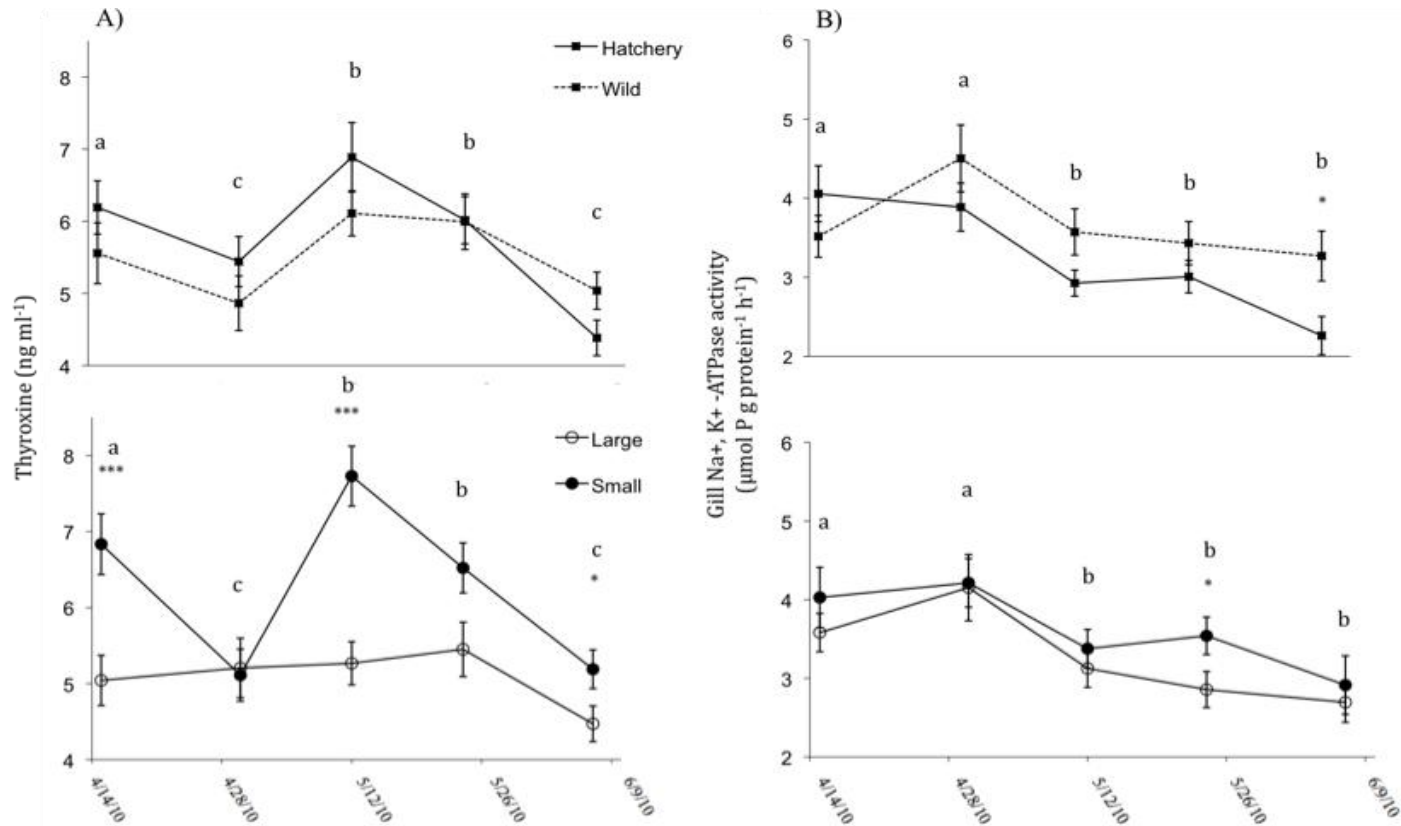
Figure 5.7: Thyroxine levels (A) and gill Na+, K+ -ATPase activity (B) of hatchery and wild steelhead trout coming from small and large eggs (mean ± SE) reared under hatchery condition*. Upper graphs show physiological differences between hatchery (plain line) and wild fish (dashed line) and lower graphs shows physiological differences between fish coming from large eggs (°) and fish coming from small eggs (•). Different letters indicate statistical differences (p < 0.05) among sampling times (Scheffe post Hoc tests). * indicates statistical differences among origin or size at each sampling time (*** p ≤ 0.001; ** p ≤ 0.01; * p ≤ 0.05).*

6 General conclusions

6.1 Intra- clutch variation in egg size

In both species that I studied, there was intra- clutch variation in egg size. Such variation was visible for a human eye but it was much more visible in Arctic charr species. It is still unclear why such variation occurs in both wild and hatchery populations of salmonids. Such variation may originate from differences in vitellogenin provisioning across the eggs during the maturation process. Another hypothesis will be that egg size is determined accordingly to the oocyte location in the ovary (i.e. near or further from the blood vessels). However these hypotheses will need to be tested and it is still unclear why intra- clutch variation in egg size exists, even in stable environment such as hatchery environment.

6.2 Short and long term effects of egg size

The overall objective of my research was to look at both short and long term effects of egg size on various phenotypic traits of two polymorphic salmonids, Arctic charr and steelhead trout. In both species I identified correlations between egg size, early development, early behavior and early growth (i.e. short term effects). In Arctic charr, long term effects of egg size were found for growth, feeding behavior and morphology. In steelhead trout I found long terms effects of egg size on growth and osmo-regulatory status in one - year - old fish. Thus, the study presents new findings that demonstrate that variability in egg size is an important source of phenotypic variation in fishes.

These results confirm that egg size affect early life history traits as previously demonstrated in other salmonids (e.g. Srivastava and Brown, 1991; Einum and Fleming, 1999; Heath *et al.*, 1999; Einum and Fleming, 2000a; 2002; Pakkasmaa and Jones, 2002). However, these studies have reported that egg size effects disappeared shortly at or a few weeks after emergence (e.g. Heath *et al.*, 1999). In most cases these studies only examine egg size effects until emergence or up to the loss of egg size positive relationship with phenotypic trait, most generally body size. In rainbow trout *O. mykiss*, egg size correlation with juvenile growth lasted longer within families (up to 15 weeks) rather than across families (up to 9 weeks; Blanc, 2002). Here, I demonstrated that the positive relationship between egg size and *O. mykiss* body size disapeared within 8 to 9 weeks post emergence (unpublished data) and in turn became negative 4 months post emergence (chapter 4). In Arctic charr the positive relationship between egg size and body weight was maintained until 4 months after emergence (chapter 3). Taken together these results indicate that 1) in early development and up to emergence, egg size positively correlates with body size of salmonids; 2) an absence of positive correlation between egg size and body weight of juveniles does not necessarily indicate that egg size does not affect further the growth of

juveniles (see chapter 4 of this volume); and 3) the egg size – body size relationship may last longer within families than across families (Blanc, 2002). In other words, the importance of egg size for the development of phenotypic traits, growth and more generally the implications of egg size for fish diversity has clearly been neglected.

It is now well accepted that the developmental pathways that salmonids undergo to reach sucessful reproduction are determined by both proximate (environmental regulators) and ultimate mechanisms (genetically determined thresholds; Thorpe *et al.*, 1998). Smolting, i.e. changes in behavior, physiology and morphology of juveniles salmonids to adapt to salt-water, is one important aspect of life history strategy of salmonids. Smolting is thresholds dependent especially in terms of body size and growth; such thresholds are genetically determined and the state of the fish and rate of change at a particular time is determined by the environmental opportunities (Thorpe *et al.*, 1998). If the threshold is exceeded fish will undergo one life history strategy if not fish will undergo different life history strategy. In both species that I studied, egg size significantly affected first year growth of juveniles (chapters 3 and 4). First year growth pattern appears to be crucial in the expression of life history strategy e.g. migration to salt-water versus residualism (Sharpe *et al.*, 2007). However, the role of egg size in first year growth of salmonids and ultimately the influence of egg size on the determination of life history and ultimately reproduction strategy has so far been neglected. Here, I have observed significant effects of egg size on first year growth, on behavior and on osmo- regulatory status of the fish. Thus, it should be considered to include egg size in future models characterizing salmonid life – history variation (e.g. Thorpe *et al.*, 1998).

6.3 Egg size and domestication

Effects of domestication on salmonids have been of interest for both hatchery practices and restoration of wild populations using hatchery fish. Large reductions in relative fitness have been observed for hatchery salmonids compared to wild ones (Berejikian and Ford, 2004). Genetic effects of domestication considerably reduce reproductive capabilities of hatchery fish when released to natural environments, i.e. 40% decrease in fitness per generation raised in hatchery (Araki *et al.*, 2007). At the same time, egg size is known to be smaller in hatchery fish when compared to wild fish (this study and chapter 1; Einum and Fleming, 2000b). In both species I studied, hatchery eggs were smaller and more variable in size when compared to those of wild fish. This was seen in Arctic charr that had been four generations in a hatchery as well as in steelhead trout that were a first generation hatchery fish. Thus, it is clear that reduction in egg size happens rapidly as I see important decrease in egg size after only one generation in domestication. These findings support the hypothesis that females experiencing high growth rate as juveniles produce large number of small eggs as adults (Jonsson *et al.*, 1996; Lobón-Cerviá *et al.*, 1997; Morita *et al.*, 1999; Fleming *et al.*, 2000; Olsen and Vøllestad, 2003; reviewed by Einum *et al.*, 2004). My data shows that egg size is likely very plastic in salmonids and may be a mechanism allowing optimization of both maternal and offspring fitness.

6.4 The importance of egg size for the evolution of fishes

Foraging behavior of steelhead trout (chapter 4) and Arctic charr juveniles (chapters 2 and 3) coming from smaller and larger eggs within family differed in a similar way. Larger siblings coming from large eggs showed more foraging at the surface whereas small ones fed of the bottom. Recently, Sturlaugsdóttir (2008) reported similar divergence of behavior between juveniles coming from small and large eggs. These fish came from small benthic and pelagic morphs of Arctic charr from Thingvallavatn Iceland. Such a difference in foraging behavior, rising from egg size differences, might be important for habitat segregation and evolution of sympatric divergence. This may be especially true for polymorphic species such as Arctic charr and steelhead trout. Skúlason *et al.* (1999) hypothesized that plasticity in foraging behavior and mobility may trigger morphs segregation. Indeed variation in behavior, stemming from small size differences at first feeding, may influence habitat and food selection.

Egg size is an important and often-studied aspect of the life history of fishes (e.g. Stearns, 1992; Einum *et al.*, 2004), and much attention has focused on the ecological factors that drive changes in egg size (e.g. Smith and Fretwell, 1974; Einum and Fleming, 2000a; b; 2002; Einum *et al.*, 2004). However, few studies have explored how egg size affects developmental pathways of fishes (but see Balon, 1999; 2002). Considering invertebrates, interest in egg size evolution was spurred by the observation that egg size is strongly associated with developmental mode: species with small eggs (i.e. small amount of yolk) have planktonic larvae, disperse, feed on plankton, and then undergo metamorphosis, whereas species with large eggs (i.e. large amount of yolk) tend to have short-lived, non feeding larvae or have no larvae stage at all (reviewed by Moran and McAlister, 2009). These two extreme modes of development respectively refer to indirect and direct development. Similar developmental trajectories linked to egg size (i.e. yolk amount) have been described in fishes (Balon, 1999). In chapter 1, I showed that egg size was positively correlated with energy content i.e. yolk amount. Balon (2002) argues that fish from the Salmonidae family develop through a transitory ontogeny (i.e. intermediate mode of development), characterized by a free swimming and feeding embryo periods with first feeding embryos still having fin folds that differ from adult morphology. Following Balon's ideas and findings from the invertebrate literature, fish embryos coming from eggs that differ in size and in energy content may differ in developmental pathway ranging between the two extremes: indirect and direct development. Thus, because of less amount of yolk embryos coming from small eggs may have to develop feeding structures earlier and/or more rapidly than embryos coming from larger eggs because they have less yolk. In short egg size variation among and within species may reflect different ways of how embryos use the energy available in individual egg. Eiríksson *et al.* (1999) showed that small benthic Arctic charr had smaller eggs and its embryos directed more energy towards bone development when compared with planktivorous charr coming from larger eggs. More research of the connection between egg size and developmental trajectories is needed to understand better how egg size promotes fish diversity. The characterization of early bone development and its genetic mechanism in morphs of Arctic charr that differ in egg size is currently ongoing at the University of Iceland (Snorrason *et al.* in preparation). Egg size can change rapidly and considerably due to environmental factor(s) (e.g. chapter 4). Baker *et al.* (2011) reported rapid and extensive changes in egg size, and clutch size presumably connected to

lake productivty in an Alaskan sticklebacks population. Egg size variation may induce developmental, behavioral and morphological changes in phenotypes of offspring, creating rapidly intra specific diversity. Thus, egg size may play an important, and until now neglected, role in the evolution of morphs, different life history strategies and new species.

The model of diversification and speciation of fishes described by Smith and Skúlason (1996) starts with a monomorphic population and ends with two sympatric species (see also Skúlason and Smith, 1995; Snorrason and Skúlason, 2004). When new habitats become available (e.g. after the last glaciation) invading fish species were provided with a number of new unexploited resources to harvest. Foraging theory predicts that in such circumstances fish population should start harvesting the most profitable resource (Pyke *et al.*, 1977; Stephens and Krebs, 1986; Perry and Pianka, 1997). As the population grows and intra specific competition increases there will be strong selection for harvesting additional resources promoting the formation of resource morphs (Skúlason and Smith, 1995). This can be followed by strong selection against hybridization, especially if morphs show clear phenotypic adaptation towards the resource they harvest and hybrids show intermediated morphology (Snorrason and Skúlason, 2004). Most commonly such reproductive isolation would come in place through assortative mating behavior. Work on threespine sticklebacks *Gasterosteus aculeatus* L. has shown the importance of assortative mating based on size (Borland, 1986; Nagel and Schluter, 1998; Ólafsdóttir *et al.*, 2006) and diet (Snowberg and Bolnick, 2008) for reproductive isolation of morphs. In this model of divergent evolution, phenotypic plasticity is the primary mechanism creating early variation between individuals of the same species. But plasticity may not be the only mechanism that creates phenotypic variation at this stage. I have shown for the first time that intra specific diversity can arise from differences in egg size among individuals of a same population. I showed that egg size, when considered within and across family, created diversity in terms of foraging behavior, mobility and agonistic behavior. Such divergence in early behaviors may then be followed by changes in growth and morphology with important long- term life-history consequences (e.g. Thorpe *et al.*, 1992; Metcalfe, 1993), than can further effect morph segregation. This diversity might be further enhanced if there is clear interaction between maternal, environmental and genetic effects (chapter 2). Diversity caused by egg size can then be reinforced by different developmental trajectories and phenotypic plasticity. Therefore, egg size effect on phenotypic traits (growth, morphology and behavior) may promote early divergence of salmonids.

In conclusion, this research focused on laboratory experiments that revealed that egg size can create intra specific diversity. Egg size affected development, physiology, behavior and morphology of salmonids. Additionally, the long term effects of egg size on growth and apparently life history choices suggest that egg size triggers a cascade of events early in life with important consequences for later stages of development and life history strategy. Environmental effects, such as rearing juvenile fish in hatchery environment for one year before being released in the natural environment, appears to have dramatic consequences for egg size with important changes in growth, behavior and development of their progeny. This research yields important and new results concerning the role of egg size for development, growth and behavior of both Arctic charr and steelhead trout. My study shows that egg size is an important epigenetic factor promoting rapid diversity of fishes that can arise in one generation.

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