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Investigating fitness consequences of hybridisation between farmed and wild Atlantic salmon

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Investigating fitness consequences of hybridisation between farmed and wild Atlantic salmon

A thesis submitted for the degree of Doctor of Philosophy

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Abstract

Farmed fish display genetic differences from wild fish in a variety of morphological, behavioural and physiological traits as a result of the domestication process and selective breeding. Farmed salmon typically outgrow wild salmon by large ratios under hatchery conditions, although observed growth differences are much less in the wild. It is possible that farmed salmon have become adapted to regulated domestic environments, while concurrently they are unable to perform as well in more variable wild environments. Escaped farmed salmon interact with wild salmon through resource competition and disease transmission, and can interbreed with wild salmon. The introduction of mal-adapted domestic genotypes into wild populations can lower their productivity. Comparative studies that assess the effects of hybridisation on life-history traits linked to fitness are important in understanding how interbreeding will affect the resilience of wild populations. The present thesis investigated the freshwater growth and survival of multiple families derived from various farmed, wild and F1 hybrid salmon populations when reared at contrasting (1) temperatures, (2) densities and rearing conditions, (3) food availabilities, and (4) diets. In all experiments farmed salmon outgrew wild and hybrid salmon, and their hybrids displayed intermediate growth. Relative growth differences detected at contrasting temperatures were population-specific; indicating that the competitive balance between conspecifics may depend upon genetic background and river temperature. Findings highlight the merits of adopting a more spatially resolved approach to risk management of wild populations. In all other experiments the relative growth differences among groups did not differ across treatments, indicating that farmed fish have retained their plasticity in response to respective experimental treatments. Although experiments were conducted under controlled conditions, findings suggest that the investigated treatments are not individually responsible for elevated growth differences observed in hatchery conditions or the lower growth differences observed between farmed and wild salmon in the wild.

Contents

List of tables

List of figures

Chapter 1: General introduction

1.1 Aquaculture vs. fisheries

The world's population is set to rise to 8.9 billion by 2050 (United Nations: Department of Economic and Social Affairs 2004), and food production will need to increase accordingly. Fish and seafood products are recognised as an important and accessible source of protein, with fish making up 16.7% of all animal protein consumed in 2010 (FAO 2014b). In 2012, approximately 58 million people were engaged in capture fisheries or the aquaculture industry (FAO 2014b), therefore fish and seafood products are not only important as a food source, but also have substantial socio-economic significance (Tidwell & Allan 2001). Increasing demand for seafood coupled with innovative fishing technologies has placed escalating pressure on wild fish stocks in recent years (Tidwell & Allan 2001). In the most recent Food and Agricultural Organisation (FAO) assessment which was based on 2011 data, 28.8% of fish stocks were estimated to be overfished, 61.3% of fish stocks were fully fished, and 9.9% of fish stocks assessed were reported as under fished (FAO 2014b). Although in 2011 global capture fisheries attained the second highest yield recorded (93.7 million tonnes) to date, the overall trend of capture fisheries is still relatively constant (Figure 1.1) (FAO 2014b). Several studies depict a negative outlook for the future of capture fisheries (Pitcher 2001; Pauly 2009; Pitcher & Cheung 2013); although others suggest that some fisheries may recover (Worm & Branch 2012). In general, however, it is clear that the potential for capture fisheries alone to meet the growing demand for seafood is limited (Tidwell & Allan 2001).

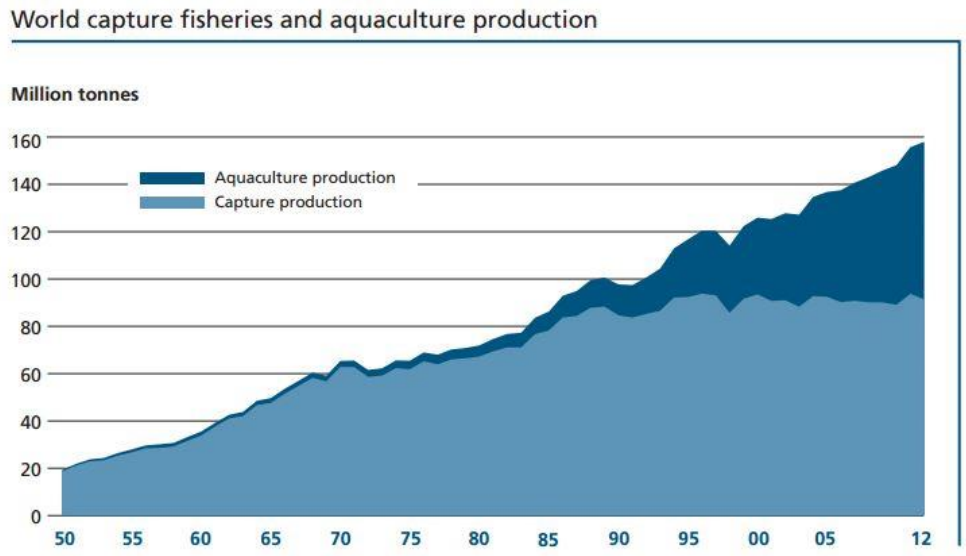

Figure 1.1: The production numbers (in million tonnes) for worldwide capture fisheries and aquaculture up to 2012 (Taken from FAO 2014b).

Aquaculture production continues to grow and expand worldwide, achieving an all-time high of over 100 million tonnes in 2014 (FAO 2016), and growing at an annual average rate of 6.2% over the last ten years (FAO 2014b). Aquaculture now supplies more than half of all fish products consumed worldwide (Naylor *et al.* 2009). While aquaculture is often touted as the solution to the stagnation facing capture fisheries, the industry is not without its unique challenges (Tidwell & Allan 2001; Soto *et al.* 2010). Several examples include the controversial use of large amounts of fish meal and fish oil in diets (Naylor *et al.* 2009; Merino *et al.* 2012), the organic waste and pollution arising from farming activities (Bannister *et al.* 2014), the spread of disease and parasites to wild conspecifics and the over-use of antibiotics (Thorstad *et al.* 2015; Chuah *et al.* 2016), and finally the ecological and genetic effects of escaped fish on wild fish populations (Jonsson & Jonsson 2006; Taranger *et al.* 2014), which is the focus of the present thesis.

In the early days of commercial carnivorous fish and shrimp farming, the industry was heavily reliant on obtaining essential dietary protein and fatty acids by including large amounts of fish meal and fish oil derived from marine sources (Davis & Arnold 2000; Torstensen *et al.* 2008). However, increasing aquafeed prices, fluctuating supply of marine protein and oil, and criticisms from the fisheries and conservation sectors have driven research in the aquaculture industry to seek other alternative sources of dietary proteins and oils, such as terrestrial plants (Kaushik *et al.* 2004; Torstensen *et al.* 2008), and even microalgae (Walker & Berlinsky 2011). There has since been a decrease in the percentage of fishmeal and fish oil included in aquaculture diets, although it is likely that low level usage of marine sources will continue for the foreseeable future (FAO 2014b). Large scale intensive fish farming has been identified as contributing to environmental pollution through the uncontrolled discharge of water or nutrient waste and habitat destruction (Eng *et al.* 1989), leading to conflict with other agricultural industries and other stakeholders over limited natural resources (Tidwell & Allan 2001). The development of commercial scale coastal aquaculture in Southeast Asia has created negative impacts on coastal ecosystems by increasing nutrient loads around fish cages and habitat destruction through the conversion of mangrove swamps to fish ponds (Eng *et al.* 1989). In an effort to mitigate further environmental damage, various new practises have been implemented. For example, new shrimp farms now retain mangrove buffer zones or encourage replanting efforts (Tidwell &

Allan 2001). Similarly, the use of integrated multi-trophic aquaculture (IMTA), where finfish are grown in close proximity to inorganic and organic extractive aquaculture species (seaweeds and mussels), is being increasingly explored as a means of recycling the waste nutrients from high trophic level species (Troell *et al.* 2009). High densities of potential hosts within farms have led to an increase in parasite and disease incidences in aquaculture, which may impact wild fish that share the same local water bodies (Middlemas *et al.* 2013; Madhun *et al.* 2014). For example, introduced farmed Atlantic salmon from Sweden unintentionally spread the parasite *Gyrodactylus salaris* into some Norwegian rivers, causing the collapse of wild salmon in these populations (Laikre *et al.* 2010). The salmon industry faces an on-going challenge to control and reduce the number of sea lice (*Lepeophtheirus salmonis* K.) on fish within farms, and it is likely that lice are being transmitted from farmed salmon to wild salmonids in the area, with detrimental effects on wild populations (Heuch *et al.* 2005; Middlemas *et al.* 2013). The use of antibiotics as treatments for a variety of fish ailments has largely begun to decrease due to better health management and the development of vaccines for certain diseases (Tidwell & Allan 2001), although this may not be the case in developing countries (Holmström *et al.* 2003; Cabello 2006). Finally, escaped farmed fish may negatively affect local conspecifics through competition for resources, disease transfer and through interbreeding and introgression of mal-adapted "hatchery genes" into wild populations (Naylor *et al.* 2000). Several studies highlight the negative effects of interbreeding between farmed and wild conspecifics (Fleming *et al.* 2000; McGinnity *et al.* 1997; 2003; Arechavala-Lopez *et al.* 2011; Skaala *et al.* 2012), and specific examples will be discussed further below.

Commercial aquaculture is a relatively young industry; while stocking and subsistence fish farming can be traced back to circa 1000 BC in China, the latter half of the 20th century was the beginning of the peak of modern aquaculture, when technological advances allowed for large-scale operations to become profitable (Nash 2011). New technologies and a focus on sustainability and research have led to various improvements within the industry (Tidwell & Allan 2001). Therefore, while the aquaculture industry faces various concerns from conservationists and environmentalists, there is an understanding that sustainability is vital for the future success of the industry. Many countries and industries are, for example, developing aquaculture certification schemes to promote best management practises in a quest to encourage consumer and market acceptance (FAO 2014b).

Aquaculture and fisheries are interrelated subsectors, with frequent interactions due to the sharing of a common environment or resources, and competition for their products worldwide (Naylor *et al.* 2000; Soto *et al.* 2010). In order to continue to supply a source of high quality protein to an ever-increasing world population, both industries must continue to develop sustainably, while ensuring that the negative effects of both can be minimised.

1.2 Aquatrace: the development of tools for tracing and evaluating the genetic impact of fish from aquaculture

Aquaculture is currently the fastest growing food sector in the world (FAO 2014b); although this growth is spatially discontinuous. Developing countries are enjoying large increases in aquaculture production, while developed areas like the USA and Europe are experiencing a decline in production and rely largely on imports (FAO 2014b). The European Common Fisheries Policy is designed to ensure that fisheries and aquaculture industries are economically, socially and environmentally sustainable (European Commission 2015), which includes the requirement to conserve the integrity of exploited fish populations and their natural environment. In 2002 the European Commission developed the Strategy for the Sustainable Development of European Aquaculture in order to promote the growth of the European aquaculture sector within a sustainable context, and they identified farmed escapees as an area that needed addressing within the environmental challenges facing aquaculture development (European Commission 2002). The European Commission is dedicated to boosting European aquaculture, and in 2009 they released a new impetus for the sustainable development of EU aquaculture, which encourages aquaculture development and highlights the key areas of focus (European Commission 2009).

In 2013, the EU approved a 7th Framework project titled Aquatrace (Grant agreement no: 311920) which funded the present thesis. With 22 partner institutions across Europe working together on 13 work packages, the overall aim of Aquatrace is the development of tools for tracing and evaluating the genetic impact of fish from aquaculture (Aquatrace Consortium 2016). Aquatrace will support the development of sustainable European aquaculture and provide recommendations of good environmental status in line with the Marine Strategy Framework Directive (Marine Strategy Framework Directive 2008).

In order to understand the potential impact of domesticated fish on wild populations, information regarding the level of hybridisation and introgression across spatial and temporal scales and the effects of hybridisation or introgression on wild population fitness is needed.

Discrimination between farmed and wild conspecifics is complicated due to there being several strains of farmed fish with different histories of selection and variable levels of documentation regarding domestication (De Innocentiis *et al.* 2004). Aquatrace partners aim to carry out extensive sampling in combination with the use of genomic technology in order to assess the levels of introgression/hybridisation for three commercially important Mediterranean aquaculture species: sea bass (*Dicentrarchus labrax* L.), sea bream (*S. auratus* L.) and turbot (*S. maximus* L.). In order to elucidate the effects of introgression/hybridisation on wild population fitness, a wider understanding of the genomic structure of the target species is needed. Experiments which assess the fitness consequences by employing genomic tools to assess impacts of interbreeding between wild and farmed conspecifics are largely lacking for the three species named above (but see Karaiskou *et al.* 2009; Loukovitis *et al.* 2012), due to inadequate genomic resources and knowledge of functional genomics. However, `model species`are available, such as Atlantic salmon (for which extensive comparative genetic studies investigating the consequences of interbreeding have taken place) for undertaking such work (for example Bekkevold *et al.* 2006)**.** Therefore, experiments which use Atlantic salmon (*Salmo salar* L.) and brown trout (*Salmo trutta* L.) will enable us to further understand the effects of escapees, and how to focus future research in other aquaculture species. The present thesis utilises Atlantic salmon within a common garden design to investigate how farmed, wild, and F1 hybrid salmon families perform under varied environmental conditions. While there are numerous studies addressing fitness consequences of genetic interactions between wild and farmed fish (McGinnity *et al.* 2009; Houde *et al.* 2010a; Besnier *et al.* 2015), our studies are among the first to involve a larger number of farmed and wild populations and to investigate fitness in these specific environmental conditions. Such studies may inform and support further research and management options within the aquaculture industry.

1.3 Atlantic salmon as a model species

1.3.1 Population structure & local adaptation

Atlantic salmon (*Salmo salar,* Linnaeus (1758)) are iteroparous fish which are native to rivers on the east and west coasts of the Atlantic Ocean in the Northern hemisphere (Klemetsen *et al.* 2003). They typically display an anadromous life cycle (Figure 1.2), although some populations spend their entire life cycle in freshwater. Spawning occurs in rivers and streams in the autumn and winter months, with hatching taking place in the spring (Thorstad *et al.* 2011). Juveniles remain in fresh water after hatching as free-swimming parr

for a variable number of years before the smoltification process begins, whereby they become adapted to saltwater and begin their migration to the sea to feed and mature (Thorstad *et al.* 2011). After a variable length of time at sea Atlantic salmon returns to freshwater to spawn (Thorstad *et al.* 2011). The typically fragmented nature of wild Atlantic salmon freshwater habitats allows for the development of genetically divergent and reproductively isolated populations on a local scale (Taylor 1991; Carvalho 1993; Verspoor 1997; Garcia de Leaniz *et al.* 2007). Their population structure is further maintained by their strong behavioural instinct to home to, and reproduce in, their natal rivers (Taylor 1991; Verspoor 1997; Thorstad *et al.* 2011). The scale of population structure can vary spatially from large distances to nearby habitats in the same river system, and temporally as populations which spawn at different times in the same stream may exhibit genetic differences between them (Taylor 1991; Verspoor 1997).

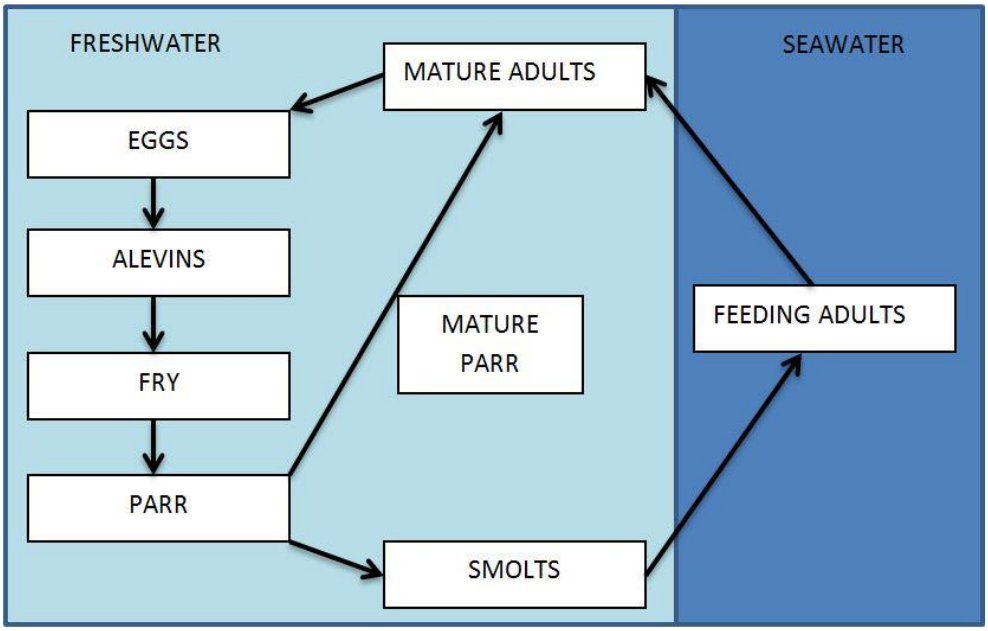

Figure 1.2: Simplified life cycle of anadromous Atlantic salmon (adapted from Solberg 2013).

Within and between populations, Atlantic salmon display significant heritable variation for many life history, behavioural and morphological traits (Garcia de Leaniz *et al.* 2007; Fraser *et al.* 2011), which are potentially associated with local adaptation. Local adaptation occurs when natural selection acts within a population to increase the occurrence of those traits that promote an individual's fitness through greater survival or reproductive success (Taylor 1991; Carvalho 1993). Therefore local individuals may have a fitness advantage in their native environment over non-local individuals (Fraser *et al.* 2011). The development and maintenance of locally adapted populations takes place through the

mechanisms of restricted gene flow and natural selection, and can occur over a relatively short time scale (Carvalho 1993; Verspoor 1997). The criteria for local adaptation (as described in Fraser *et al.* 2011) are: (i) the population must demonstrate different fitness across different environments; (ii) the population must have higher fitness in its local environment than a foreign population in the same environment; and (iii) the fitness differences among populations must be heritable and a result of selection and not genetic drift. However the extent of local adaptation in salmonids is a contentious issue (Adkison 1995). Salmonids are known to exhibit many traits which are only partially heritable and primarily influenced by the environment, therefore even genetically similar populations may exhibit different locally adaptive morphological or behavioural characteristics in different environments (known as phenotypic plasticity) (Adkison 1995). Phenotypic plasticity is the ability of an organism's genotype to change the expression of its phenotype along an environmental gradient (Bradshaw 1965). Adaptive variation (phenotypic plasticity) has long been recognised as important for fitness within and among salmon populations (Taylor 1991); whether this variation has a genetic basis is vital to determining whether it represents local adaptation.

Most evidence for local adaptation in salmonids is indirect and unequivocally documenting it represents a challenge (Garcia de Leaniz *et al.* 2007; Fraser *et al.* 2011). There are numerous experimental approaches which can be used to investigate adaptation among populations, although they vary in the amount of information they yield and their applicability in uncovering local adaptations (Garcia de Leaniz *et al.* 2007; Fraser *et al.* 2011). The most promising designs are reciprocal transplants, translocation studies, and common garden experiments, all of which can be used to investigate local adaptations among populations (Fraser *et al.* 2011). The recent advances in quantitative genetics and genomic techniques allow for the detection of selection on specific traits (*i.e.* quantitative trait loci (QTL) studies) at the molecular level (Garcia de Leaniz *et al.* 2007), and for identifying molecular level variation among populations. Despite the challenges of yielding evidence for local adaptation, examples in wild salmon populations include adaptive variation for traits related to life history, morphology, physiology, behaviour, and disease resistance (see reviews by Taylor 1991; Verspoor 1997; Garcia de Leaniz *et al.* 2007; Fraser *et al.* 2011). For example, O'Toole *et al* (2015) investigated the fitness of offspring from local and foreign salmon and their reciprocal F1 hybrid crosses reared communally in the wild native environment of the local population. Overall lifetime success of the foreign and hybrid

populations relative to the local population were found to be 31% and 40% respectively, indicating a genetic basis for fitness among populations. They were thus able to demonstrate a higher fitness of the local population over the foreign population, indicative of local adaptation; although it was acknowledged that the study did not constitute a full reciprocal transplant (O'Toole *et al.* 2015).

Understanding the level of local adaptation and adaptability in general within salmon populations is of practical importance to conservationists and fisheries scientists. Bio-complexity, or the portfolio effect, is a concept in fisheries management whereby the conservation of subpopulations which exhibit diverse life history characteristics or local adaptation is encouraged in order to ensure the resilience of the species as a whole (Hilborn *et al.* 2003). Local adaptation and the adaptive potential of wild populations are increasingly important concepts due to anthropogenic environmental changes, including climate change (Jensen *et al.* 2008; McGinnity *et al.* 2009). It is thought that genetic or biological diversity may act as a buffer to environmental change, and so enhance the long-term sustainability of a population (Schindler *et al.* 2010).

1.3.2 Domestication of Atlantic salmon for commercial production

The commercial farming of Atlantic salmon began in Norway towards the end of the 1960s (Gjedrem *et al.* 1991). Initially, broodstock were gathered from 40 rivers across Norway to ensure high genetic diversity for the subsequent selection of optimal strains for domestication (Gjedrem *et al.* 1991). Selection was primarily focused on high potential growth rates and late maturation (Gjedrem *et al.* 1991). Today, commercial Atlantic salmon farming is practised worldwide, with the main farming activities in Norway, Scotland, Ireland, Canada and Chile. Salmon breeding programs have expanded to include direct selection for a number of traits including growth, late maturation (to ensure that energy for flesh growth is not wasted on gonad development), disease resistance, flesh colour, and body composition (Gjøen & Bentsen 1997). The genetic gain on growth rate from direct selection through these breeding programs has been estimated at 10 to 15% per generation (Gjedrem 2000). In addition to changes in the traits directly targeted by selection, farmed salmon also exhibit changes in various behavioural traits which are not directly selected for, such as decreased stress at high densities and increased boldness or aggression (Fleming *et al.* 1997; Houde *et al.* 2010b; Bourret *et al.* 2011). Such changes may occur as a result of the indirect responses to artificial selection and the relaxed natural selection within the domestic environment (Ruzzante 1994; Weber & Fausch 2003). Similarly, the low mortality associated

with domestic environments may result in phenotypes persisting in captive environments that would not survive in the natural environment (Huntingford 2004). Genetic drift may also cause random and rapid changes in allele frequencies in domestic populations with limited effective population sizes (Mignon-Grasteau *et al.* 2005).

The early Atlantic salmon breeding programs sought to maximise the available genetic diversity within breeding populations by utilising brood fish from multiple wild populations, however reduced genetic variation has since been documented in neutral genetic markers (Clifford *et al.* 1998; Norris *et al.* 1999; Skaala *et al.* 2004; Karlsson *et al.* 2011). Reduced genetic variation is most likely the result of genetic drift and founder effects (Gjedrem *et al.* 1991), and that the breeding program has since been closed to new individuals for over ten generations (Gjøen & Bentsen 1997). Farmed salmon are thought to exhibit their own form of local adaptation, in that generations of domestic selection for various commercially important traits has led to an increase in so-called 'hatchery alleles' – those that are specifically associated with the domestic rearing environment. In most circumstances, such hatchery reared individuals may be mal-adapted to wild conditions, and may have a reduced performance in nature (Stringwell *et al.* 2014).

1.4 Wild and farmed interactions within fish

In the most recent ICES assessment of wild salmon stocks it was estimated that the total nominal catch of wild Atlantic salmon in the North Atlantic was 1285t in 2015 (ICES 2016). In stark contrast, the provisional production of farmed Atlantic salmon in the North Atlantic area for 2015 was estimated at around 1648 000t and the worldwide production of farmed Atlantic salmon is estimated to be 1900 times the reported nominal catch of wild Atlantic salmon (ICES 2016). Wild salmon are in decline in most of their native ranges; this has primarily been attributed to low marine survival, freshwater habitat destruction, climate change, marine predation and ecological and genetic interactions with farmed conspecifics (Parrish *et al.* 1998; ICES 2016).

Globally, large numbers of farmed Atlantic salmon escape from sea or freshwater net pens each year. For example, in 2014 the Norwegian Fisheries Directorate reported that 271, 000 Atlantic salmon escaped from commercial farms throughout Norway; in 2014, the Scottish Government received reports of over 184, 500 escapees, while in Ireland, 230. 000 salmon escaped during a single event (Norwegian Fish Directorate 2014; The Scottish Government 2014; The Fishsite 2014). The salmon farming industry in Europe and Canada

have high levels of accountability for escape events, including mandatory reporting of escapees (Taranger *et al.* 2014). Researchers caution, however, that published numbers could be an under-estimation of escapees due to unreported escape events (Glover 2010; Skilbrei *et al.* 2014). In the Mediterranean, the scale of escape events of other marine aquaculture species is largely unknown as mandatory reporting is not yet required (Arechavala-Lopez *et al.* 2013). Although there are no official statistics available on the number of escapes in the Mediterranean, Jackson *et al* (2015) used data from fish farm insurance companies to imply the level of escape events. They found that from 2001 to 2005, 36% of the total value of all insurance claims by Greek fish farmers was due to stock losses resulting from storms (Jackson *et al.* 2015), indicating that escape events are also a common occurrence in the Mediterranean. In most cases, escapes occur due to adverse weather resulting in structural damage, or due to operational or mechanical failures resulting from, for example, collisions with boats (Jensen *et al.* 2010). There are also biological causes of escapes, which include net damage due to predation or biting and, in broadcast spawning species, through the release of fertilised eggs into the surrounding water column (Jørstad *et al.* 2008; Somarakis *et al.* 2013). Jackson *et al* (2015) compiled 3 years of data (2007-2009) relating to escape incidents within 6 countries in Europe (Norway, Ireland, Scotland (UK), Spain, Greece, and Malta). Almost 9 million fish escaped from 242 incidents, with an estimated total income loss of €47.5 million per annum (Jackson *et al.* 2015). Escape events can occur as large-scale, acute events such as whole cage losses, or through chronic leakage of few individuals during harvesting or maintenance (Baskett *et al.* 2013). Escapes can occur at all life stages, and such age-specific variation may also influence the impacts of escapes on wild conspecifics (Baskett *et al.* 2013). Target life history stage differs depending on the production practises of the country in which it is farmed. For example in Norway salmon mostly escape at the adult sea water stage as most smolts are produced in land-based tanks, whereas in other countries, such as Scotland, the fish may escape from freshwater loch cages as juveniles too (ICES 2016). It is important to note that interactions between domesticated and wild conspecifics are not limited to farm escapes, and can occur when hatchery fish are intentionally released through stocking (Baskett & Waples 2012), however for the purposes of this thesis the focus will be on accidental escapes of domesticated salmon from aquaculture facilities.

1.4.1 Farm vs. wild phenotypes

Farmed salmon display significant differences to wild salmon for a range of fitness related traits due to domestication resulting from direct selection, indirect selection, founder

effects and genetic drift, and origin-based differences (Huntingford 2004). Growth differences between farmed and wild salmon are probably the most documented trait differences, and growth has been used as a proxy for fitness in various comparative studies (Besnier *et al.* 2015; Reed *et al.* 2015). Several studies have shown large differences in growth between farmed and wild salmon (Glover *et al.* 2009; Solberg *et al.* 2013a; 2015) with as much as a 5-fold difference found under hatchery conditions (Solberg *et al.* 2013b) although growth differences are typically much lower under natural conditions (Fleming *et al.* 2000; Skaala *et al.* 2012). Selection has probably resulted in changes to the endocrine system controlling growth, as studies have shown higher levels of growth hormone (GH) (Fleming *et al.* 2002) and insulin-like growth factor I (IGF-I) (Solberg *et al.* 2012; but see Bicskei *et al.* 2014) in farmed compared to wild salmon. Growth hormone is not only important for growth, but may also influence a variety of behavioural traits in salmonids, including appetite, feed conversion efficiency, foraging behaviour, aggression and metabolism (Björnsson 1997; Neregård *et al.* 2008a; 2008b). Several studies have highlighted differences in the aforementioned behavioural traits among farmed and wild conspecifics (Fleming & Einum 1997; Thodesen *et al.* 1999), indicating that selection has influenced farmed salmon endocrinology. Studies show that farmed salmon are more risk-prone (Fleming & Einum 1997; Einum & Fleming 1997; although see Solberg *et al.* 2015) and display lowered anti-predator responses than their wild counterparts (Houde *et al.* 2010b). Farmed salmon and their hybrids also appear less responsive to predator (Debes & Hutchings 2014) and environmental stress than wild conspecifics (Solberg *et al.* 2013a). Several differences have also been found at the molecular level, including differences in allelic diversity and heterozygosity (Norris *et al.* 1999; Skaala *et al.* 2004; 2005), and gene transcription profiles associated with immunity and metabolic pathways (Roberge *et al.* 2008; Debes *et al.* 2012; Bicskei *et al.* 2014; 2016). Along with the morphological and behavioural changes observed in farmed fish relative to wild conspecifics, farmed fish often exhibit lower survival and reproductive success in the wild (Fleming *et al.* 2000; McGinnity *et al.* 2004).

Farmed salmon males often display divergent spawning success and behaviour compared to wild males, resulting in farmed males being less reproductively successful than their wild counterparts (Fleming *et al.* 2000; Weir *et al.* 2004). It has thus been suggested that the principle route of gene flow would be wild males mating with farmed females (Fleming *et al.* 2000). Interestingly, it was found that mature male parr originating from farms had a higher reproductive success than both wild and hybrid mature male parr, therefore this could

also be an important alternative route through which farmed genes enter wild populations (Garant *et al.* 2003). The gamete breeding potential of farmed relative to wild salmon was investigated using *in vitro* comparisons of their sperm and eggs and no differences in function were found between the two groups (Yeates *et al.* 2014). It is possible that an adjustment time before spawning could improve the escaped salmon's reproductive behaviour, resulting in successful fertilisation between wild and farmed salmon (Yeates *et al.* 2014). The resulting hybridisation will yield offspring which may have sub-optimal phenotypes in the wild and may potentially disrupt local adaptation within wild populations (Randi 2008).

Intermediate F1 hybrid growth has been documented in comparative studies of Atlantic salmon in hatchery (Glover *et al.* 2009; Fraser *et al.* 2010), semi-natural (Solberg *et al.* 2013b), and wild conditions (Einum & Fleming 1997; McGinnity *et al.* 2003). Hybrid vigour was detected in F1 Atlantic salmon hybrids able to dominant their parental strains in pairwise contests (Einum & Fleming 1997). Outbreeding depression has been found for egg survival in F2 Atlantic salmon hybrids under natural conditions (McGinnity *et al.* 2003). Hybrid vigour or heterosis is when hybrids perform better relative to their parents while outbreeding depression is when hybrids display reduced fitness due to the breakdown of co-adapted gene complexes, respectively. First generation hybrids commonly display either intermediate trait values or hybrid vigour, while backcrosses and F2 hybrids exhibit outbreeding depression (McGinnity *et al.* 2003); therefore studies which utilise backcrosses or F2 generation hybrids are vital to understanding how introgression with farmed salmon will affect a wild population.

1.4.2 Implications of interbreeding

While most escaped fish fail to survive or recruit, successful spawning between farmed and wild conspecifics has been documented in Canadian (Carr *et al.* 1997), Irish (Crozier 1993; Clifford *et al.* 1998), and Norwegian rivers (Lura & Saegrov 1991; Saegrov *et al.* 1997). Significant genetic changes have been found in wild Atlantic salmon populations from rivers in Canada (Bourret *et al.* 2011), Norway (Skaala *et al.* 2006; Glover *et al.* 2012; 2013) and Ireland (Clifford *et al.* 1998), driven by gene flow from escaped farmed salmon. The estimated cumulative introgression levels in 20 wild populations of Norwegian Atlantic salmon were as high as 47% in some populations while others were not significantly affected, with some of the inter-population variation being attributed to wild population densities (Glover *et al.* 2013). The level of gene flow from farmed fish will depend on the size and frequency of the escape events, their successful reproduction with wild fish, and the

subsequent survival of their offspring (Verspoor 1997). The size of the wild population and the degree of mal-adaptation of the farmed fish will also affect the fitness consequences of interbreeding between farmed and wild fish (Baskett *et al.* 2013). If wild salmon populations are locally adapted to their native conditions, the loss of genetic diversity through introgression or hybridisation with mal-adapted invading conspecifics would result in an overall loss of population productivity and viability (Adkison 1995). Despite the reduced growth differences observed in the wild, the higher growth rates of farmed salmon may provide a competitive and survival advantage compared to wild salmon. Farmed Atlantic salmon juveniles are able to displace (McGinnity *et al.* 2003) wild salmon under certain environmental conditions.

Genetic interactions are not the only consequence of escapees. Ecological interactions between wild and farmed fish could potentially influence population fitness, with escaped farmed salmon competing with wild salmon for resources and spreading pathogens (Fleming *et al.* 2000; Naylor *et al.* 2005; Madhun *et al.* 2014). Aquaculture pens containing high numbers of salmon may be sources of sea lice (*Lepeophtheirus salmonis* K), an ecto-parasite which has been linked to negative effects on wild Atlantic salmon and sea trout (*Salmo trutta* L.) populations (Krkošek *et al.* 2012; Skilbrei *et al.* 2013). Large numbers of farmed fish escaping into rivers with vulnerable wild populations could influence population density and density dependent factors such as competition, predation, and resource availability. As mentioned previously, studies show that farmed fish are able to displace wild fish under certain conditions (McGinnity *et al.* 1997), and the higher growth exhibited by the offspring of farmed fish may be advantageous in competitive situations (Skaala *et al.* 2012). For example, it was found that the productivity of a wild Norwegian Atlantic salmon population was decreased by 30% when artificially invaded by a farmed salmon population (Fleming *et al.* 2000). Therefore, ecological interactions could have serious implications for the viability of wild salmon populations through the decrease in wild smolt productivity and displacement of potential wild spawners (Fleming *et al.* 2000; McGinnity *et al.* 2003; Skaala *et al.* 2012).

Atlantic salmon is the most studied species regarding farm-wild interactions, however, there are few studies which investigate the genetic and ecological interactions between farmed and wild Mediterranean aquaculture species (see review by Arechavala-Lopez *et al.* 2013; Grigorakis & Rigos 2011). For example, Karaiskou *et al* (2009) investigated the genetic diversity of Greek farmed and wild sea bream using microsatellite markers. They found significant population differentiation between farmed and wild

conspecifics and lower allelic richness in the farmed population (Karaiskou *et al.* 2009). Similarly, Loukovitis *et al* (2012) found reduced allelic diversity within farmed relative to wild populations of sea bream within the Mediterranean. Investigations of the post-escape behaviour of farmed sea bass and sea bream revealed the potential for resource competition and spread of disease as an overlap was found in diet and habitat use among farmed and wild conspecifics (Arechavala-Lopez *et al.* 2012; 2014). Therefore, studies of farm-wild interactions in salmonids are also important for understanding the potential effects of escapes in other aquaculture species. Similarly, studies which explore the effects of domestication on fitness-related traits may shed some light on how divergence in these traits may affect hybridisation between farmed and wild conspecifics. Farmed fish may be able to maximise their growth under hatchery conditions due to adaptation to the regulated rearing conditions of the domestic environment. Similarly, it is possible that the growth and behavioural differences discussed above and differences in food availability and composition between the hatchery and the wild environment may interact to produce the reduced growth of the offspring of farmed fish observed in the wild.

1.5 Using reaction norms and common garden studies to quantify hybridisation and introgression

The biological consequences of hybridisation and introgression can be investigated by examining differences in functional traits between farmed, hybrid and wild populations. Most functional traits are the product of more than one gene, known as polygenic effects, making it more difficult to determine the process behind any changes in population traits. Growth is a key life-history trait in salmon and is strongly related to fitness (Jonsson & Jonsson 2006; Garcia de Leaniz *et al.* 2007). Other important life-history traits influencing fitness in salmonids are survival, age and size at maturity, and fecundity (Hutchings 2004).

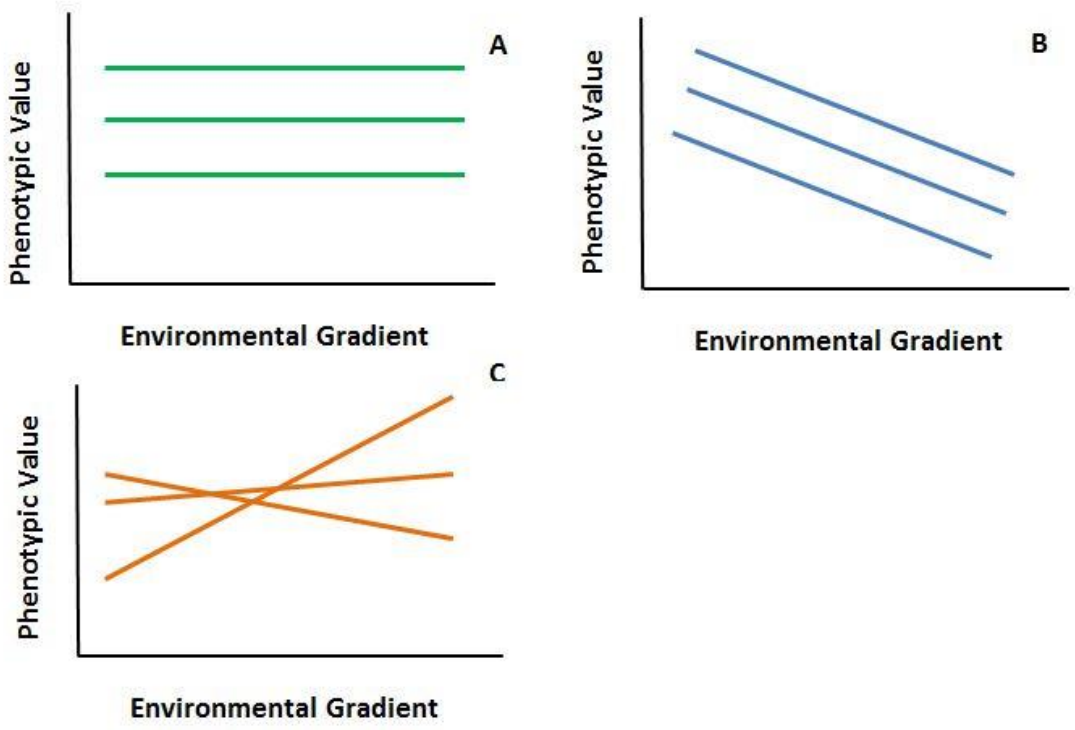

Figure 1.3: Reaction norms graphically describe the variation within a trait along an environmental gradient. Each line represents how a genotype changes its phenotype value over a changing environment. Figure A represents a lack of variation within a trait for each genotype. The phenotypic value of the trait does not change along an environmental gradient. Figure B represents a change in trait value with the environment; however there is no indication of G x E (genotype by environment) interactions between the three genotypes as the slopes are identical. Figure C represents both variations in the trait and G x E interactions. The three genotypes converge to similar phenotypes in the middle of the gradient, but diverge to different values at the extremes of the gradient. (Adapted from Hutchings 2004; Garcia de Leaniz *et al.* 2007).

As mentioned previously, phenotypic plasticity is the generation of different phenotypes from a single genotype along an environmental gradient (Bradshaw 1965), and may be a result of selection but does not necessarily represent genetic change (Hutchings 2004). Reaction norms are graphical measures of the scope of trait variation (phenotypic plasticity) and they visualise how a phenotype changes along an environmental gradient (Figure 1.3) (Hutchings 2004; Ghalambor *et al.* 2007; Hutchings 2011). Studying reaction norms allows one to examine trait variation among populations and can be used to investigate how selection, including artificial selection influences response to environmental change (Hutchings 2004). Reaction norms convey information about the size of trait plasticity, whether there are genotype by environment (G x E) interactions, and how the additive genetic variance of the trait changes as the environment changes (Hutchings 2004). The slope of a

reaction norm represents the amount of variation within a trait (phenotypic plasticity), thus steeper slopes reflect a highly plastic trait (Hutchings 2004). A change in the reaction norm within a population subject to different environments could represent a change in local adaption or the fitness of a population (Darwish & Hutchings 2009). Crossing reaction norms (as in Figure 1.3C) indicate the presence of a G x E interaction, which in turn suggests that genetic variation is present between the study units (Hutchings 2004). Various studies have used comparisons of reaction norms between farmed, hybrid and wild salmon populations to look for evidence of trait variation between groups (Fraser *et al.* 2008; Morris *et al.* 2011; Solberg *et al.* 2013a). Reaction norm studies conducted using common garden designs allow for the environmental variation of any phenotypic variation to be controlled for, meaning that any subsequent variation is implied to have a genetic basis.

Common garden studies involve rearing fish from all origins in communal tanks, ensuring that all fish are exposed to the same environment. Molecular markers such as microsatellites (Hansen *et al.* 2001; Skaala *et al.* 2004; Manel *et al.* 2005), or single nucleotide polymorphisms (SNPs) (Liu & Cordes 2004; Helyar *et al.* 2011) can be used in combination with varied statistical approaches and software programs to assign fish within communal tanks back to their family of origin. In a closed system, such as that used in the subsequent chapters, all parental genotypes involved in the experimental cohort are able to be genotyped. The performance of parental assignment methods are influenced by the number of potential parent pairs and the information contained within the genotypes, with assignment performance decreasing with the former and increasing with the latter (Liu 2007). Performance can also be influenced by the relatedness and sexing of the parents, and the accuracy of genotype scoring (Liu 2007). Co-dominant markers like microsatellites are the preferred choice for parentage assignment (Liu 2007). The two main statistical approaches on which most software are based are the exclusion principle and maximum likelihood theory (Freeland 2005). Exclusion-based parental assignment is the simplest, and involves discounting all parents whose genotypes do not match the potential offspring's genotype until a single matching parental set remains (Freeland 2005; Liu 2007). Maximum likelihood methods involve calculating likelihood ratios (or probabilities) for each possible parental set based on the expected degree of allele sharing between parents and offspring and the frequency of these alleles in the population (Freeland 2005). Likelihood ratios are calculated for each locus and the overall likelihood that a given individual is the parent is obtained by multiplying all the likelihoods across loci (Freeland 2005). The present thesis used

microsatellites and the Family Analysis Program (FAP), a program which uses exclusion-based probabilities to assign offspring back to their parents (Taggart 2007). This program has been used in several comparative studies to assign offspring back to their parents (Ferguson *et al.* 1995; McGinnity *et al.* 2003; Glover *et al.* 2004). For information about the microsatellite multiplexes used in the present study please see Table S1.1 in Appendix 1.

1.6 Aims and objectives of this thesis

The present thesis aims to improve the current knowledge of the genetic impacts of aquaculture on native salmon populations by investigating trait variation among wild, farmed, and first generation (F1) hybrid salmon for key life-history traits, specifically growth and survival across several environmental gradients. Findings have the potential to contribute towards the conservation and management of wild salmon populations and to improve and promote sustainable aquaculture practises. Specifically, by examining how phenotypic growth reaction norms of wild, farmed and F1 hybrid salmon respond to varied environmental parameters this thesis aims to:

1. Quantify whether domestication selection has resulted in a reduced range of tolerance for extreme temperatures by examining differences in the growth reaction norms for families of farmed, wild, and F1 hybrid salmon across a range of temperatures in a common garden hatchery setting (Chapter 2/Paper I: Harvey AC, Glover KA, Taylor MI, Creer S, Carvalho GR (2016) A common garden design reveals population-specific variability in potential impacts of hybridization between populations of farmed and wild Atlantic salmon, *Salmo salar* L. *Evolutionary Applications*, 1-15. doi:10.1111/eva.12346).
2. Quantify whether domestication selection has resulted in a change in the reaction norms for survival and growth between farmed, wild, and F1 hybrid salmon across a range of contrasting densities and along an environmental gradient ranging from hatchery conditions to a semi-natural environment (Chapter 3/Paper II: Harvey AC, Juleff G, Carvalho GR, Taylor MI, Solberg MF, Creer S, Dyrhovden L, Matre IH, Glover KA. 2016. Does density influence relative growth performance of farmed, wild, and F1 hybrid Atlantic salmon in semi-natural and hatchery common garden conditions? *Royal Society Open Science*. doi: 10.1098/rsos.160152).
3. Quantify whether domestication selection has resulted in a reduced tolerance of variable feed availability by examining differences in the survival and growth reaction

norms for farmed, wild, and F1 hybrid salmon reared separately (Chapter 4/Paper III: Harvey AC, Solberg MF, Glover KA, Taylor MI, Creer S, & Carvalho GR. 2016. Plasticity in response to feed availability - does feeding regime influence the relative growth performance of domesticated, wild and hybrid Atlantic salmon Salmo salar parr? *Journal of Fish Biology*. doi: 10.1111/jfb.13076)

4. Quantify whether domestication selection has resulted in farmed salmon becoming adapted to the nutritional profile and physical shape of a commercial salmon diet by examining differences in the survival and growth reaction norms for families of farmed, wild, and F1 hybrid salmon fed contrasting diets in a common garden hatchery setting (Chapter 5/Paper IV: Harvey AC, Solberg MF, Troianou E, Carvalho GR, Taylor MI, Creer S, Dyrhovden L, Matre IH, Glover KA. Growth reaction norms of farmed, hybrid and wild Atlantic salmon: has domestication led to genetic divergence in diet preference? *BMC Evolutionary Biology* - submitted).

1.7 Summary of papers/chapters

1.7.1 Chapter 2: A common garden design reveals population-specific variability in potential impacts of hybridisation between populations of farmed and wild Atlantic salmon, *Salmo salar* L.

Paper I: Harvey AC, Glover KA, Taylor MI, Creer S, Carvalho GR (2016) A common garden design reveals population-specific variability in potential impacts of hybridization between populations of farmed and wild Atlantic salmon, Salmo salar L. Evolutionary Applications, 1-15. doi:10.1111/eva.12346.

The relative freshwater growth of 9 populations comprised of 35 families of farmed (2 populations), wild (5 populations), and F1 hybrid (2 populations) salmon was investigated at three contrasting temperatures: 7°C (low treatment), 12°C (control), and 16°C (high treatment). On average, farmed fish from both populations outgrew wild and hybrid salmon, and the hybrid populations displayed intermediate growth. A significant temperature-by-population effect was found, indicating that the growth differences were population-specific, where some wild populations performed better than others relative to hybrid and farmed populations at certain temperatures. Therefore the competitive balance between farmed and wild salmon may depend on the thermal profile of the river and the genetic background of the

respective populations. While limited to F1 hybridisation, results indicate that risk management of local fish populations could benefit from a more spatially resolved approach.

1.7.2 Chapter 3: Does density influence relative growth performance of farm, wild, and F1 hybrid Atlantic salmon in semi-natural and hatchery common garden conditions?

Paper II: Harvey AC, Juleff G, Carvalho GR, Taylor MI, Solberg MF, Creer S, Dyrhovden L, Matre IH, Glover KA (2016) Does density influence relative growth performance of farmed, wild, and F1 hybrid Atlantic salmon in semi-natural and hatchery common garden conditions? Royal Society Open Science. doi: 10.1098/rsos.160152.

The relative growth differences between farmed, wild and F1 hybrid salmon were studied at three contrasting densities within a hatchery environment and two contrasting densities within a semi-natural environment. Mortality was low for all groups in the hatchery environment, and was highest for all groups in the low density semi-natural treatment. Farmed salmon significantly outgrew hybrid and wild salmon in all treatments. Within the hatchery environment, growth of all experimental groups decreased with an increase in fish density. Importantly however, the reaction norms for growth were similar across treatments for all groups. Thus, we found no evidence to suggest that the offspring of farmed salmon have adapted to higher fish densities than wild salmon as a result of domestication. Consequently, the substantially higher growth rate of farmed salmon observed in the hatchery compared to wild salmon does not appear to be caused by differences in their ability to grow in high density hatchery scenarios.

1.7.3 Chapter 4: Plasticity in response to feed availability - does feeding regime influence the relative growth performance of domesticated, wild and hybrid Atlantic salmon *Salmo salar* parr?

Paper III: Harvey AC, Solberg MF, Glover KA, Taylor MI, Creer S, & Carvalho GR (2016) Plasticity in response to feed availability - does feeding regime influence the relative growth performance of domesticated, wild and hybrid Atlantic salmon Salmo salar parr? Journal of Fish Biology. doi: 10.1111/jfb.13076.

Growth was compared between farmed, wild and F1 hybrid salmon when reared at three contrasting feeding regimes in order to understand how varying levels of food availability affects relative growth. Groups were reared in single strain tanks and the treatments consisted of standard hatchery feeding (*ad libitum*), access to feed for four hours every day, and access to feed for twenty-four hours on three alternate days in a week.

Mortality was low in all treatments, and food availability had no effect on survival of all groups. As expected, the offspring of farmed salmon significantly outgrew the wild fish, while hybrids displayed intermediate growth. Furthermore, the relative growth differences between the farmed and the wild salmon did not change across feeding treatments, indicating a similar plasticity in response to feed availability. Although undertaken in a hatchery setting, these results suggest that food availability may not be the sole driver behind the observed reduced growth differences found between farmed and wild fish under wild conditions.

1.7.4 Chapter 5: Growth reaction norms of farmed, hybrid and wild Atlantic salmon: has domestication led to genetic divergence in diet preference?

Paper IV: Harvey AC, Solberg MF, Troianou E, Carvalho GR, Taylor MI, Creer S, Dyrhovden L, Matre IH, Glover KA. Growth reaction norms of farmed, hybrid and wild Atlantic salmon: has domestication led to genetic divergence in diet preference? BMC Evolutionary Biology - submitted.

Growth and survival differences between farmed, wild and F1 hybrid salmon fed three contrasting diets were investigated under hatchery conditions. The diet treatments consisted of a commercially available pelleted salmon diet, a commercially available pelleted carp diet, and a diet consisting of varying amounts of invertebrates commonly found in Norwegian rivers (a natural diet). There was an overall effect of treatment on growth, and all the groups grew differently to each other, however all groups responded similarly relative to each other by displaying similar growth reaction norms across the treatments. Thus, similar plasticity towards differing diets was detected in salmon of all origins, and no indication of genetic-based adaptation to the shape or content of commercial diets was detected in the farmed salmon.

Chapter 2: A common garden design reveals population-specific variability in potential impacts of hybridisation between populations of farmed and wild Atlantic salmon, *Salmo salar* L.

Abstract

Released individuals can have negative impacts on native populations through various mechanisms including competition, disease transfer and introduction of maladapted gene-complexes. Previous studies indicate that the level of farmed Atlantic salmon introgression in native populations is population-specific. However few studies have explored the potential role of population diversity or river characteristics, such as temperature, on the consequences of hybridisation. We compared freshwater growth of multiple families derived from two farmed, five wild, and two F1 hybrid salmon populations at three contrasting temperatures (7°C, 12°C, and 16°C) in a common garden experiment. As expected, farmed salmon outgrew wild salmon at all temperatures, with hybrids displaying intermediate growth. However, differences in growth were population-specific and some wild populations performed better than others relative to the hybrid and farmed populations at certain temperatures. Therefore, the competitive balance between farmed and wild salmon may depend both on the thermal profile of the river and the genetic characteristics of the respective farmed and wild strains. While limited to F1 hybridisation, the present study shows the merits in adopting a more complex spatially resolved approach to risk management of local populations.

Published in *Evolutionary Applications:* doi:10.1111/eva.12346

2.1 Introduction

The long-term evolutionary effects of both intentional and unintentional releases of domestic conspecifics on wild populations are of growing concern to conservationists and commercial forestry, fishing and wildlife stakeholders (Rhymer & Simberloff 1996; Laikre *et al.* 2010). Successful interbreeding between domestic and wild conspecifics may result in negative genetic effects such as loss of native population genetic structure, loss of genetic variation, and the breakdown of local adaptations (McGinnity *et al.* 2003; Laikre *et al.* 2010). Fitness loss can occur when alleles important for local adaptation are replaced by maladaptive or non-local alleles through hybridisation (observable in the F1 generation) (Randi 2008; Laikre *et al.* 2010), and by the loss of locally adapted gene complexes through introgression (generations of hybridisation and back-crossing) (McClelland & Naish 2007), a mechanism of outbreeding depression. Ultimately the local population genetic composition may be partly or completely replaced by that of the captive individuals (Sušnik *et al.* 2004; Meldgaard *et al.* 2007). Even if gene flow does not occur, native populations can be negatively affected by released individuals through direct competition for resources (Arechavala-Lopez *et al.* 2011), potential disease transmission (Villanúa *et al.* 2008; Madhun *et al.* 2014), and wasted reproductive effort (*i.e.* non-viable offspring) (Rhymer & Simberloff 1996). Collectively, such impacts can lower native population productivity and may affect genetic diversity by decreasing effective population sizes (Hindar *et al.* 2006; Laikre *et al.* 2010). These detrimental ecological and genetic effects are particularly problematic for wild populations with low population sizes or at risk from extinction (Baskett *et al.* 2013).

A valuable tool for understanding the effects of interbreeding is to investigate how wild and farmed populations and their hybrids perform relative to each other when exposed to differing environments, for example using reaction norm studies (Leger & Rice 2003; Hutchings 2004; Darwish & Hutchings 2009). Reaction norms illustrate how a phenotype responds to environmental change, and such studies may expose genotype by environment (G x E) interactions which can indicate that genetic variability for a phenotype or trait exists among conspecifics when exposed to different environments (Hutchings 2004). Common garden design studies, where individuals from all origins are reared communally and exposed to the same treatment(s) to eliminate random environmental effects, provide a way of investigating the genetic basis of phenotypic differences between groups (Hutchings 2011). Advances in molecular genetic technologies allow such comparative studies to elucidate genetic effects of hybridisation and introgression for a variety of fitness related traits in

disparate species (Fleming & Einum 1997; Leger & Rice 2003; Meldgaard *et al.* 2007; Colautti *et al.* 2009; Goedbloed *et al.* 2013). Within agriculture and forestry, studies on hybridisation generally focus on crop-wild interactions, and have provided valuable insights into how genetic changes within wild populations impact local plant population resilience and transgenic crop risk assessments (Adler *et al.* 1993; Viard *et al.* 2002; Mercer *et al.* 2007). In wildlife management, research has centred on interactions among wild and captive-bred or feral conspecifics, with the aim of evaluating the risks of outbreeding depression in subsequent hybridised or introgressed populations (Walker *et al.* 2004; Randi 2008). From a fisheries perspective, the majority of hybrid-wild interaction studies have focussed on the effects of intentional stocking (Vasemagi *et al.* 2005; Hamasaki *et al.* 2010) or accidental escapes from commercial fish farms (McGinnity *et al.* 2003).

Arguably, the best studied species in terms of monitoring genetic impacts of escapees is the Atlantic salmon (*Salmo salar* L.). Recent decades have witnessed a marked increase in commercial production of Atlantic salmon in several countries with the global production of Atlantic salmon from aquaculture exceeding 2 million tonnes in 2012 (FAO 2014a). Such rapid expansion has led to concern about potential negative environmental interactions imposed on native stocks by escaped farmed fish (Naylor *et al.* 2005; Weir & Grant 2005; Taranger *et al.* 2014). Ecological and genetic impacts of interactions between wild and farm escapees are compounded by difficulties in containing detrimental consequences due to the extent and scale of open marine systems (Naylor *et al.* 2000). Escape events are a common occurrence, and often involve the accidental release of large numbers of farmed individuals (Soto *et al.* 2001; Morris *et al.* 2008; Norwegian Fish Directorate 2014; The Scottish Government 2014). In most countries where salmon farming is practised, it is a legal requirement to report any production losses, however the reported numbers of escapees are most likely an underestimate of the true number as cases often go unreported (Glover 2010; Madhun *et al.* 2014; Taranger *et al.* 2014). Catch statistics from experimental studies estimate that the number of escaped Atlantic salmon in the wild in Norway alone is in excess of a million individuals annually (Skilbrei *et al.* 2014).

The potential negative impacts of farmed fish on native populations stem from the genetic differences accrued in farmed stocks over the last few decades. Atlantic salmon aquaculture is based upon rearing fish that originate from selective breeding programs (Gjedrem *et al.* 1991; Gjedrem 2000; Gjedrem 2010). While a variety of commercially important traits have been selected for in domestic populations, growth rate and size have

been the most consistently selected traits since breeding programs were first initiated in the early 1970´s (Gjedrem 2000). Growth in salmonids displays high heritability estimates, and the genetic gain for this trait has been estimated at 10 to 15% per generation (Gjedrem 2000). At present, the most advanced farmed populations have undergone more than 10 generations of directional selection, and as a result, their offspring display significantly higher growth rates than offspring of wild salmon under farmed conditions (Fleming & Einum 1997; Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b). Furthermore, it has been observed that in under farmed conditions, heritability estimates for growth are reduced in farmed relative to wild salmon (Solberg *et al.* 2012; 2013a). These results suggest the loss of genetic variation for growth, which is in accordance with genetic studies that have demonstrated reductions in allelic diversity at highly polymorphic genetic markers in farmed populations compared to wild conspecifics (Norris *et al.* 1999; Skaala *et al.* 2004; Solberg *et al.* 2012; 2013a). Body size is known to influence fitness and reproductive success in fish (Jonsson & Jonsson 2006; Garcia de Leaniz *et al.* 2007), and has been used as a proxy for fitness in other salmonid comparative studies (Einum & Fleming 1997; Solberg *et al.* 2013a). It is also known to influence the outcomes of resource and social competition (Post *et al.* 1999).

Wild Atlantic salmon are characterised by genetically distinct local populations; a product of their typically isolated freshwater habitats and their ability to home to their natal rivers to spawn (Taylor 1991; Verspoor 1997; Garcia de Leaniz *et al.* 2007; Carvalho 1993). The morphological and ecological divergence seen among wild salmon populations can to some degree reflect local adaptation to their native environments (Hindar *et al.* 1991; Taylor 1991; Carvalho 1993; Garcia de Leaniz *et al.* 2007; Houde *et al.* 2011; O'Toole *et al.* 2015), and likely underpin population resilience in changing environments (Hilborn et al., 2003; Schindler et al., 2010). Maintaining diversity both within and among populations can help to ensure the long term stability of populations against environmental change (Hilborn *et al.* 2003; Schindler *et al.* 2010). Several common garden studies have highlighted population specific genetic differences in early development in grayling (*Thymallus thymallus* L.) (Haugen & Vøllestad 2000) and brown trout (*Salmo trutta* L.) (Jensen *et al.* 2008), and between farmed and wild conspecifics and their hybrids or back-crosses for a variety of life-history traits, including compensatory growth (Morris *et al.* 2011) and early development (Darwish & Hutchings 2009) in Atlantic salmon. However, there have been few studies which highlight the potential role of such population diversity on impacts of hybridisation or introgression (Normandeau *et al.* 2009), and none under common garden conditions.

Recent studies have quantified introgression of farmed salmon escapees in 20 wild populations (Glover *et al.* 2012; 2013). These studies indicate that introgression levels are strongly population-dependent, and that the frequency of escapees is only modestly correlated with levels of introgression. Using a modelling approach on these empirical data, it has been subsequently demonstrated that population size, together with frequency of escapees, is a better predictor of introgression levels (Heino *et al.* 2015). Nevertheless, much of the variation in the levels of introgression of farmed salmon among native populations remains population-specific. That is, the characteristics of each interacting farmed and native population may determine the degree and impacts of hybridisation and introgression. While it has been suggested that the density of wild fish within an environment, and thus the level of competition between wild and farmed fish, is a significant factor influencing the relative success of farmed escapees among rivers (Glover *et al.* 2012; 2013), it is possible that other environmental or river- specific factors may influence relative competitive success of farmed, hybrid and wild salmon in the wild. Water temperature is a key environmental factor that varies between rivers within and among regions. Temperature is also a key determinant of developmental and growth rates (Jonsson & Jonsson 2011b; Forseth *et al.* 2011), and is therefore likely to be associated with adaptation of wild populations to natal rivers (Garcia de Leaniz *et al.* 2007). However, thus far, the relative variance in growth rate of different farmed salmon strains and wild salmon populations exposed simultaneously to a range of controlled temperatures has not been fully evaluated.

Studies that investigate genetic differences among farmed and wild conspecifics and their interaction in hybrid individuals are essential in understanding the mechanisms driving observed population-level variance across a divergent set of environmental conditions. Understanding the potential effects of outbreeding depression and ecological interactions between farmed and wild conspecifics is necessary in order to underpin contemporary and future management strategies in a growing aquaculture industry, and for the formulation of conservation risk assessments (Randi 2008; Fraser *et al.* 2010; Laikre *et al.* 2010). Therefore, we investigated freshwater growth of multiple families derived from farmed, wild and hybrid salmon populations under three strongly contrasting temperature regimes to estimate variation in growth among populations. Three divergent temperatures were chosen to represent temperatures which approach the lower and upper boundaries for growth in Atlantic salmon, and a temperature which is intermediate.

2.2 Materials and methods

Contribution statement

This experiment was designed by Gary Carvalho, Kevin Glover and Martin Taylor. Initial experimental set-up was performed by Kevin Glover, Lise Dyrhovden and Ivar-Helge Matre. The sampling was carried out by Lise Dyrhovden, Ivar-Helge Matre, Alison Harvey and Samantha Beck. All laboratory work, statistical analysis and manuscript preparation was performed by Alison Harvey.

Experimental crosses

Adult brood fish collected from a total of five wild populations and two commercial farmed strains were used to produce the experimental families (Fig. 2.1). The two commercial farmed strains used were Mowi and Salmobreed. Mowi is the Marine Harvest strain, and is the oldest Norwegian commercial strain (Gjedrem *et al.* 1991). Salmobreed was established in 1999, and is based upon genetic material from several older Norwegian farmed strains. Both strains are extensively used in commercial aquaculture in Norway and internationally. Strain ID was not the focus here, and both were thus anonymized randomly as Farm 1 and Farm 2 and are referred to as the farm populations throughout. Wild parental fish upon which the families were produced were either sampled directly in rivers (Vosso, Figgjo, Arna) and verified as wild based upon reading scale characteristics (Lund & Hansen 1991), or alternatively collected from the Norwegian Gene Bank for wild Atlantic salmon (Driva and Skibotn). The sire of family 17 had a tag when caught in the river Figgjo, which indicated that the specific fish originated from the nearby River Ims. The Norwegian Gene Bank is a program that conserves wild salmon populations regarded as under threat from disease or extinction. Individuals are taken from the rivers and are then reared in the Gene Bank where genetic structure is monitored. Gametes from first and third generation Driva and first and second generation Skibotn gene bank strains were collected at the gene bank hatchery and transported back to Matre. Wild salmon from the River Figgjo (58°81'N, 5°55'E) are predominantly one-sea-winter fish with some two and three winter fish (Friedland *et al.* 2009). The River Vosso (60°64'N, 5°95'E) is characterised by its large multi-sea-winter salmon, and the Norwegian Gene Bank conserves this population, thus fish from this strain have been reared in a local hatchery before release into the fjord at the smolt stage. The River Arna (60°24'N, 5°29'E) is a small river in Western Norway, with a variable-age spawning population. The River Skibotn (69°38'N, 20°26'E) population in northern

Norway is conserved by the Norwegian Gene Bank due to repeated infestation by the parasite *Gyrodactylus salaris*. The River Driva (62°40'N, 8°34'E) population in mid-Norway is also conserved by the Norwegian Gene Bank due to infestation by *G. salaris.* Hydrographical data pertaining to river water temperature was accessed through the Norwegian Water Resources and Energy Directorate (2015). The average monthly water temperatures for each river are presented in Figure 2.2. There was no data available for Arna, thus the nearby Oselva River was used as a temperature reference. The highest temperature recorded was 16.7°C in Oselva, and the lowest recorded temperature was 0.0°C in Skibotn.

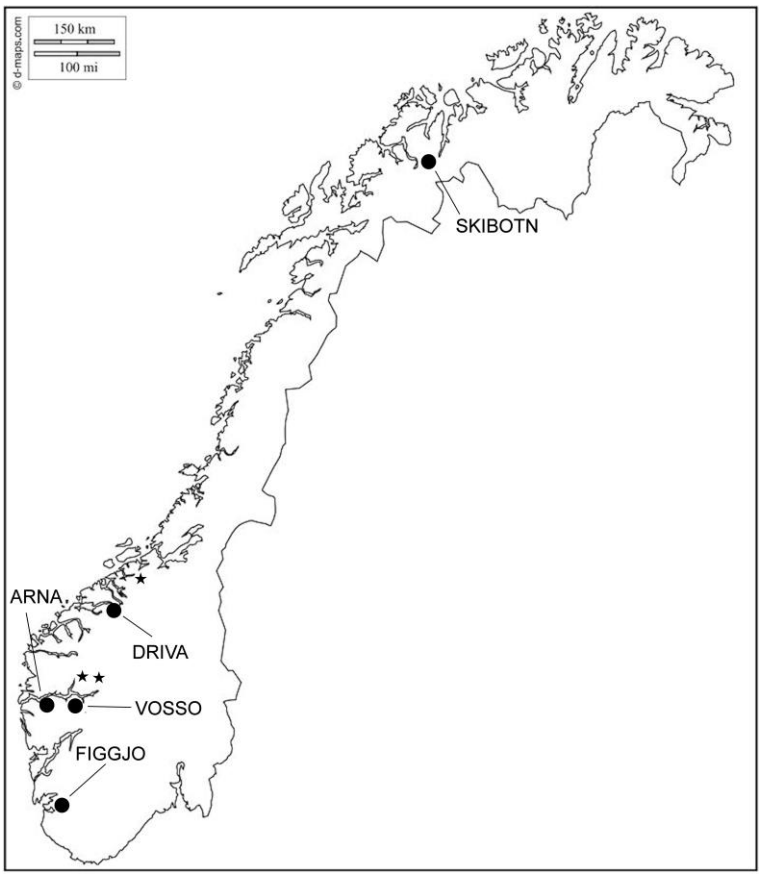

Figure 2.1: Map showing origin of wild populations. Wild fish collected from five river populations were included in this study. Gametes from the Vosso, Skibotn and Driva populations were collected from the Norwegian Gene Bank for Atlantic salmon. The * represents Haukvik, the Norwegain Gene Bank hatchery from which the Skibotn and Driva populations were collected, and the ** represents the Eidfjord Norwegian Gene bank hatchery were the Vosso population was collected.

All 35 experimental families were established at the Matre experimental field station located on the west of Norway in weeks 46-47 of 2012. The five wild populations and two farmed populations were used to create farmed, F1 hybrid and wild families as follows: 8

farmed families consisting of Farm 1 and Farm 2; 8 hybrid families consisting of two F1 hybrid populations; and 19 wild families consisting of fish from five wild populations. Figgjo females were crossed with Farm 1 males to produce the Hybrid 1 families, and Farm 2 females were crossed with Vosso males to produce the Hybrid 2 families. The full crossing design is presented in Table S2.1. All nine experimental groups are hereon referred to as the experimental populations. All nine populations were represented by 4 families each with the exception of Driva, which consisted of just 3 families (Appendix 2: Table S2.1).

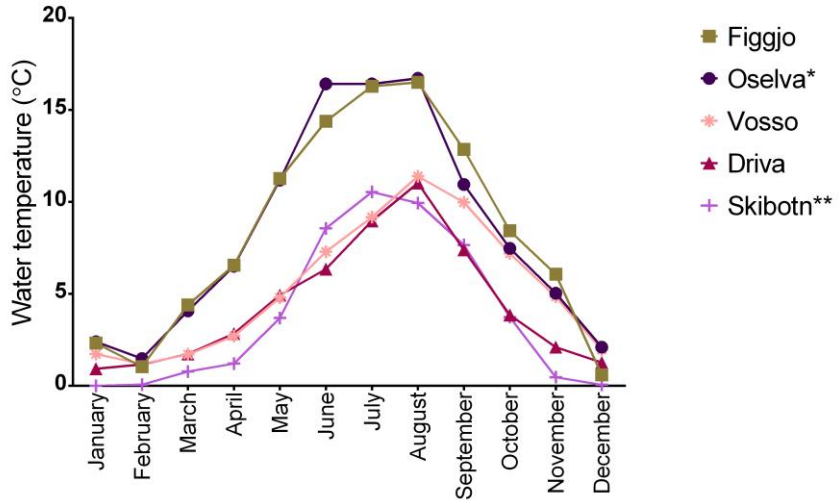

Figure 2.2: Average monthly water temperature for each of the rivers from which the experimental fish originated. Daily logger data from 2012 was used to calculate average monthly temperatures (and SD) within the rivers Figgjo, Oselva, Vosso, and Driva. **Skibotn river water temperature was only available sporadically for years before 1986, and thus the most complete data set (1986) was used to calculate average monthly water temperature in Skibotn. *There was no data available for the Arna River, thus data from the nearby Oselva was used.

Experimental design

A common garden experimental design was used to investigate relative growth differences between farm, wild and hybrid F1 crosses of Atlantic salmon at three different temperatures. Salmon from a total of 35 families of farmed, wild and F1 hybrid origin were reared in communal tanks under standard hatchery conditions at three different water temperatures: the control treatment consisted of two replicate tanks at 12^{o}C, while the treatments consisted of two replicate tanks at 7^{o}C (low treatment) and 16^{o}C (high treatment) respectively (Table 2.1). The temperatures were chosen to represent a representative range

experienced by *S. salar* populations: 12°C is typically experienced by farmed salmon in a hatchery environment, 7°C and 16°C represent two contrasting experienced within the natural salmonid temperature range. Temperature regimes were maintained throughout the experiment, from transferral to tanks on 2 April until experiment termination on 23-27 September 2013. At the end of experiment, individual growth measurements of wet weight and fork length were recorded and adipose fin or tail samples for DNA analysis were taken from a subset of individuals in each tank.

Table 2.1: Overview of the experimental design.

Treatment	**Low temperature (7°C)**		**Control temperature (13°C)**		**High temperature (16°C)**	
Initial number of fish	Tank 1 (35 families of 30 fish = 1050)	Tank 2 (35 families of 30 fish = 1050)	Tank 3 (35 families of 30 fish = 1050)	Tank 4 (35 families of 30 fish = 1050)	Tank 5 (35 families of 30 fish = 1050)	Tank 6 (35 families of 30 fish = 1050)
Sampled	700	700	700	700	700	700
Genotyped	688	692	697	686	694	697

Rearing conditions

Experimental replicates were established in week 4 of 2013, when 30 eyed-eggs from each of the 35 families were sorted into six identical hatchery trays (thus each replicate contained 1050 fish from the 35 families). At time of sorting, egg diameter was recorded for each family. In week 14 the hatched and ready-to-start feeding fry were transferred to six tanks, and the experiment was started. All tanks were 1m in diameter and flow rate was 27L/min. The fish were reared under standard hatchery conditions with a 24 hour light regime as per standard hatchery conditions. The fish were fed *ad libitum* with a commercial pelleted diet (Skretting), and pellet size was adjusted according to manufacturer's tables, whereby a random sample of fish from each tank were measured at regular intervals to estimate average weight. Due to the high growth rate of fish at elevated temperatures, the high temperature replicates were split into two tanks each on 9 July, these were further split into 6 tanks on 8 August and then 8 tanks on 3 September. Thus, at the end of the experiment each high temperature replicate consisted of 4 tanks. On 28 August the control replicates were split into 2 tanks each. Mortality was low within all tanks, ranging between 8% -12%.

Sampling, genotyping & parentage assignment

Upon termination, 700 fish were randomly sampled from each of the replicate tanks (4200 in total). Where tank replicates had been split, an equal number of fish were randomly sampled from each split tank to make up a total of 700 fish per replicate. All individuals in each tank were euthanized following standard guidelines with an overdose of Finquel® Vet anaesthetic (ScanVacc, Årnes, Norway). The fish were wet-weighed, fork length measured and fin clipped for DNA analysis. Fins were placed individually into labelled tubes of 100% ethanol.

DNA-based parentage testing was used to identify between 686 and 697 of sampled fish from each replicate back to respective family of origin. DNA was extracted in 96 well plates using a variation of the salt extraction method (adapted from Aljanabi & Martinez 1997). Parental DNA was extracted and genotyped twice to ensure consistent genotyping. Each plate contained 2 randomly placed negative controls (blank wells) to ensure unique identification of each plate. Five microsatellite loci were amplified in one PCR multiplex: *SsaF43* (Sanchez *et al.* 1996), *Ssa197* (O'Reilly *et al.* 1996), *SSsp3016* (Genbank # AY372820), *MHCI* (Grimholt *et al.* 2002) and *MHCII* (Stet *et al.* 2002). PCR products were resolved on an ABI Applied Biosystems 3731 Genetic Analyser and sized using a 500LIZ standard (Applied Biosystems). Genemapper Version 4.0 was used to score alleles manually. Individuals were then assigned back to family using the Family Assignment Program (FAP) (v3.6) (Taggart 2007), an exclusion-based assignment program that is routinely used in other studies for the purpose of parentage assignment in comparative studies of salmonids (Solberg *et al.* 2013b; Glover *et al.* 2004; Skaala *et al.* 2013).

Statistical analysis

Statistical analysis was carried out using R version 3.2.1(R Core Team 2015) with all critical p-values set to 0.05. In order to test for differences in family representation among the split replicate tanks, a chi-square (X^2) test based on numbers of fish in each family was performed. A linear mixed effect model (LME) was used to investigate the variation in weight at termination between the populations among the treatments, and covariates analysed. The response variable was the continuous variable of log-transformed weight at termination. Variation between split tanks and the replicate treatment tanks was controlled for by including split tank nested within replicates further nested within treatments in the model as random intercept factor effects with 14 levels. Variation within families across the

treatments was controlled for by including family nested within strain as a random intercept effect (35 levels) with differing slopes for the effect of treatment.

Relative growth between the strains

The LME model was fitted using *lmer* from the *lme4* package in R (Bates *et al.* 2014). The full model was fitted with treatment (T) and population (P) as fixed factor covariates with 3 and 9 levels respectively, egg size (E) as a fixed continuous covariate, and all two-way interactions between the fixed covariates: treatment and population (TP); treatment and egg (TE); and population and egg (PE) as fixed covariates, with tank (t) and family (*f*) as random covariates (as described above).

The fit of the full model was investigated by plotting the model residuals against all covariates, and the normality of the model residuals was visually confirmed using a histogram. The distributions of the random effects were investigated visually using quantile-quantile plots. The *lmerTest* package in R allows for automatic model selection using the *step* function (Kuznetsova *et al.* 2014). The function performs backwards selection on both the fixed and random effects to determine the simplest best-fitting model (Kuznetsova *et al.* 2014).

It first performs backwards selection on the random elements of the model using likelihood ratio tests, with a significance level of 0.1 as a default, before performing backwards selection on the fixed elements in the model (Kuznetsova *et al.* 2014). The *anova* function from the *lmerTest* package was used to obtain p-values for the fixed covariates of the model and are calculated using an F-test based on Satterthwaite's approximation and the significance level is set to 0.05 (Kuznetsova *et al.* 2014). The final model fit was confirmed by investigating plots of the model residuals against the covariates included in the model as well as those which were not included in the model. Normality of the final model residuals was confirmed visually using histograms. The full and final models, as given by the step function output, are presented in Table 2.2.

Post-hoc multiple comparisons were carried out for the interaction term of population by treatment using the function *pairs* in the *lsmeans* package with a Tukey adjustment for multiple comparisons, which calculates the differences of least squares means (Lenth 2016). The test computes all pair-wise comparisons and reports p-values and confidence intervals (Lenth 2016).

Table 2.2: Full model investigating weight variation.

			Random effects				**Fixed effects**					
Model	N	Response	Variable	Chi.sq	Chi Df	P	Variable	Sum Sq	Num Df	Den Df	F	P
Growth	4154	Log Weight	**Tank**	69.41	1	**<1e-07**	**Treatment**	79.67	2	16.55	2292	**<1e-07**
			Family	157.04	5	**<1e-07**		2.65	8	25	19	**<1e-07**
							Population					
							Egg	0.09	1	25	4.9	**0.036**
							T X P	2.38	16	24	8.5	**0.000**
							T x E	0.17	2	25	4.9	**0.016**
							P x E	0.12	8	17	0.89	0.547

The interactions terms included in the full model: treatment : population (T x P), treatment : egg size (T x E), and population : egg size (P x E). N; number of individuals. Chi.sq; the value of the Chi square statistics. Chi Df; the degrees of freedom for the test. P; the p-values of fixed and random effects. Sum.Sq; sum of squares. Num Df, numerator degrees of freedom. Den Df; denominator degrees of freedom based on Sattherwaithe's approximations. F; F-value.

Ethical statement

Temperatures experienced by the experimental fish were within the natural temperature ranges experienced by Atlantic salmon, and, the rearing conditions were otherwise as in standard Atlantic salmon farming; therefore approval of the experimental protocol by the Norwegian Animal Research Authority was not required. However all welfare and use of experimental animals was performed in strict accordance with the Norwegian Animal Welfare Act 2010. In addition all personnel involved in this experiment had undergone training approved by the Norwegian Food Safety Authority, which is mandatory for all personnel running experiments involving animals included in the Animal Welfare Act.

2.3 Results

Genotyping & parentage assignment

Of the 4200 individuals sampled, 34 individuals (<1% of the total) could not be assigned unambiguously back to a single family using the microsatellite multiplex and were removed from the dataset prior to analysis. Twelve individuals were identified as outliers due to extreme condition factors (< 0.7 or > 1.9, where it is obvious a recording error has occurred) attributed to human recording error and subsequently removed from the dataset prior to analysis. The final dataset for analysis contained a total of 4154 individuals.

Statistical analysis

Growth between treatments

Final weight at termination was significantly different between each of the three treatments, being highest in the high temperature treatment, lowest in the low temperature treatment, and intermediate in the control treatment (Table 2.2; Fig. 2.3). Growth in the low temperature treatment was very low for all strains, probably due to the low growth potential for salmon at this temperature. Within each split replicate, families were represented within their expected frequencies (X^2 = 388.46, df = 442, P = 0.968), as expected with random sampling.

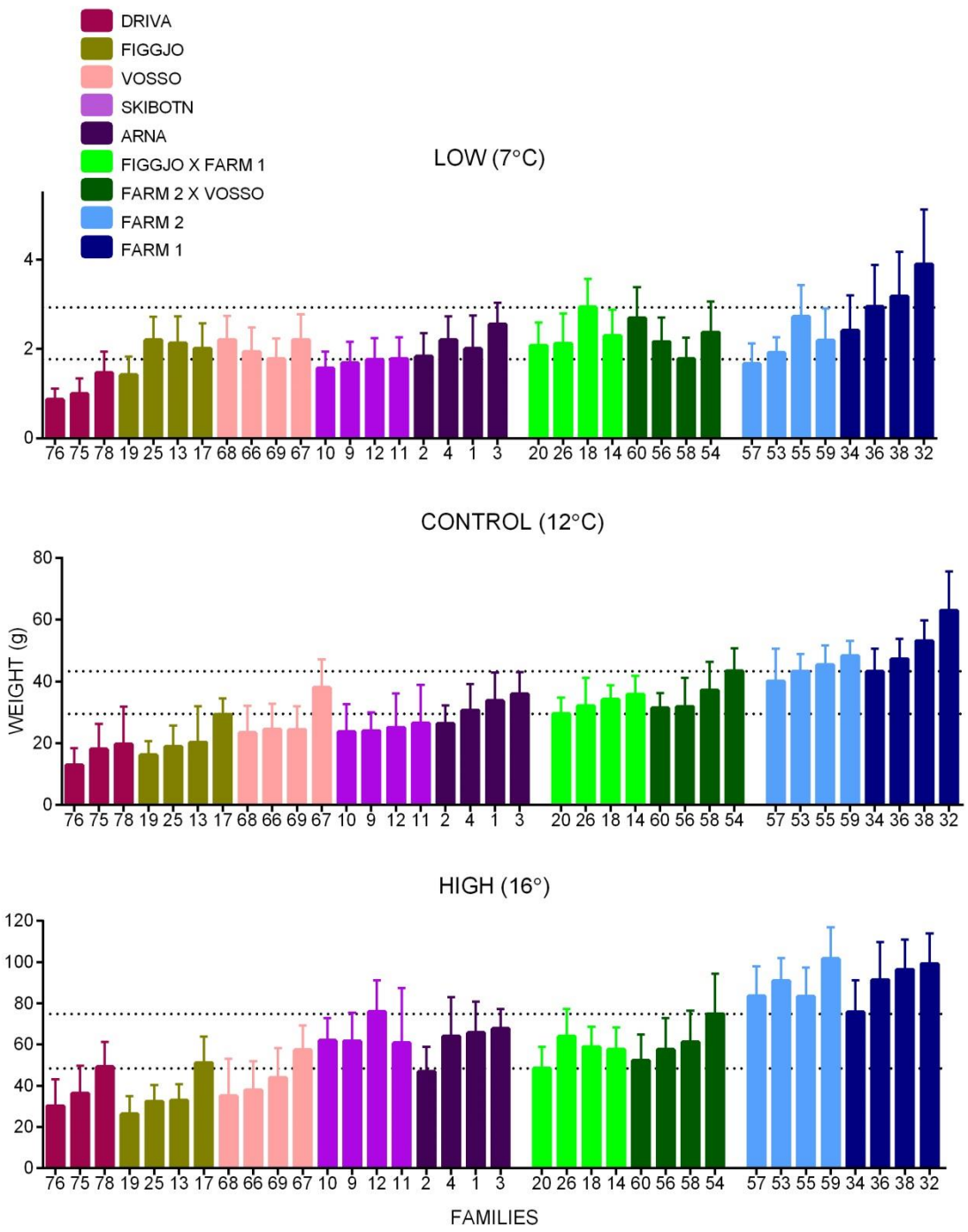

Figure 2.3: Average weight of each family within each population for the three treatment temperatures. The error bars represent the standard deviation. Certain families within populations performed better than other families within the same populations under certain temperature conditions. The populations performed differently across treatments. The dotted lines show the mean weight of the smallest and largest hybrid families. Hybrid crosses are labelled as maternal x paternal.

Relative growth differences between strains

The final model included all the covariates described above, apart from the interaction between population and egg size (Table 2.2). The fixed effect of population had a significant effect on weight at termination (Table 2.2). Adjusted pairwise comparisons between each population within each treatment are given in Table 2.3, with the significance level set to 0.05. On average, the farmed populations performed better than the hybrid and wild populations, while the hybrid performance was intermediate. It was evident, however, that some wild populations performed as well as or better than, the hybrid and farm populations within particular treatments (Table 2.3; Fig 2.3). The largest growth differences were seen between Farm 1 and Driva in the control temperature treatment where the farmed population grew three times more than the wild population. The smallest growth difference was observed in the low temperature treatment where the Farm 2 population growth was equal to both Arna and Vosso populations. In the control treatment, the smallest growth difference between farmed and wild populations was found between Farm 2 and Arna, where the relative growth ratio was just 1:1.4.The two farmed salmon populations were not significantly different from each other in growth rate in any treatment (Table 2.3). There was a visible, although not significant, trend of growth differences between the farm populations at the low temperature (Fig. 2.3). In the high temperature treatment, Skibotn and Arna had the highest wild population growth (Table 2.3). Driva grew significantly different to the other populations in at least one temperature treatment, apart from Skibotn (Table 2.3). On average, Driva displayed the lowest growth in the low and control temperature treatments, while Figgjo had the lowest growth at high temperatures. The largest growth differences detected in the wild populations were between Arna and Driva where the relative growth ratio was 1:1.9 in the low and control treatments. Growth in the hybrid populations was not significantly different to each other in any treatment. Hybrid 1 displayed relatively intermediate growth to both its parental populations for all treatments (Table 2.3, Fig. 2.3). Hybrid 2 displayed similar relative growth to both its parental populations at low temperatures, while growth was intermediate between the parental populations in the other two treatments (Table 2.3, Fig. 2.3).

Table 2.3: (A) P-values for the Tukey-adjusted pairwise comparison of populations across treatments and (B) relative weight differences between each population at each treatment temperature.

A	Driva			Figgjo			Skibotn			Vosso			Arna			Hybrid 1			Hybrid 2			Farm 2		
	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C
Driva	-	-	-																					
Figgjo	**	ns	ns	-	-	-																		
Skibotn	ns	ns	ns	ns	ns	ns	-	-	-															
Vosso	**	.	ns	ns	ns	ns	ns	ns	ns	-	-	-												
Arna	**	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-	-									
Hybrid 1	***	**	ns	ns	.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-	-						
Hybrid 2	***	**	ns	ns	.	.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-	-			
Farm 2	**	*	***	ns	**	*	ns	**	ns	ns	.	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	-	-
Farm 1	*	*	***	ns	*	*	**	*	ns	ns	**	***	ns	.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

B	Driva			Figgjo			Skibotn			Vosso			Arna			Hybrid 1			Hybrid 2			Farm 2		
	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C	7°C	12°C	16°C
Ave. Weight (g)	1.13	16.71	39.7	1.94	21.8	36.6	1.69	24.7	64.4	2.02	27.4	43.6	2.14	31.6	61.9	2.34	33.0	57.5	2.23	35.5	60.4	2.09	44.32	89.6
CV	0.40	0.55	0.38	0.31	0.42	0.37	0.27	0.40	0.28	0.27	0.37	0.39	0.29	0.27	0.26	0.29	0.20	0.21	0.30	0.25	0.29	0.33	0.18	0.17
Driva	-	-	-																					
Figgjo	1:0.8	1:0	1:1.2	-	-	-																		
Skibotn	1:1.1	1:0	1:0.9	1:0.9	1:0	1:1	-	-	-															
Vosso	1:0.7	1:0	1:1.3	1:0.9	1:0	1:1.4	1:0.7	1:0	1:1.4	-	-	-												
Arna	1:0.5	1:0	1:0.8	1:0.9	1:0	1:1	1:0.7	1:0	1:1	1:0.8	1:0	1:0.9	-	-	-									
Hybrid 1	1:0.4	1:0	1:0.7	1:0.9	1:0	1:0.8	1:0.7	1:0	1:0.8	1:0.8	1:0	1:0.7	1:1.1	1:0	1:0.7	-	-	-						
Hybrid 2	1:0.5	1:0	1:0.9	1:1	1:0	1:1.1	1:0.8	1:0	1:1.1	1:0.8	1:0	1:1	1:1.1	1:0	1:1	1:1.5	1:0	1:1	-	-	-			
Farm 2	1:0.2	1:0	1:0.5	1:1.1	1:0	1:0.6	1:0.8	1:0	1:0.6	1:0.9	1:0	1:0.6	1:1.2	1:0	1:0.6	1:1.7	1:0	1:0.6	1:1.3	1:0	1:1.1	-	-	-
Farm 1	1:0.2	1:0	1:0.6	1:0.1	1:0	1:0.7	1:0	1:0	1:0.7	1:1	1:0	1:0.7	1:1.3	1:0	1:0.7	1:1.8	1:0	1:0.7	1:0.9	1:0	1:0	1:0.1	1:0	1:0

The p values are represented as significance codes whereby '***' <0.0001, '**' <0.001, '*' <0.01, '.' ≤0.5 and 'ns' denotes not significantly different.
The populations were organised using the average weights from the control treatment and ordered from lowest average weight to highest average weight.
Average weight of Farm 1 (not shown due to space constraints): 7°C: 3.13g; 12°: 51.49g; 16°C: 90.8g. Each population is compared to all other populations

within each treatment. Ratios were calculated by dividing the average weights of the column populations by the row populations along the horizontal axis from right to left. Thus the bigger fish were most commonly the numerator to ensure ratios of >1. CV: coefficients of variation for each population within each treatment. CV of Farm 1: 7°C: 0.36; 12°C: 0.22; 16°C: 0.20.

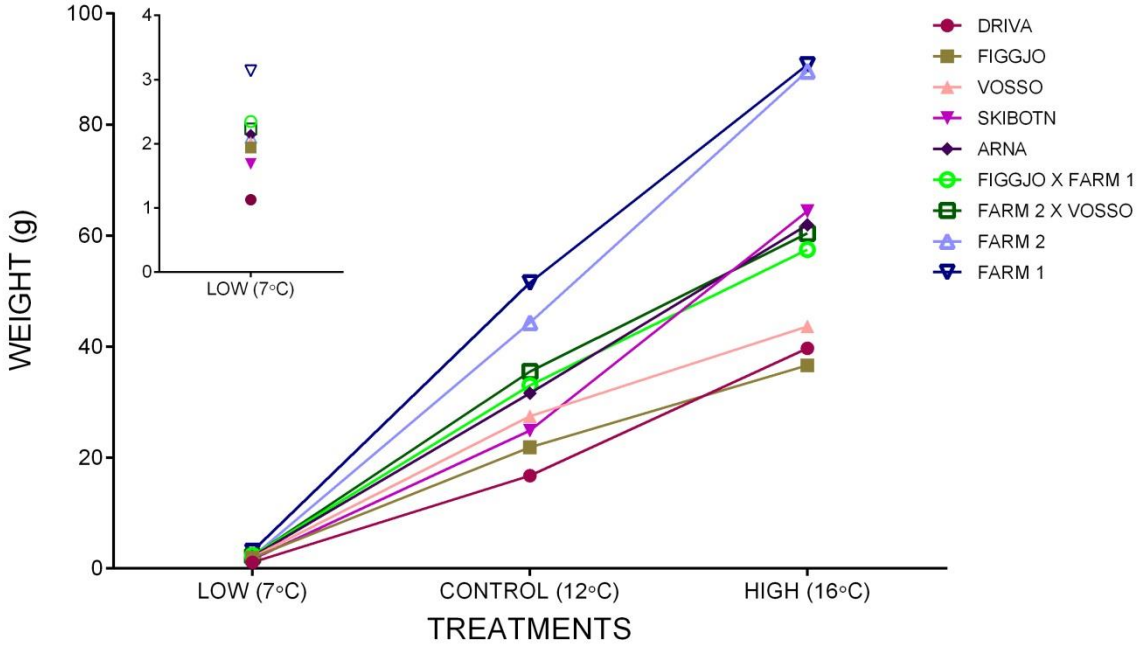

Figure 2.4: Growth reaction norms of each population. Average weight norms of reaction across the three treatment temperatures: low (7°C), control (12°C), and high (16°C). Replicate tanks have been pooled. The significant genotype by environment interaction is visible as the crossing lines between the populations across the treatments. For clarity, the inset graph represents the average weights of each population at the low temperature treatment.

In order to investigate further whether the observed differences in growth between the populations were changing between the treatments, an interaction term was included in the model. The interaction of population and treatment was retained in the final model (Table 2.2), indicating a population-by-temperature effect on final weight. Thus the slopes of the reaction norms of the populations changed across the temperatures relative to the other populations, as evident in Figure 2.4. The populations thus responded differently relative to each other to the different temperature treatments, indicating that population plays a role in salmon growth at varied temperatures.

A positive effect of egg size on final weight was detected, thus families with a larger average egg size grew larger than those with a smaller average egg size (Table 2.2). The interaction between treatment and egg size had an effect on weight at termination (Table 2.2). Further analysis of the effect of egg size at the treatment level revealed that egg size was found to be a significant covariate in the low temperature treatment, and marginally insignificant in the high temperature treatment. The outputs, as given by the step function, for the models run to investigate significance of egg size on growth are presented in Table S2.2.

The above LME was run with population replaced by group (wild, farmed, and hybrid) and all other covariates as presented above. The growth of the groups was significantly different between treatments. Farmed salmon were larger than both the hybrid and the wild salmon, with hybrid salmon displaying intermediate growth. In the low temperature treatment farmed and hybrid growth did not differ significantly, although farmed salmon outgrew hybrids by 1.15 and both grew significantly more than wild salmon. The final model output, as presented by the step function, is given in Table S2.3.

2.4 Discussion

In the present study, growth of two farmed, five wild and two F1 hybrid populations were investigated at three different temperatures using a common garden experimental design. Our study is the first to compare the growth of several different populations of wild, hybrid and farmed salmon in such an experimental setting across a temperature gradient. Overall, we found: (i) on average, farmed salmon outgrew wild salmon at all temperatures, with hybrids displaying intermediate growth (ii) at the population level, there was significant variation among populations in growth rate to the extent that there was an overlap in weight between some wild populations and the hybrid and farm populations; (iii) there was a significant population-by-temperature interaction detected; and (iv) egg size (*i.e.*, a maternal

effect) was a significant predictor of size attained in the low temperature treatment but not in the control and high temperature environments.

Temperature effects

For all populations, growth was greater in the high temperature treatment, intermediate in the control treatment, and lowest in the low temperature treatment (Fig. 2.3). For most of the wild populations the relative growth differences between the farmed and wild populations increased as the temperature increased (Table 2.3B), indicating that, although all the populations grew larger at higher temperatures, there are potentially larger growth differences between farmed and wild fish at higher temperatures, which may further influence the competitive balance between farmed and wild fish in rivers with warm thermal profiles, and may have implications for hybridisation success under climate change.

To control for maternal effects, average family egg size was included in the LME. It was found that egg size was a significant predictor of growth at the low temperature treatment and marginally non-significant in the high temperature treatment. Such a pattern may derive from slow development at low temperatures whereby egg size influences early growth directly at this stage (Dunham 2004).

Population effects

Populations investigated here are different to those used in previous growth studies; however, growth in some populations (Figgjo, Farm 1, and Hybrid 1) have been compared under different environmental parameters and displayed similar growth ratios to those seen previously (see Solberg *et al.* 2013b). Thus the present study confirms earlier studies (Glover *et al.* 2009; Solberg *et al.* 2013a), that growth in farmed salmon relative to wild salmon has been significantly increased through selection extending over ten generations in commercial breeding programs. The magnitude of growth differences seen in our study is however, on average, less than previously reported (Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b). It is possible that the higher growth typical of farmed salmon under aquaculture conditions may further increase the growth differences observed between farmed and wild salmon due to competition interactions. Solberg et al (2013b) investigated growth differences between farmed and wild conspecifics in mixed and single-group tanks under controlled conditions. They found no difference in the relative growth across experimental designs, indicating that

social interaction is not responsible for inflating the growth differences observed in aquaculture conditions (Solberg *et al.* 2013b).

Population by temperature effects

On average, farmed salmon were significantly larger than wild salmon at all three experimental temperatures. However when examined at the population level, the magnitudes of the growth differences were more variable than expected, and influenced strongly by population (Table 2.3B, Fig. 2.3). Certain wild and hybrid strains grew as well or better than other wild and farm populations in some of the treatments (Table 2.3B, Fig. 2.4). For example, while Farm 2 was larger than the wild and hybrid populations in the control and high treatments, certain wild and hybrid populations were larger, on average, than Farm 2 in the low temperature treatment (Figure 2.4), and while Driva exhibited the lowest average growth overall in the low and control treatments, Driva outgrew Figgjo in the high temperature treatment (although this difference was non-significant after correction for multiple comparisons). Growth represents the genetic trait that has been documented to differ greatest between farmed and wild salmon, and previous comparative studies show that under aquaculture conditions, farmed salmon significantly outgrow wild salmon (Einum & Fleming 1997; Fleming & Einum 1997; Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b). While the present study also found similar differences, significant growth variation was also detected among the wild populations (Table 2.3, Fig.2.3). Hybrids displayed mostly intermediate growth relative to their respective parental populations (Table 2.3, Fig. 2.3). Intermediate hybrid growth relative to their parental populations has been documented for Atlantic salmon in comparative studies in aquaculture (Solberg *et al.* 2013a; Glover *et al.* 2009), semi-natural (Solberg *et al.* 2013b), and wild conditions (Einum & Fleming 1997; Skaala *et al.* 2014). Intermediate manifestations of a variety of traits have been documented for other species, including Helmeted guineafowl (*Numida meleagris* L.) (Walker *et al.* 2004), sticklebacks (*Gasterosteus aculeatus*L.) (Hatfield & Schluter 1999) and eucalypts (*Eucalyptus* spp.) (Dungey *et al.* 2000).

Temperature plays an important role in maintaining adaptive population variation of developmental rates and survival in early life-history stages in salmonid populations (Taylor 1991; Garcia de Leaniz *et al.* 2007). Studies have highlighted differences in populations for time of emergence and embryonic and larval survival that may be linked to local temperature regimes (reviewed in Taylor 1991). Temperature is also strongly linked to growth rates,

which in turn, influence important life-history traits such as size and age at maturity and smolting (Jonsson & Jonsson 2006), and can influence competition (Post *et al.* 1999). Darwish & Hutchings (2009) investigated the genetic variation in early life history traits between farmed and wild backcrossed F2 Atlantic salmon under three different temperature regimes. They found genetic variation between populations for key life history traits such as time to hatch and post hatch survival. The results of the present study provide evidence for a genotype-by-environment interaction of an observable fitness related trait; namely, growth across different temperatures. Thus the competitive balance, exhibited as growth, between farmed and wild fish may be influenced by the origin of the farmed and wild fish.

Farmed salmon generally experience less variation in environmental parameters during production than wild salmon, such as low feeding competition, lack of predators and otherwise homogenous environmental conditions. During the early freshwater phase, for example, during start-feeding, the water temperature in the hatchery is typically elevated to 10 degrees or more in order to increase growth rates and produce a higher number of 0+ or 1+ smolts, depending upon the production strategy (Fjelldal *et al.* 2009). It could be expected therefore, that farmed fish might not grow optimally in lower temperatures. Here, there was no evidence that the farmed fish grew any worse than expected at low temperatures, indeed there was an overall lack of growth for all strains, and it is likely that the variability within and between the strains and the low growth observed derives from reduced growth across all strains. Thus, there was insufficient evidence found for thermal adaptation in the wild and farmed strains.

2.5 Conclusions and recommendations

The population-specific differences in growth demonstrated here represent analogous genetically-based population diversity. Jensen *et al* (2008) found population level differences in four wild brown trout populations for early life-history traits at different temperatures and suggest that these populations are locally adapted to their native water temperature. While Jensen *et al* (2008) focused on wild populations; Normandeau *et al* (2009) compared gene expression of backcrossed Atlantic salmon farm-wild hybrids and their respective wild and farmed parent strains using 2 wild strains and 1 fourth generation farmed strain. They found significant population-specific differences in liver gene expression of various transcripts between the strains, and concluded that the consequences of introgression with farm genes will depend on the genetic architecture of the wild population (Normandeau *et al.* 2009). McGinnity *et al* (2009) used a regression model to predict that influxes of hatchery genes into

wild native salmon populations coupled with increasing water temperature due to climate change could negatively impact the local populations' ability to adapt. Therefore understanding how populations perform in variable temperatures is important for understanding how local populations might adapt to climate change (Jensen *et al.* 2008). Although limited to F1 hybridisation effects, the present study clearly shows the merits in adopting a more complex spatially resolved approach to risk management of local populations. This is especially true of species where populations are likely to be locally adapted to their native environmental conditions or are at risk from outbreeding due to hybridisation with nonlocal conspecifics. A study to investigate genetic structure in a historically genetically distinct lineage of grayling in the north Adriatic found that this critically threatened population has become heavily introgressed with a more homogenous Danubian grayling population due to indiscriminate stocking efforts (Sušnik *et al.* 2004). Gharrett *et al* (1999) investigated outbreeding depression in F2 hybrids of pink salmon (*Onchorhynchus gorbuscha* W.) derived from two genetically distinct strains which are isolated based on even- and odd-year life cycles. They found that fewer F2 hybrids survived relative to F2 controls (Gharrett *et al.* 1999). Although the studies above do not involve domestic vs. wild interactions, they serve to reinforce the potential negative effects of hybridisation with genetically distinct or non-local populations.

Investigating the consequences of outbreeding and hybrid fitness on population integrity is vital to understand how wild populations will respond to hybridisation and introgression over time (Fraser *et al.* 2010). While hybridisation with novel farmed populations may initially cause an increase in genetic diversity (Glover *et al.* 2012), ultimately outbreeding depression via introgression may cause a loss of locally adapted wild population diversity and homogenisation with farmed genotypes, which could threaten population stability and potential to adapt to on-going environmental change. The success of introgression of escaped farmed salmon varies among rivers (Skaala *et al.* 2006; Glover *et al.* 2012; 2013). Thus, while our study focuses on F1 hybridisation, the significant population by temperature interaction observed, coupled with natural variation in river temperature may affect the level of hybridisation and competition when there are large differences in body size between farm and wild conspecifics. Here, we found no differences in growth between the two hybrid populations for all temperatures, and their growth was intermediate between wild and farmed parental populations. Harbicht *et al* (2014) investigated the effects of hybridisation on adaptive potential after multiple generations of selection in the wild in

transplanted combinations of wild, hybrid, and domesticated brook trout (*Salvelinus fontinalis* M.) in new environments. Following several generations, it was concluded that introduced foreign genes were lost, and the hybrid populations came to resemble the wild population (Harbicht *et al.* 2014). Fraser *et al* (2010) compared differences for a number of traits between farmed and wild Atlantic salmon and their multigenerational hybrids under common garden conditions. They found that wild backcrossing of hybrids did not completely restore trait distributions to their wild states (Fraser *et al.* 2010). Thus the consequences of multiple generations of hybridisation remains unclear, and further studies which investigate the effects of, specifically, multigenerational hybridisation on population fitness are required.

Comparative studies that use Atlantic salmon as a model species to investigate the consequences of hybridisation and introgression are important for the development of risk assessments and understanding of impacts for other aquaculture species. As aquaculture continues to expand worldwide through new production species, it will be important to focus on monitoring local populations to elucidate the integrity of genetic structure present, and how escapees might affect this. Despite constraints arising from the limited number of families per population examined here and the incomplete range of farm-wild hybrid crosses, clear trends in performance were evident among populations across treatments. Thus while we were able to document a G x E interaction, we acknowledge that further studies based on additional families and crosses would be beneficial. Studies which link the phenotypic differences observed in important life-history traits to their underlying genetic structure, such as through linkage mapping, will likely advance management and conservation of both wild populations and their farmed conspecifics.

Acknowledgements

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Supplementary Tables

Table S2.1: Experimental crosses. Nine different populations were used to make three experimental groups: 8 farmed families consisting of two pure commercial populations; 8 hybrid families consisting of two F1 hybrid populations; and 19 wild families consisting of five wild populations. In this table and throughout the study the hybrid crosses are referred to as maternal x paternal.

Family	Dam	Sire	Group	Population
1	A1	A9	Wild	Arna
2	A2	A10	Wild	Arna
3	A3	A11	Wild	Arna
4	A4	A12	Wild	Arna
9	Ski1	Ski3	Wild/Genebank	Skibotn
10	Ski1	Ski4	Wild/Genebank	Skibotn
11	Ski2	Ski3	Wild/Genebank	Skibotn
12	Ski2	Ski4	Wild/Genebank	Skibotn
13	F1	F11	Wild	Figgjo
14	F1	Farm1.11	Hybrid	Figgjo x Farm 1
17	F3	F13	Wild	Figgjo
18	F3	Farm1.13	Hybrid	Figgjo x Farm 1
19	F4	F14	Wild	Figgjo
20	F4	Farm1.14	Hybrid	Figgjo x Farm 1
25	F7	F17	Wild	Figgjo
26	F7	Farm1.17	Hybrid	Figgjo x Farm 1
32	Farm1.1	Farm1.11	Farm	Farm 1
34	Farm1.2	Farm1.12	Farm	Farm 1
36	Farm1.3	Farm1.13	Farm	Farm 1
38	Farm1.4	Farm1.14	Farm	Farm 1
53	Farm2.3	Farm2.11	Farm	Farm 2
54	Farm2.3	V11	Hybrid	Farm 2 x Vosso
55	Farm2.4	Farm2.12	Farm	Farm 2
56	Farm2.4	V12	Hybrid	Farm 2 x Vosso
57	Farm2.5	Farm2.13	Farm	Farm 2
58	Farm2.5	V13	Hybrid	Farm 2 x Vosso
59	Farm2.6	Farm2.14	Farm	Farm 2
60	Farm2.6	V14	Hybrid	Farm 2 x Vosso
66	V2	V10	Wild/ranched genebank	Vosso
67	V3	V11	Wild/ranched genebank	Vosso
68	V4	V12	Wild/ranched genebank	Vosso
69	V5	V13	Wild/ranched genebank	Vosso
75	Dr2	Dr7	Wild/ranched genebank	Driva
76	Dr2	Dr3	Wild/ranched genebank	Driva
78	Dr5	Dr7	Wild/ranched genebank	Driva

Table S2.2: Full model investigating egg size variation between populations at the different treatment temperatures. The variables in bold were retained in the final models for each treatment. Egg size is only retained in the low temperature treatment. The interaction term represents population: egg size (P x E).

			Random effects				**Fixed effects**					
Model	**N**	**Response**	**Variable**	**Chi.sq**	**Chi Df**	**P**	**Variable**	**Sum Sq**	**Num Df**	**Den Df**	**F**	**P**
Low (7°C)	1380	Log Weight +1	**1|Tank**	55.47	1	**<0.000**	P x E	0.064	8	17	0.52	0.827
			1|Fam	133.89	1	**<0.000**	**Population**	1.31	8	25	11.26	**0**
							Egg	0.12	1	25	7.6	**0.010**
Control (12°C)	1383	Log Weight +1	**1|Tank**	11.42	1	**0.001**	P x E	0.062	8	17	0.36	0.928
			1|Fam	105.07	1	**<0.000**	**Population**	3.55	8	26	20.63	**<0.000**
							Egg	0.018	1	25	0.84	0.368
High (16°C)		Log Weight +1	**1|Tank**	3.32	1	**0.068**	P x E	0.13	8	17	0.96	0.497
			1|Fam	135.27	1	**<0.000**	**Population**	1.82	8	26	13.47	**0.000**
							Egg	0.071	1	25	4.22	0.051

Table S2.3: Full model investigating weight variation where population is replaced by group. The variables in bold were retained in the final models for each treatment. The interactions included in the full model were: group: egg size (G x E), group : treatment (G x T), and treatment : egg size (T x E).

			Random effects				**Fixed effects**					
Model	N	Response	Variable	Chi.sq	Chi Df	P	Variable	Sum Sq	Num Df	Den Df	F	P
Group effects	4154	Log Weight	**1|Tank**	69.38	1	**<0.000**	G x E	0.027	2	28	0.76	0.475
			1|Family	432.35	1	**<0.000**	**Group**	0.87	2	31	25.24	**0.00**
							Treatment	53.03	2	26	1526	**<0.000**
							Egg size	0.057	1	31	3.26	0.081
							G x T	0.63	4	30	9.1	**0.0001**
							T x E	0.25	2	31	7.13	**0.003**

Chapter 3: Does density influence relative growth performance of farm, wild, and F1 hybrid Atlantic salmon in semi-natural and hatchery common garden conditions?

Abstract

The conditions encountered by Atlantic salmon, *Salmo salar* L., in aquaculture are markedly different from the natural environment. Typically, farmed salmon experience much higher densities than wild individuals, and may therefore have adapted to living in high densities. Previous studies have demonstrated that farmed salmon typically outgrow wild salmon by large ratios in the hatchery, but these differences are much less pronounced in the wild. Such divergence in growth may be explained partly by the offspring of wild salmon experiencing higher stress and thus lower growth when compared under high density farming conditions. Here, growth of farmed, wild and F1 hybrid salmon were studied at contrasting densities within a hatchery and semi-natural environment. Farmed salmon significantly outgrew hybrid and wild salmon in all treatments. Importantly however, the reaction norms were similar across treatments for all groups. Thus, the present study was unable to find evidence that the offspring of farmed salmon have adapted more readily to higher fish densities than wild salmon as a result of domestication. It is suggested that the substantially higher growth rate of farmed salmon observed in the hatchery compared to wild individuals may not solely be caused by differences in their ability to grow in high density hatchery scenarios.

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3.1 Introduction

Captive populations undergo various morphological, physiological and behavioural changes during domestication (Schütz *et al.* 2001). Adaptation to the domestic environment occurs through two routes: environmentally induced changes to developmental processes within a single generation and genetic change across generations (Ruzzante 1994; Price 1999). Relaxed natural selection can also result in domestic individuals that are more variable than wild conspecifics for certain traits which have adaptive value in the wild but less so in captivity (Mignon-Grasteau *et al.* 2005). For example, low mortality associated with domestic environments results in phenotypes persisting where they would not have persisted in the wild (Weber & Fausch 2003; Huntingford 2004). Genetic and morphological change occurs through direct and indirect responses to artificial selection and natural selection within the domestic environment contrasted with the wild environment (local adaptation in wild populations), and the differential mortality described above (Ruzzante 1994; Weber & Fausch 2003; Huntingford 2004). Random changes in allele frequencies can also arise through genetic drift in domestic populations with limited effective population sizes (Mignon-Grasteau *et al.* 2005). Thus, many domestic populations have become adapted to their captive environment, and may have reduced fitness in natural or novel environments when compared to wild individuals (Price 1999; Mignon-Grasteau *et al.* 2005). A loss of adaptive potential through domestication can negatively influence wild populations if domesticated individuals interbreed with wild conspecifics, such as when farmed individuals are released for restocking or are accidentally released through escape events.

Domesticated fish experience environments which differ vastly from those in nature in several ways (Weber & Fausch 2003; Huntingford 2004). Compared to the wild, hatchery environments typically display reduced environmental variation, fish densities are much higher, food is provided in excess, predation is absent, and there is no competition for mates (Einum & Fleming 2001; Jonsson & Jonsson 2006). Furthermore, there is often strong directional selection for a variety of commercially valuable traits such as growth rate and delayed maturation (Thodesen & Gjedrem 2006; Gjedrem 2010). The outcome is that domestic fish are different to wild fish for several behavioural, morphological and physiological traits (Weber & Fausch 2003), likely underlain by genetically-based as well as phenotypic plasticity (Jonsson & Jonsson 2006).

Atlantic salmon (*Salmo salar*, Linnaeus (1758)) are iteroparous fish native to rivers on the east and west coasts of the Atlantic Ocean in the Northern hemisphere (Klemetsen *et*

al. 2003). They typically display an anadromous life cycle, although some populations spend their entire life cycle in freshwater. Stream-dwelling populations of wild Atlantic salmon (*Salmo salar* L.) typically exhibit territoriality (Imre *et al.* 2005), and individual growth and survival are regulated through exploitative (indirect competition for communal resources) and interference (direct resource competition through dominance or fighting) competition (Post *et al.* 1999). The density of salmon tends to vary greatly among and within river systems (Webb *et al.* 2007). When densities are high, competition is exacerbated and the population is regulated by density-dependent mortality, emigration or displacement (Imre *et al.* 2005). Less commonly the territory size of an individual will decrease, causing individual growth to decrease. Thus, population regulation occurs through density dependent growth (Post *et al.* 1999), though this type of population regulation is more common in lake-dwelling fish where emigration is not possible (Imre *et al.* 2005). Studies show that when density in the wild is increased, individual growth decreases due to density-dependent factors (Imre *et al.* 2005; Bohlin *et al.* 2002).

Growth is an important component of fitness (Jonsson & Jonsson 2006), and body size is known to influence the outcome of social and resource competition (Post *et al.* 1999; Byström & García-Berthou 1999). Farmed Atlantic salmon have been under direct selection for fast growth for more than ten generations, and consequently the offspring of farmed salmon typically outgrow wild salmon by up to several fold under communal hatchery conditions (Fleming *et al.* 2002; Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b). In the wild, however, growth differences are far less pronounced (Fleming *et al.* 2000; Skaala *et al.* 2012; Reed *et al.* 2015). The lower growth and survival of farmed fish within wild environments may be due to the high metabolic costs associated with increased aggression or maladapted foraging behaviour of farmed escapees (Weber & Fausch 2003), or their inability to adapt to variable feed in the natural environment (Sundt-Hansen *et al.* 2012). Conversely high growth differences observed between farmed and wild fish in the hatchery might derive from adaptation of farmed salmon to high densities, typically fed to excess. Reduced response to stress relative to their wild conspecifics has been documented in domestic salmon (Solberg *et al.* 2013a) and sea trout (anadromous *Salmo trutta* L.) (Lepage *et al.* 2001). While the increased stress, competition and social interaction associated with high densities would intuitively result in decreased growth as described above, it is thus possible that the domestication process has resulted in farmed strains that maintain high growth at high densities.

Understanding how changing environmental conditions such as density affect growth and survival in domestic and wild conspecifics, and their hybrids, can increase our knowledge of the risks associated with escapees of farmed fish and the consequences of hybridisation. Here, a common garden design was used to investigate the growth of farmed, wild and F1 hybrid Atlantic salmon offspring at three contrasting densities within a hatchery, and at two contrasting densities under semi-natural conditions. The aim was to investigate whether differences in growth rates between farmed, wild and F1 hybrid salmon displayed similar reaction norms at different densities in the two environments. Specifically, the hypothesis tested was that the relative growth difference between farmed and wild salmon would be higher in the high density conditions as a result of adaptation of farmed salmon to those conditions.

3.2 Materials and methods

Contribution statement

This experiment was designed by Gary Carvalho, Kevin Glover and Martin Taylor. Initial experimental set-up was performed by Monica Solberg, Lise Dyrhovden, Ivar-Helge Matre and Alison Harvey. The sampling was carried out by Lise Dyrhovden, Ivar-Helge Matre, Alison Harvey, Gareth Juleff, Monica Solberg and Stian Morken. Subsets of samples were genotyped by Gareth Juleff (outdoor tank samples) and Laila Unneland (unassignable individuals). All other samples were genotyped by Alison Harvey. The statistical analysis and manuscript preparation was performed by Alison Harvey.

Family production

All families used in this experiment were established in November 2013 at Matre, the Institute of Marine Research's (IMR) experimental fish-farm in Norway. Atlantic salmon from the commercial farmed strain Mowi and wild caught Atlantic salmon from the River Etne (59°40'N, 5°56'E) were used to produce five pure farmed, five pure wild, and five F1 hybrid families (15 families in total) (Table S3.1). Mowi is the oldest Norwegian commercial strain and is used by Marine Harvest (Gjedrem *et al.* 1991). Mowi was established in the late 1960s primarily using fish from the River Vosso and the River Aaroy, whose populations are known to contain large multi-sea winter fish (Glover *et al.* 2009). The main traits that have been under selection in the Mowi strain are growth, late maturation and fillet quality. The farmed salmon used in this study had undergone over 10 generations of selection. The salmon population from the River Etne is the largest salmon population in Hordaland, western

Norway. A 2004 report estimated that the smolt production for the River Etne was around 30 000 individuals in a 15km area (Otterå *et al.* 2004). A study conducted using snorkelling observations and catch statistics for the period 2004-2011 estimated that the median number of wild fish in the Hardangerfjord river system (including the River Etne) was estimated to be 3.5 fish per 10 000m^2 (Vollset *et al.* 2014). The wild parental salmon were collected directly from the river in the autumn of 2013 by angling and transferred to the local hatchery where they were held until gametes were stripped from the fish. Fish scales were read from these individuals in order to ensure that they were wild fish and not farmed escapes (Lund & Hansen 1991). Population genetic analyses have revealed introgression of farmed salmon in a number of Norwegian populations, including the population in the river Etne (Glover *et al.* 2013; 2012). Therefore, although the wild fish used in this study were indeed born in the wild (based upon scale reading), it is not possible to completely exclude the possibility that some of those individuals used as broodstock may have admixed ancestries at some level.

All F1 hybrids were produced by crossing a farmed Mowi female with a wild Etne male (Mowi x Etne). The hybrids were thus maternal and paternal half-siblings with the farmed and wild families, respectively. From here on group refers to the origin of each cross-type, *i.e.* farmed, wild and hybrid.

Eyed eggs were sorted into hatchery trays representing the treatment replicates in week 5 of 2014 (where week 1= first week of January). The replicates were all incubated under standard hatchery conditions until transfer to tanks. Dead eggs were removed when necessary. In the hatchery treatments, the control and high density replicates initially consisted of 30 eggs each from the 15 families (n = 450 per tank) while each low density replicate consisted initially of 15 eggs each family (n = 225 per tank). In the semi-natural treatments the low density replicates consisted of 30 eggs from each family (n = 450 per tank) and the high density replicates consisted of 90 eggs per family (n = 1350 per tank). Egg volume measurements were taken from each family in order to calculate average family egg diameter. Egg diameter was calculated as 25cm divided by the number of eggs counted on a 25cm rule.

Experimental design

In order to investigate the effect of density and environment on growth and survival in salmon of farmed, wild and hybrid origin, fish were reared in communal fish tanks (*i.e.*, common garden) at three densities in a hatchery environment and at two densities in a semi-

natural environment. These treatment densities were chosen to represent densities that farmed and wild fish may not typically experience in their respective local environments, where typically the farming environment is characterised by much higher densities than the wild environment. For an overview of the experiment see Table 3.1. The treatments consisted of five differing rearing conditions: three hatchery treatments further differentiated into high, low, and control densities, and two semi-natural treatments consisting of high and low densities. Treatment from here on refers to the five different rearing conditions as described below.

Table 3.1: Details of the experimental design.

Treatment	**Hatchery**			**Semi-natural**	
	Low	**Control**	**High**	**Low**	**High**
Replicates (n)	2	2	2	2	2
Initial fish per replicate	225	450	450	450	1350
Families per replicate	5 farmed : 5 hybrid : 5 wild in all treatment replicate tanks				
Total fish	450	900	900	900	2700
Water level	55cm	55cm	13.5cm	25cm	25cm
Volume (m^3)	1.2375	1.2375	0.30375	7.85	7.85

Initial numbers of eggs per family within each replicate treatment and the water level and volumes of each treatment.

Hatchery treatments

Three treatments were set-up within a hatchery environment to represent (i) low density (~ 0.16 fish/L) (ii) a control density (~ 0.36 fish/L) which represented a standard hatchery density (iii) a high density environment (~1.5 fish/L). These are hereon referred to as the low, control, and high hatchery treatments. Each treatment consisted of two replicate tanks with 6 experimental hatchery tanks in total. The low density treatment was established by initially using half the number of fish used in the control and high treatments. The high density treatment consisted of the same initial number of fish as the control treatment with a water level 25% of the control water level (55cm) to simulate a high density environment.

Unfed-fry were transferred from the hatchery incubators to the experimental tanks in week 17, when treatment conditions commenced. The fish were reared in 1.5m^2 tanks with a maximum flow rate of 35L/min at ambient water temperature. Temperature was recorded daily and ranged from 4.5 to 14.4°C. Start feeding began in week 18, and fish were fed a commercial pellet diet (Skretting) *ad libitum*. Pellet size was adjusted according to

manufacturer's tables, and the fish were kept on 24 hour photoperiod throughout the experiment as is standard in salmon hatcheries.

Semi-natural treatments

The semi-natural environment consisted of replicate donut-shaped 7.85m^3 tanks (outer diameter 7m, inner diameter 3m) filled with gravel (of variable sizes to reflect a natural river bed, no larger than ~ 30cm in diameter) and situated outdoors (these are more fully described in Solberg *et al.* 2013b; 2015). Water level was kept at 25cm in both treatments. The density conditions were imposed by adding three times as many fish into the high density treatment (~ 0.11 fish/L) compared to the low density treatment (~ 0.05 fish/L). The treatments are from here on referred to as the low and high semi-natural treatments. Each treatment consisted of two replicate tanks; therefore there were 4 experimental semi-natural tanks.

Fish were planted out as fry into the semi-natural environment in week 17, when treatment conditions commenced. Automatic feeders were situated near the water inlet and fish were fed *ad libitum* as in the hatchery experiment. The fish experienced natural light conditions and ambient water temperature which ranged from 4.6 to 14.4°C across the experimental period (Supplementary Figure S3.1). Average daily temperature was used to calculate the degree days (cumulative average daily temperature over the experimental period) for the hatchery and semi-natural treatments. The semi-natural tanks were predator-free, *i.e.* no predators were explicitly placed within the tanks.

Sampling & data

The experiment ran for 20 weeks and was terminated in calendar week 37 of 2014. Mortality was recorded daily for each hatchery treatment replicate and was used to estimate total mortality at experiment termination. Average biomass within the hatchery treatments was estimated each month by measuring 100 randomly sampled fish in each replicate, which allowed for the estimation of stocking density within these treatments as the experiment progressed (Figure 3.1). It was not possible to record daily mortality in the outdoor semi-natural tanks, however total mortality was estimated using the number of surviving fish sampled at the end of the experiment. Mortality data are presented in Table 3.2. All remaining fish were euthanized with an overdose of Finquel Vet anaesthetic following standard guidelines (Årnes, Norway). Individual growth measurements of wet weight and fork length were recorded and adipose or caudal fin samples were taken from each individual

and stored in 100% ethanol. A total of 2105 individuals were sampled in the hatchery tanks and a total of 1883 individuals were sampled in the semi-natural tanks.

Table 3.2: Weight and mortality within treatments at experiment termination.

Treatment	Tank	N - sampled	Weight (g)		Density (kg/1000L)		Mortality (%)
			Mean	SD	First	Last	
Hatchery Low	1	205	35.06	11.28	0.16	5.99	9.9
	2	212	36.03	11.1			5.8
Hatchery Control	3	421	33.01	11.16	0.32	11.35	6.5
	4	422	33.72	11.94			6.7
Hatchery High	5	424	26.07	9.78	1.41	35.66	5.8
	6	421	25.2	8.57			6.5
Semi-natural Low	7	85	16.92	7.89	NA	0.33	81.2
	8	98	11.29	5.63			78.3
Semi-natural High	9	861	13.79	6.02	NA	2.8	36.3
	10	839	12.01	5.37			37.9

High and low correspond to the density of fish in the treatments, while control represents an intermediate density. First and last correspond to the first density calculated from average biomass per treatment taken in week 23 and the final density measurement calculated from final weight data taken in week 37.

Genotyping and Parentage Assignment

DNA-based parentage testing was used to assign individual fish from the hatchery and semi-natural treatments respectively back to their family of origin. DNA was extracted in 96 well plates using a variation of the salt extraction method (adapted from Aljanabi & Martinez 1997). Parental DNA was extracted and genotyped twice to ensure consistent genotyping. Each plate contained 2 randomly placed negative controls (blank wells) to ensure unique identification of each plate. Five microsatellites were amplified in a single PCR multiplex: *SsaF43* (Sanchez *et al.* 1996), *Ssa197* (O'Reilly *et al.* 1996), *SSsp3016* (Genbank # AY372820), *MHCI* (Grimholt *et al.* 2003), and *MHCII* (Stet *et al.* 2002). There were 38 individuals from the hatchery experiment and 82 individuals from the semi-natural experiment which could not be unambiguously assigned back to one family using the original multiplex. These samples were genotyped using additional loci (Supplementary Table S3.2) in order to unequivocally identify their families. PCR products were resolved on an ABI Applied Biosystems 3731 Genetic Analyser and sized using a 500LIZ standard (Applied Biosystems). Genemapper Version 4.0 was used to score alleles manually. Individuals were

then assigned back to family using the Family Analysis Program (FAP) (v3.6) (Taggart 2007).

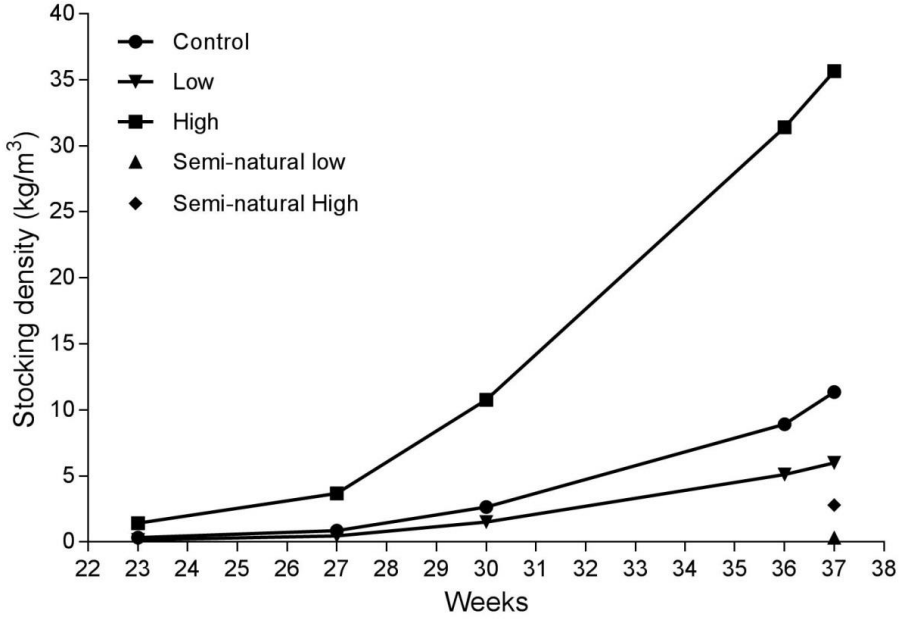

Figure 3.1: Average stocking density of the treatments. The stocking density was calculated by estimating average biomass per replicate by weighing a random sample of 100 fish from each tank at specific time points within the experiment duration. This was only possible for the hatchery tanks, and therefore only the stocking density at experiment termination is presented for the semi-natural tanks.

Statistical analysis

Statistical analysis was carried out using R version 3.1.3 (R Core Team 2015), and all critical p-values were set to 0.05 unless otherwise stated.

Growth

A linear mixed effect model (LME) was used to investigate the variation in weight at termination. The response variable was the continuous variable of log-transformed weight at termination. The LME model was fitted using *lmer* from the lmerTest package in R (Kuznetsova *et al.* 2014). The full model was fitted with treatment (T) and group (G) as fixed categorical factors, egg size (E) as a continuous fixed effect, and all two-way interactions between the fixed covariates: treatment and group (TG); treatment and egg (TE); and group and egg (GE) as fixed effects. Tank replicates (t) nested within treatments were included as a random intercept effect (10 levels), and family (f) was included as a random intercept effect (15 levels) with differing slopes for the effect of treatment:

$$Y = \beta_0 + \beta_1 T + \beta_2 G + \beta_3 E + \beta_4 TG + \beta_5 TE + \beta_6 GE + b_t + b_f + \varepsilon \text{ where } \varepsilon \sim N(0,\sigma^2) \quad (3.1)$$

where β_0 is the intercept and ε is the normally distributed error term. The lmerTest package in R allows for automatic model selection using the *step* function (Kuznetsova *et al.* 2014). The function performs backwards selection on both the fixed and random effects to determine the simplest best-fitting model (Kuznetsova *et al.* 2014):

$$Y = \beta_0 + \beta_1 T + \beta_2 G + \beta_3 E + \beta_4 TE + b_t + b_f + \varepsilon \text{ where } \varepsilon \sim N(0,\sigma 2) \quad (3.2)$$

It first performs backwards selection on the random elements of the model using likelihood ratio tests, with a significance level of 0.1 as a default, before performing backwards selection on the fixed elements in the model (Kuznetsova *et al.* 2014). The p-values generated for the fixed part of the model are calculated using an F-test based on Sattethwaite's approximation and the significance level is set to 0.05 (Kuznetsova *et al.* 2014). Both the full and final model fits were confirmed by investigating plots of the model residuals against the covariates included in the model as well as those which were not included in the model. Normality of the model residuals was confirmed visually using histograms. The full and final model with parameter estimates as given by the *lme4* output with overall covariate p values generated from the *step* function is presented in Table 3.3. Pair-wise comparisons of log weight between treatments and between groups were performed using the *glht* function in the *multcomp* package with Tukey adjustments for multiple comparisons (Table S3.3) (Hothorn *et al.* 2008). Pair-wise comparisons of egg size among the groups were performed using the *glht* function as above (Table S3.3). Relative growth differences comparing the average weight in grams and log weight of farmed to wild and hybrid to wild fish are presented for each treatment in Table 3.4.

Mortality

In order to investigate whether survival differed between treatments, a generalized linear mixed effect model (GLMM) was fitted using the *glmer* function in the lme4 package (Bates *et al.* 2014). The full model included the fixed factor covariates of treatment (T) and group (G), the continuous effect of egg size (E), and two-way interactions between the fixed covariates: treatment and egg (TE), treatment and group (TG) and group and egg size (GE). In order to control for any differences in mortality between replicates and families the variables tank (t) and family (f) were included in the model as random intercept covariates:

Table 3.3: Parameter estimates of the full model for the linear mixed model investigating log weight variation.

Covariate	Fixed effects	Parameter estimate	Std. error	t value	P value	Overall p value
	Intercept	1.64	0.05	35.51	0.00	
Treatment	**Hatchery Control**	**-0.02**	**0.06**	**-0.35**	**0.74**	
	Hatchery High	**-0.12**	**0.06**	**-2.01**	**0.09**	**0.00**
	Semi-natural High	**-0.45**	**0.06**	**-7.12**	**0.00**	
	Semi-natural Low	**-0.401**	**0.07**	**-5.92**	**0.00**	
Group	**Hybrid**	**-0.07**	**0.04**	**-1.99**	**0.07**	**0.00**
	Wild	**-0.27**	**0.04**	**-7.60**	**0.00**	
Egg size	**Egg size**	**-0.02**	**0.03**	**-0.68**	**0.51**	**0.00**
Treatment	Hatchery Control * Hybrid	-0.01	0.03	-0.25	0.80	
* Group	Hatchery High * Hybrid	-0.01	0.03	-0.16	0.87	
	Semi-natural High * Hybrid	0.00	0.04	-0.01	0.99	
	Semi-natural Low * Hybrid	0.03	0.06	0.45	0.66	0.58
	Hatchery Control * Wild	-0.03	0.03	-1.03	0.32	
	Hatchery High * Wild	-0.09	0.04	-2.31	0.04	
	Semi-natural High * Wild	-0.05	0.05	-1.11	0.29	
	Semi-natural Low * Wild	-0.05	0.06	-0.90	0.39	
Treatment	**Hatchery Control * Egg size**	**0.01**	**0.01**	**1.07**	**0.30**	
*** Egg size**	**Hatchery High * Egg size**	**0.03**	**0.02**	**2.22**	**0.05**	**0.03**
	Semi-natural High * Egg size	**0.07**	**0.02**	**3.40**	**0.01**	
	Semi-natural Low * Egg size	**0.07**	**0.02**	**2.91**	**0.01**	
Group	Hybrid * Egg size	0.05	0.04	1.27	0.24	0.48
* Egg size	Wild * Egg size	0.30	0.03	0.90	0.39	
	Random effects	**Variance**	**Std. dev**			
Family	Hatchery Low	0.002	0.044			
	Hatchery Control	0.001	0.025			
	Hatchery High	0.002	0.041			
	Semi-natural High	0.004	0.065			
	Semi-natural Low	0.004	0.062			
Tank		0.003	0.055			
Residual		0.020	0.150			

The final model (equation 3.2) covariates are presented in bold. The final column gives single p values estimated for each covariate in the full model using the *step* function in the lmerTest package by an F-test based on Sattethwaite's approximation. The significance level is set to 0.05 unless otherwise stated. Std. error; standard error of the parameter estimates. Std. dev; standard deviation of the variance estimates of the random effects.

$$\text{logit}(Y) = \beta_0 + \beta_1 T + \beta_2 G + \beta_3 E + \beta_4 TE + \beta_5 TG + \beta_6 GE + b_t + b_f + \varepsilon \qquad (3.3)$$

where β_0 is the intercept and ε is the error term. The response variable, survival, was binary, and thus the binomial distribution was used with the default logit link function and the model was fitted using the Laplace approximation. The random effect structure was investigated by fitting the full model with only one random effect at a time and plotting the 95% prediction intervals of the random effect using the *dotplot* function in the lattice package (Deepayan 2008). If all the prediction intervals of the random effect overlapped zero then this effect was removed from the final model. The mixed function from the afex package was used to investigate the significance of the fixed covariates (Singmann & Bolker 2014). The p –values of the fixed effects are presented in Table 3.5. The function calculates type 3-like p-values for each fixed covariate based on parametric bootstrapping (Singmann & Bolker 2014). The final model included covariates which yielded the best fit:

$$\text{logit}(Y) = \beta_0 + \beta_1 T + \beta_4 TE + b_f + \varepsilon \qquad (3.4)$$

Table 3.4: Relative weight and log weight differences between each group within each treatment.

	Treatment	Origin	W (g)	Relative W (g) difference		Log W	Relative Log W difference	
				to Wild	to Hybrid		to Wild	to Hybrid
Hatchery	Low	Farm	45.2	1.8	1.2	1.64	1.2	1.1
		Hybrid	36.45	1.5		1.55	1.1	
		Wild	24.74			1.37		
	Control	Farm	42.95	1.9	1.2	1.62	1.2	1.1
		Hybrid	34.53	1.5		1.52	1.2	
		Wild	22.71			1.32		
	High	Farm	33.51	2	1.2	1.51	1.3	1.1
		Hybrid	26.85	1.6		1.41	1.2	
		Wild	16.68			1.19		
Semi-natural	Low	Farm	19	2	1.3	1.24	1.3	1.1
		Hybrid	15.04	1.6		1.13	1.2	
		Wild	9.3			0.92		
	High	Farm	16.68	1.9	1.3	1.19	1.3	1.1
		Hybrid	13.15	1.5		1.08	1.2	
		Wild	8.99			0.92		

The relative growth differences were calculated by dividing the average weight (W) in grams of the farmed fish by the wild and hybrid fish respectively, and the average weight of the hybrid fish by the wild fish within each treatment. The relative log weight (Log W) differences were calculated as above using the average log weights (Log W) of each group within each treatment.

Table 3.5: Parameter estimates of the glmm model investigating variation in survival and overall p values of each model covariate.

Covariate	Fixed effects	Parameter estimate	Std. error	Z value	P value	Overall p value
Treatment	Intercept	2.46	0.33	7.56	0.00	**0.00**
	Hatchery High	**0.18**	**0.31**	**0.59**	**0.55**	
	Hatchery Low	**-0.73**	**0.36**	**-0.20**	**0.84**	
	Semi-natural High	**-1.97**	**0.22**	**-8.79**	**<2e-16**	
	Semi-natural Low	**-4.40**	**0.27**	**-16.13**	**<2e-16**	
Group	Hybrid	1.08	0.52	2.08	0.04	0.59
	Wild	-0.03	0.46	-0.07	0.95	
Egg size	Egg size	-4.90	80.79	-0.06	0.95	0.86
Treatment * Group	Hatchery High * Hybrid	-0.19	0.51	-0.38	0.71	0.08
	Hatchery Low * Hybrid	0.21	0.66	0.32	0.75	
	Semi-natural High * Hybrid	-0.78	0.38	-2.06	0.04	
	Semi-natural Low * Hybrid	-0.43	0.43	-1.01	0.31	
	Hatchery High * Wild	0.13	0.45	0.28	0.78	
	Hatchery Low * Wild	-0.27	0.51	-0.53	0.60	
	Semi-natural High * Wild	-0.07	0.33	-0.22	0.83	
	Semi-natural Low * Wild	0.75	0.38	1.97	0.05	
Treatment * Egg size	**Hatchery High * Egg size**	**53.54**	**31.76**	**1.69**	**0.09**	**0.00**
	Hatchery Low* Egg size	**-20.76**	**32.41**	**-0.64**	**0.52**	
	Semi-natural High * Egg size	**-4.02**	**21.54**	**-0.19**	**0.85**	
	Semi-natural Low * Egg size	**-84.36**	**25.48**	**-3.30**	**0.00**	
Group * Egg size	Hybrid * Egg size	74.19	107.60	0.69	0.49	0.89
	Wild * Egg size	47.56	82.28	0.58	0.56	
	Random effects	**Variance**	**Std. dev**			
	Tank	0.00	0.00			
	Family	**0.24**	**0.49**			
	Deviance	5331.30				

Covariates in bold were retained in the final model (equation 3.4). The final column gives single p values estimated for each covariate within the final model estimated using the *mixed* function in the afex package by parametric bootstrapping. Std. error; standard error of the parameter estimates of the fixed effects. Std. dev; standard deviation of the variance estimates of the random effects.

Ethical statement

The experimental (permit number 64472) was officially approved March 26 2014, by the Norwegian Animal Research Authority (NARA). All welfare and use of experimental animals was performed in strict accordance with the Norwegian Animal Welfare Act. In addition all personnel involved in this experiment had undergone training approved by the Norwegian Food Safety Authority, which is mandatory for all personnel running experiments involving animals included in the Animal Welfare Act.

3.3 Results

Genotyping & parentage assignment

Of the 3988 individuals sampled, 11 individuals (<0.001% of the total) could not be assigned unambiguously back to a single family using the microsatellite multiplexes. A further 4 individuals were identified as outliers due to extreme condition factors attributed to human recording error and subsequently removed from the dataset prior to analysis. Thus, a total of 3973 individuals were used in the analysis.

Statistical analysis

Growth

Treatment, group, egg size and the interaction of egg size and treatment were retained as significant effects in the growth model (Table 3.3). All genetic groups grew significantly different to each other across the treatments, with farmed fish being larger than hybrid and wild fish, and hybrid fish being larger than wild fish (Supplementary Table S3.3; Figure 3.2). On average, all fish grew larger in the hatchery density treatments and growth of all groups was lowest in the semi-natural density treatments (Figure 3.2). The interaction between treatment and group was not significant, indicating that all groups responded equally relative to the other groups across the treatments, indicated by the similar relative growth differences in Table 3.4 and the reaction norms in Figure 3.3. Within the hatchery treatments, growth of all three genetic groups decreased as density increased, with the lowest growth observed in the high density hatchery treatment, although the difference in growth between the hatchery treatments was not significant (Supplementary Table S3.3). Similarly growth was not significantly different between the two semi-natural treatments, although it was visibly lowest in the semi-natural high density treatment (Figure 3.2). The final model (Equation 3.2, Table 3.3) retained an effect of egg size and a significant interaction between egg size and treatment. Egg size was significantly different among the groups (Supplementary Table S3.3) and was found to be negatively correlated to weight. It was found that egg size was only a significant predictor of weight in the semi-natural treatments, as the fish in these treatments displayed the lowest weights, possibly due to a slower development compared to the hatchery treatments (Supplementary Table S3.4). There was a difference in degree days between the hatchery (1796 degree days) and the semi-natural treatments (1586 degree days) due to different ambient temperatures between the indoor (hatchery) and outdoor (semi-natural) tanks (Supplementary Figure S3.1). Egg size was also significant in the hatchery high density

treatment, where growth was also low. The random effects of tank replicate and family were retained in the final model in order to control for any variation within these variables.

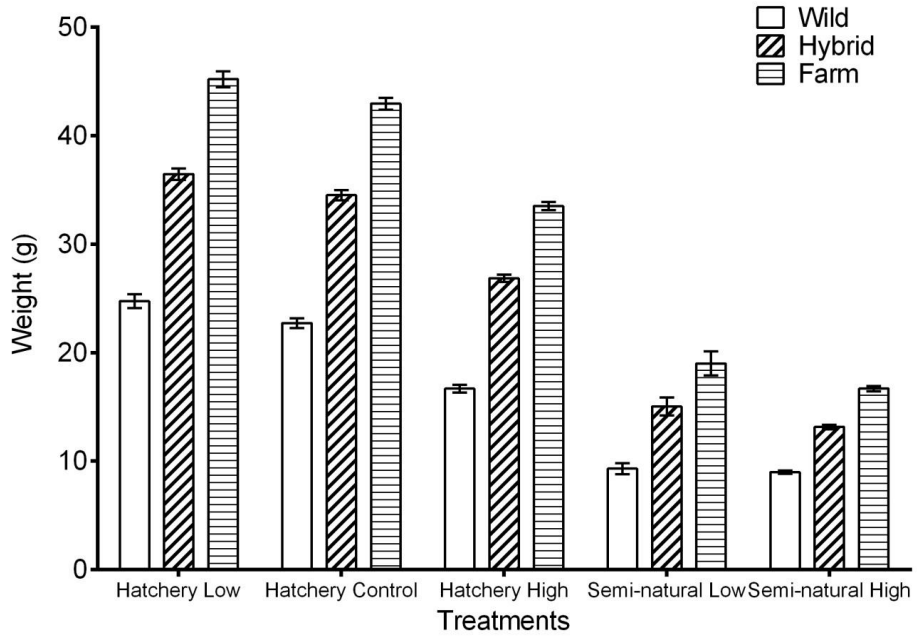

Figure 3.2: Average weights of each group within each treatment. Bars represent the standard error of the mean weight of each group within the treatments.

Mortality

Percentage survival was highest in the hatchery treatments, with no significant differences among treatments observed (Table 3.2, Figure 3.4). Within the semi-natural treatments for all groups, survival was highest in the high density treatment (Table 3.2, Figure 3.4). The low survival observed in the semi-natural low density treatment was not a result of high mortality in one specific replicate: the random effect of tank was excluded from the final model due to its non-significant effect; therefore mortality was insignificantly different between replicates within each treatment. The final model retained a significant effect of treatment and an interaction between egg size and treatment, while egg size alone was not significant (Equation 3.4, Table 3.5). On further analysis of the data split into each treatment, it was found that egg size was only significant in the hatchery high density treatment (Supplementary Table S3.5).

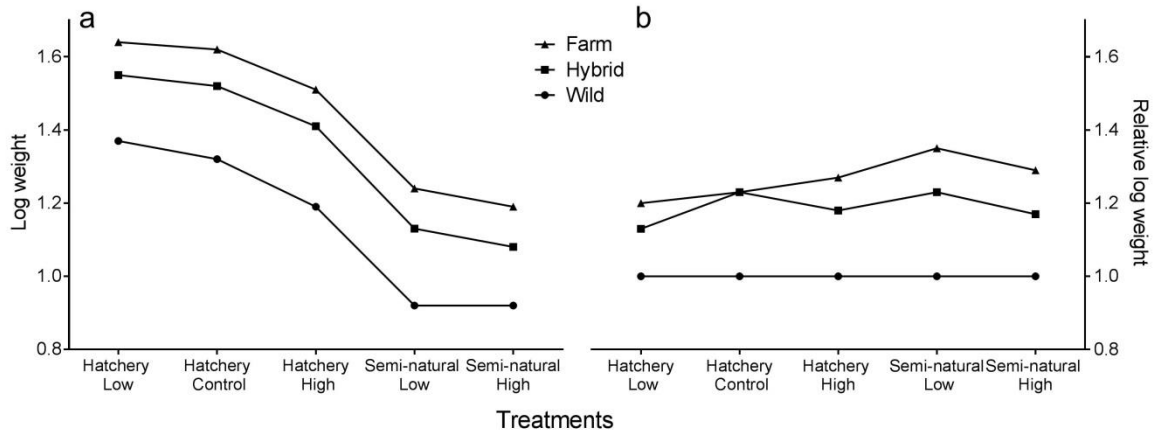

Figure 3.3: (a) Phenotypic growth reaction norms for each group across the treatments (showing average log weight) and (b) the average log weights relative to the wild group. In figure (b) the hybrid and farmed groups are compared to the wild group within each treatment. The x-axis shows the treatments.

3. 4 Discussion

Growth and survival of fish is influenced by density and availability of food (Refstie & Kittelsen 1976; Holm *et al.* 1990). The offspring of farmed Atlantic salmon generally outgrow wild salmon two-fold or more under hatchery conditions (Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b), possibly due to adaptation to high densities through domestication. Therefore, it was hypothesised that farmed salmon may be able to maintain higher growth than their wild conspecifics in high density environments, potentially explaining the elevated growth differences observed between farmed and wild conspecifics under hatchery conditions. Here, it was found that density influenced growth of all genetic groups equally, with all groups exhibiting decreased growth at higher densities; farmed salmon had the highest average growth within each treatment while wild fish had the lowest growth within each treatment; and the mortality of all groups was similar for all the treatments. Thus the present study was unable to find evidence of adaptation of farmed fish to high densities using the present treatment densities, tentatively suggesting that high density adaptation is not solely driving the divergence in growth observed between farmed and wild salmon under hatchery conditions.

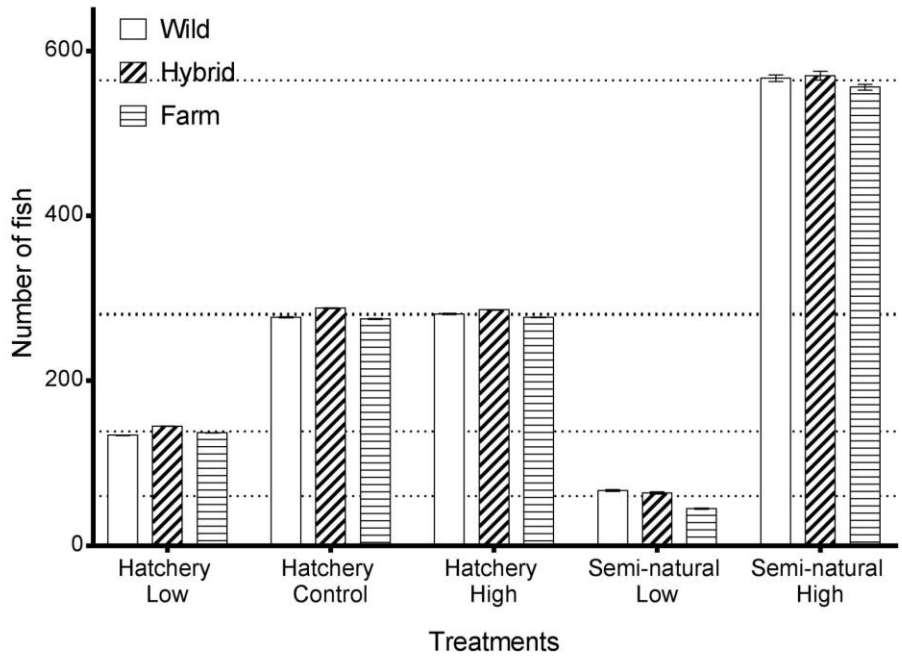

Figure 3.4: Average number of fish surviving for each group within each treatment. Dotted horizontal lines represent the expected number of surviving fish per group in each treatment based on average mortality. Error bars represent the standard error of the average family variation per group within each treatment.

Growth

High density conditions are known to lead to behavioural changes, induce stress behaviours and lower feed utilisation, all of which can decrease growth among fish (Montero *et al.* 1999). Refstie & Kittelsen Bohlin (1976) found that under controlled conditions with excess feed the growth of two domesticated populations of Atlantic salmon decreased as density increased. The negative effect of higher densities on growth has also been observed in other fish species (Bohlin *et al.* 2002; M'balaka *et al.* 2012), and has been attributed to an increase in intraspecific competition and agonistic behaviour at high densities (Jørgensen *et al.* 1993; Imre *et al.* 2005). In natural systems density dependent growth will also be controlled by the number of predators and by the competition for limited resources (Post *et al.* 1999). As there were no predators in the present study, and the available food was not limiting, it is likely that the lower growth observed at the higher densities could be the result of higher crowding stress in all groups which may have caused the fish to feed less effectively relative to the other treatments..

Growth and body size are important factors determining competition and reproductive success in fishes (Jonsson 1997). Directional selection for growth has resulted in farmed salmon displaying higher growth rates than wild salmon when compared under hatchery conditions (Solberg *et al.* 2013a), and this growth may give the offspring of escaped farmed salmon a competitive advantage over wild conspecifics in the wild, although often the growth differences observed in the wild are much lower (Fleming *et al.* 2000; Skaala *et al.* 2012). Under standard hatchery conditions the relative growth differences between farmed and wild conspecifics has been documented to be as high as 3-fold (Solberg *et al.* 2013a) and even 5-fold (Solberg *et al.* 2013b), with Glover *et al* (2009) observing that farmed salmon were twice as large as their wild conspecifics at the end of a full aquaculture production cycle. In the present study farmed salmon grew significantly larger than wild salmon in all treatments, although this growth difference was much lower than previously observed in a hatchery study using the same strains (Solberg *et al.* 2013a). Interestingly, Reed *et al* (2015) reported relatively moderate differences (5-20%) between farmed and wild salmon parr for size-at-age in the wild, and they found that their observed growth differences were similar in the hatchery environment as in the wild, contrasting the results of previously cited studies. They attribute these differences to the difference in historical selection regimes and generation time between the farmed strain used in their study (Irish farm strain derived from the Norwegian Mowi strain in 1983) compared to the other studies (more recent Norwegian Mowi strain) (Reed *et al.* 2015).

Solberg *et al* (2013a) found that juvenile farmed salmon exhibited a lower response to stress than their hybrid and wild conspecifics when exposed to a twice-daily stressor of lowered water levels, indicating that domestication has resulted in farmed salmon which are able to maintain a higher level of growth under stressful conditions. Elevated stress due to crowded conditions has been shown to negatively influence appetite and growth performance in Atlantic salmon (McCormick *et al.* 1998) and brown trout (Pickering & Stewart 1984). It is possible that the process of domestication may have adapted farmed salmon to higher growth under stressful high density conditions. Thus, farmed salmon in the present study would be expected to maintain a higher growth relative to the wild salmon at high densities within the hatchery treatment due to a relaxed response to crowding stress. However, this was not the case here. No evidence was found for an interaction between group and treatment (genotype by environment interaction) (Table 3.3), and the similar relative growth differences between groups among the treatments indicate that each group is responding to the treatments

similarly relative to the other groups (Table 3.4, Figure 3.3). It is acknowledged that the treatments used in the present study may not have been different enough to elicit a growth divergence response due to density adaptation and that such adaptations may manifest at higher densities; however the findings suggest that the higher growth differences observed in the hatchery are probably solely not the result of farmed fish being more adapted to growth at higher densities than wild fish.

The ability of an individual to adapt its behavioural strategy (plasticity) can influence fitness and competition (Brännäs *et al.* 2004). Many salmonids exhibit behavioural plasticity depending on the circumstance, for example exhibiting territorial behaviour in low densities, and schooling behaviour in high densities (Brännäs *et al.* 2004; Sundstrom *et al.* 2003). At certain densities it becomes too metabolically costly to defend a territory (Sundstrom *et al.* 2003; Brännäs *et al.* 2004). Under controlled conditions Brännäs et al (2004) found that interspecific competition among stocked brown trout depended on a variety of factors including competitive ability, food availability and prior residency. They found that growth of all groups was depressed at higher densities and it was advantageous to be less aggressive at high densities and also to be a larger individual (Brännäs *et al.* 2004). Farmed salmon are generally observed to be more aggressive than wild salmon, possibly inadvertently through selection for increased growth or because they have not been able to establish social or dominance hierarchies under hatchery conditions and may not understand the trade-off between aggression and its energetic cost in certain situations (Weber & Fausch 2003). Higher levels of growth hormone (GH) may also influence aggression in salmonids (Einum & Fleming 1997), and may also affect foraging behaviour and metabolic demands (Byström & García-Berthou 1999). These behavioural and hormonal changes within farmed salmon may partly explain their lower relative growth observed in the wild. Solberg *et al* (2013b) found that growth differences between farmed and wild conspecifics decreased along an environmental gradient from hatchery to semi-natural conditions with restricted feed. They suggest that the lower growth observed in wild studies could be caused by a combination of negative and positive size-selective mortality, whereby faster growing individuals can outcompete smaller individuals for resources (negative size selection) and where faster growing individuals are more prone to predation over smaller individuals (positive size selection), resulting in fish of all origins being of a similar size (although positive size selection was not explicitly tested in their study) (Solberg *et al.* 2013b).

In the present study growth was low among all groups in the semi-natural treatments (Figure 3.2), despite these two treatments having the lowest densities among all treatments. In the wild, salmonids are territorial and establish a social hierarchy among individuals which influence individual growth, with low-ranking fish having reduced access to feed and displaying reduced growth relative to the dominant individuals (Jørgensen *et al.* 1993). If the semi-natural environment induced territorial or dominance effects among the fish, one would then expect to see distinct size classes representing the larger, dominant fish, and the smaller, less dominant individuals. However, such trends were not observed. There was a difference in degree days between the hatchery and semi-natural treatments;it is therefore likely that other environmental conditions such as the naturally varying water temperature or ambient light conditions were responsible at least partly for the low growth observed in the semi-natural treatments. It is possible that the densities imposed on these semi-natural tanks were not sufficient to affect growth divergence among the groups. Jørgensen *et al* (1993) investigated the effects of density on hatchery-reared Arctic charr (*Salvelinus alpinus* L.) under controlled conditions. Interestingly, they found depressed growth rates in the low density treatment, and observed schooling behaviour of fish in their medium and high density tanks (Jørgensen *et al.* 1993). In the present study schooling behaviour was observed within the high density semi-natural replicates. While the low water temperature is probably the main reason behind the low growth observed in the semi-natural treatments, it is possible that the increased swimming behaviour and social interaction may have influenced growth.

In similar comparative studies of Atlantic salmon, hybrids often display intermediate levels of growth compared to their farmed and wild parental strains (Einum & Fleming 1997; McGinnity *et al.* 2003; Glover *et al.* 2009; Solberg *et al.* 2013b). Hybrid vigour commonly occurs when one or both of the parental strains are inbred, whereas a decreased performance observed in hybrids relative to their parents may occur via outbreeding depression (Einum & Fleming 1997). In the present study hybrids grew significantly different to both wild and farmed conspecifics, however there was an observable non-significant trend of hybrid relative growth being more similar to their farmed parents in each treatment (1.5-1.6:1 for hybrid to wild and 1.2-1.3:1 for farmed to hybrids using the average raw weight in grams) (Table 3.4, Figure 3.3). A study which used the same parental strains as the present study also observed intermediate hybrid growth and the same trend of more similar growth with the farmed parents (Solberg *et al.* 2013a). It is not thought that the growth levels observed in the present study represent hybrid vigour, as growth was significantly different among the groups

(Supplementary Table S3.3) and the relative growth differences between the hybrids and their parental groups are still intermediate (Table 3.5, Figure 3.3), indicating additive effects. It is acknowledged, however, that a more complete hybrid group design (*i.e.* reciprocal crosses) would allow for the unambiguous conclusion of additive hybrid growth effects. Several studies comparing gene transcription between farmed and wild salmonids observed some level of non-intermediate (non-additive) gene expression in hybrids (Roberge *et al.* 2008; Bicskei *et al.* 2014; although see Debes *et al.* 2012), and this may be population specific (Normandeau *et al.* 2009; Bougas *et al.* 2010). Bicskei *et al* (2014) examined gene transcription in farmed, F1 hybrid and wild Atlantic salmon at two life stages, and found fewer significantly differentially expressed transcripts between farmed and hybrid individuals than between hybrid and wild individuals. Their hybrid crosses were generated from the farmed females, and suggest that maternal effects might account for this bias (Bicskei *et al.* 2014). They found that the heritability patterns of many of the differentially expressed transcripts in the hybrid fish were either intermediate or maternally dominant (Bicskei *et al.* 2014), highlighting the need for reciprocal hybrid crosses in comparative studies. Maternal effects, such as egg size or maternal body size, can greatly influence offspring development and fitness (Mousseau & Fox 1998). Often maternal effects are taken into account in order to avoid overestimating or confusing genetic effects with environmental maternal effects (Heath *et al.* 1999). In the present study, the maternal effect of egg size was controlled for by including it as a covariate in the growth model.

Overall, egg size was found to be negatively influencing growth, due to the larger average egg sizes of the wild families used in the present study coupled with their lower growth compared to the farmed and hybrid families. Generally, a larger egg size is expected to convey a positive size advantage to offspring (Einum & Fleming 1999), however negative maternal effects have been observed in Chinook salmon (*Oncorhynchus tshawytscha* L.), whereby the initial positive effect of large egg size on growth was reversed after a period of time (Heath *et al.* 1999). The authors attribute this switch in egg size effect to variation in growth rate among families with different egg sizes (Heath *et al.* 1999). In the present study the growth model identified the interaction between egg size and treatment as a significant predictor of growth (Equation 3.2, Table 3.4), and when egg size was included in the models for growth at each treatment, it was found that it was only significant in the semi-natural treatments and the hatchery high density treatment. It is possible that the lack of degree days

meant the smaller fish had had less time to develop and had not yet overcome the effect of egg size, which is known to decrease with offspring development (Dunham 2004).

Mortality

Mortality within the hatchery treatments was low, and did not differ between treatments or between the groups (Table 3.5, Figure 3.4). There was high mortality observed within the low density semi-natural replicates (81.2 and 78.3 %, Table 3.2), and moderate mortality within the high density semi-natural tanks (36.3 and 37.9 %, Table 3.2). It is not possible to determine when the majority of this mortality occurred, or whether it was a gradual or acute event. It is therefore not possible to say how this may have influenced growth as the experiment continued. In natural conditions salmonids are territorial (Post *et al.* 1999) and this may impose a density dependent effect on mortality. Within a stream environment as population density increases past the carrying capacity for territories several processes can occur: territory size may decrease and influence growth through density dependence or those who are unable to acquire a territory and access to food may emigrate or die (Imre *et al.* 2005; Post *et al.* 1999). Generally, mortality is observed to be positively related to stocking density (Jørgensen *et al.* 1993), therefore it is unclear why it was the low density semi-natural replicates which suffered such high mortalities. There was no effect of group origin on mortality, indicating that all groups suffered similar relative mortalities (Figure 3.4). Interestingly, both replicates from each of the semi-natural treatments experienced similar mortality, indicating no influence of tank effects on mortality (Equation 3.4, Table 3.2). There was no observed predation from birds (I-H. Matre, pers. comm.). The mortality model identified treatment and the interaction between treatment and egg size as predictors of survival (Equation 3.4, Table 3.5). When the effect of egg size on mortality was investigated for each treatment, it was found that egg size was only significant within the high density hatchery treatment.

3.5 General implications

While comparing the relative growth of farmed, hybrid and wild salmon families under different densities, there was no evidence found to suggest that farmed salmon have adapted to higher stocking densities. Although the possibility cannot be excluded that higher and lower densities than those used in this study may elicit such effects, our treatments nevertheless elicited a response in modifying growth of all salmon reared here. The lack of interaction between density and relative growth of farmed, hybrid and wild salmon observed

here tentatively suggests that differences in relative growth between farmed, hybrid and wild salmon between the hatchery environment and the wild is caused by a complex of other factors, and not solely attributable to density. Competitive experiments in the wild at differing densities have suggested that farmed salmon display relatively greater mortality than wild salmon under higher densities (Skaala *et al.* 2012), and population genetic studies have demonstrated that the success of farmed salmon in the natural environment is also determined by native population density (Glover *et al.* 2012; 2013; Heino *et al.* 2015). It has been suggested that wild populations with lower densities (low population numbers) may be more at risk of the negative effects of hybridisation and introgression from farmed fish (Glover *et al.* 2012; Heino *et al.* 2015; Hansen *et al.* 2007). Comparative studies within a natural setting are needed in order to further understand what drives the growth differences between wild and farmed salmon in the wild. Furthermore, comparative studies at more varied densities are encouraged in order to further elucidate the effects of density on growth differences between farmed and wild conspecifics.

Studies investigating the performance of hybrids are crucial for understanding how hybridisation between farmed and wild conspecifics influences wild population dynamics. Farmed escapees can successfully interbreed with wild salmon, producing F1 hybrid offspring, and the subsequent performance of these hybrids will likely determine the future success of the wild population (McGinnity *et al.* 2003). Here, the hybrid growth was observably more similar to their farmed parents than their wild parents, which may influence their subsequent fitness in the wild. The hybrids in the present study were maternal half siblings to the farmed fish; therefore it is possible that maternal effects influenced growth patterns. It is important therefore to understand how hybrids respond to changing environmental conditions for future salmonid conservation and management, and to include reciprocal hybrids in order to differentiate between the effect of maternal egg size and the effects of domestication. Further studies which investigate the performance of backcrosses and reciprocal hybrids with wild fish will further elucidate the impacts of introgression on local population fitness.

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Supplementary tables and figures

Table S3.1: Family crosses for the experiment.

Family	Dam	Sire	Group
1	M1	M9	Farm
2	M1	E11	Hybrid
3	M2	M10	Farm
4	M2	E12	Hybrid
5	M3	M11	Farm
6	M3	E13	Hybrid
7	M4	M12	Farm
8	M4	E14	Hybrid
13	M7	M15	Farm
14	M7	E17	Hybrid
17	E1	E11	Wild
18	E2	E12	Wild
19	E3	E13	Wild
20	E4	E14	Wild
23	E7	E17	Wild

Table S3.2: Details of the microsatellite multiplexes used to assign the un-assignable individuals back to family.

Multiplex	Primers	Dye	Allele Size	No. Alleles	Direction	Sequences	References
1	SSsp2210	6FAM	124-176	14	F	AAG TAT TCA TGC ACA CAC ATT CAC TGC	Paterson et al. 2004
					R	CAA GAC CCT TTT TCC AAT GGG ATT C	
	SSspG7	PET	119-207	22	F	CTT GGT CCC GTT CTT ACG ACA ACC	Patterson et al. 2004
					R	TGC ACG CTG CTT GGT CCT TG	
	SsaD144	NED	102-254	37	F	TTG TGA AGG GGC TGA CTA AC	King et.al 2005
					R	TCA ATT GTT GGG TGC ACA TAG	
	Ssa202	6FAM	230-298	18	F	CTT GGA ATA TCT AGA ATA TGG C	O'Reilly et al. 1996
					R	GTT CAT GTG TTA ATG TTG CGT G	
	Sp2201	PET	227-367	33	F	TTA GAT GGT GGG ATA CTG GGA GGC	Patersson et al. 2004
					R	CGG GAG CCC CAT AAC CCT ACT AAT AAC	
	SsaD157	NED	271-411	35	F	ATC GAA ATG GAA CTT TTG AAT G	King et.al 2005
					R	GCT TAG GGC TGA GAG AGG AAT AC	
2	Ssa289	PET	112-134	10	F	CTT TAC AAA TAG ACA GAC T	McConnell et al. 1995
					R	GTC ATA CAG TCA CTA TCA TC	
	Ssa14	NED	134-146	6	F	CCT TTT GAC AGA TTT AGG ATT TC	McConnell et al. 1995
					R	CAA ACC AAA CAT ACC TAA AGC C	
	Ssa171	NED	197-255	26	F	TTA TTA TCC AAA GGG GTC AAA A	O'Reilly et al. 1996
					R	GAG GTC GCT GGG GTT TAC TAT	
	Sp2216	6FAM	190-270	21	F	GGC CCA GAC AGA TAA ACA AAC ACG C	Paterson et al. 2004
					R	GCC AAC AGC AGC ATC TAC ACC CAG	
	Sp1605	PET	216-268	22	F	CGT AAT GGA AGT CAG TGG ACT GG	Paterson et al. 2004
					R	CTG ATT TAG CTT TTT AGT GCC CAA TGC	

Table S3.3: Pair-wise comparisons of log weight conducted between groups and between treatments, and of the average egg size among groups. The p values have been adjusted for multiple comparisons using a Tukey adjustment. The Significance column denotes the p values as significance codes whereby '***' <0.0001, '**' <0.001, '*' <0.01, '.' ≤0.5 and 'ns' denotes not significantly different.

Contrast	Estimate	Std. Error	z value	P value	Significance
Hybrid - Farm	-0.09138	0.02071	-4.413	3.62e-05	***
Wild - Farm	-0.33787	0.02216	-15.245	1.00e-05	***
Wild - Hybrid	-0.24649	0.02254	-10.936	1.00e-05	***
Hatchery Control - Hatchery Low	-0.03282	0.05549	-0.591	0.9764	ns
Hatchery High - Hatchery Low	-0.15115	0.05672	-2.665	0.0593	ns
Semi-natural Low - Hatchery Low	-0.40929	0.0585	-6.997	<0.001	***
Semi-natural High - Hatchery Low	-0.4667	0.05722	-8.156	<0.001	***
Hatchery High - Hatchery Control	-0.11833	0.05605	-2.111	0.215	ns
Semi-natural Low - Hatchery Control	-0.37647	0.05844	-6.442	<0.001	***
Semi-natural High - Hatchery Control	-0.43388	0.05613	-7.729	<0.001	***
Semi-natural Low - Hatchery High	-0.25814	0.05718	-4.515	<0.001	***
Semi-natural High - Hatchery High	-0.31554	0.05655	-5.58	<0.001	***
Semi-natural High - Semi-natural Low	-0.0574	0.0577	-0.995	0.8577	ns
Hybrid egg size – Farm egg size	-0.00063	0.00021	-2.96	0.0087	**
Wild egg size – Farm egg size	0.0056	0.00021	26.28	<1e-04	***
Wild egg size – Hybrid egg size	0.0063	0.00021	29.61	<1e-04	***

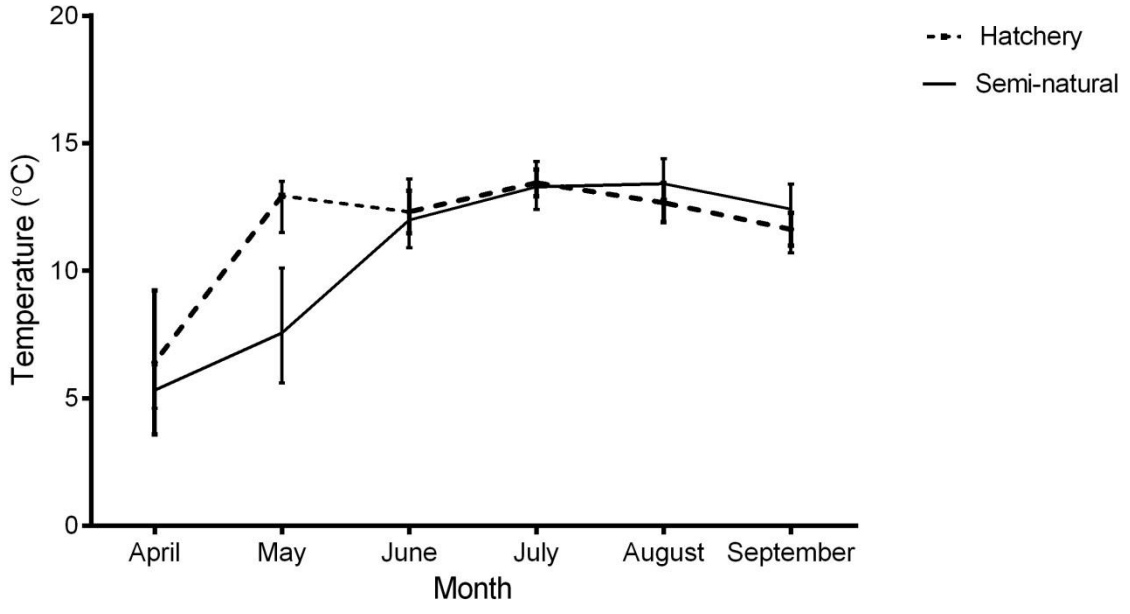
Table S3.4: Full models investigating relationship between weight and egg size variation at the different treatments. The variables in bold were retained in the final models for each treatment. Egg size is only retained in the semi-natural and hatchery high treatments.

			Random effects				**Fixed effects**					
Model	**N**	**Response**	**Variable**	**Chi.sq**	**Chi Df**	**P**	**Variable**	**Sum Sq**	**Num Df**	**Den Df**	**F**	**P**
Hatchery Low	416	Log Weight	**Family**	**243.58**	**1**	**<0.00**	Egg size	0.016	1	12.97	1.24	0.29
							Group	**1.10**	**2**	**11.94**	**43.08**	**0**
Hatchery Control	840	Log Weight	**Family**	**434.43**	**1**	**<0.00**	Egg size	0.013	1	11.16	1.80	0.21
							Group	**1.57**	**2**	**12.04**	**38.76**	**0**
Hatchery High	844	Log Weight	**Family**	**559**	**1**	**<0.00**	**Egg size**	**0.23**	**1**	**11.40**	**12.35**	**0.005**
							Group	**3.36**	**2**	**11.21**	**88.81**	**0**
Semi-natural Low	181	Log Weight	Family	1.1	1	0.29	**Egg size**	**0.86**	**1**	**NA**	**21**	**<0.00**
							Group	**3.47**	**2**	**NA**	**42.39**	**<0.00**
Semi-natural High		Log Weight	**Family**	**783.94**	**1**	**<0.00**	**Egg size**	**0.61**	**1**	**11.2**	**22.13**	**0.00006**
							Group	**2.35**	**2**	**11**	**42.74**	**0**

Table S3.5: Full models investigating relationship between survival and egg size variation at the different treatments. Egg size is only retained in the hatchery high density treatment.

Model	**Variable**	**Estimate**	**SE**	**Z**	***P value***
Hatchery Low	Intercept	2.5	0.18	14.04	<2e-06
	Egg size	-0.006	0.18	-0.035	0.97
Hatchery Control	Intercept	2.8	0.22	12.6	<2e-06
	Egg size	0.19	0.21	0.89	0.37
Hatchery High	Intercept	2.94	0.2	14.25	<2e-06
	Egg size	0.64	0.22	2.95	**0.003**
Semi-natural Low	Intercept	0.58	0.17	3.48	0.0005
	Egg size	0.17	0.17	1.01	0.29
Semi-natural High	Intercept	-1.48	0.17	-8.75	<2e-06
	Egg size	-0.19	0.17	-1.14	0.254

Figure S3.1: Average monthly water temperature for the indoor hatchery tanks and outdoor semi-natural tanks during the experimental period. Water temperature was recorded daily and is presented as monthly mean + range.



Chapter 4: Plasticity in response to feed availability - does feeding regime influence the relative growth performance of domesticated, wild and hybrid Atlantic salmon *Salmo salar* parr?

Abstract

Growth of farmed, wild and F1 hybrid Atlantic salmon parr, *Salmo salar,* was investigated under three contrasting feeding regimes in order to understand how varying levels of food availability affects relative growth. Treatments consisted of standard hatchery feeding (*ad libitum*), access to feed for 4h every day, and access to feed for 24h on three alternate days weekly. Mortality was low in all treatments, and food availability had no effect on survival of all groups. The offspring of farmed *S. salar* significantly outgrew the wild *S. salar,* while hybrids displayed intermediate growth. Furthermore, the relative growth differences between the farmed and wild *S. salar* did not change across feeding treatments, indicating a similar plasticity in response to feed availability. Although undertaken in a hatchery setting, these results suggest that food availability may not be the sole driver behind the observed reduced growth differences found between farmed and wild fishes under natural conditions.

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4.1 Introduction

Aquaculture is undergoing rapid expansion on a global scale. However, there is increasing evidence of a diverse array of negative consequences on both the natural environment and wild fish stocks (Naylor *et al.* 2000; McGinnity *et al.* 2003; Heuch *et al.* 2005). To ensure the sustainability of aquaculture, especially at a time when many natural populations continue to decline, greater understanding of the threats to wild populations and potential mitigation strategies is required. Specifically for Atlantic salmon *Salmo salar* (Linnaeus 1758) aquaculture, one of the world´s most socio-economically important farmed fishes, several challenges to sustainability have been identified, including, parasitic sea lice *Lepeophtheirus salmonis* (Krøyer 1837) and farm escapees (Taranger *et al.* 2014).

Each year, numerous farmed *S. salar* escape into the wild. While most escapees fail to recruit (Skilbrei *et al.* 2014), some enter rivers and attempt to spawn with wild *S. salar* (Lura & Saegrov 1991; Webb *et al.* 1993; Saegrov *et al.* 1997). Following successful spawning, genetic changes in native salmonid populations have been demonstrated in Ireland (Crozier 1993; Clifford *et al.* 1998), Canada (Bourret *et al.* 2011) and Norway (Skaala *et al.* 2006; Glover *et al.* 2012; 2013). Wild salmonid populations may be locally adapted to their native rivers (Taylor 1991; Garcia de Leaniz *et al.* 2007; Fraser *et al.* 2011), and experimental studies have demonstrated that offspring of farmed *S. salar* display significantly reduced survival in the wild compared to wild *S. salar* offspring (McGinnity *et al.* 1997; 2003; Fleming *et al.* 2000; Skaala *et al.* 2012). Such findings indicate that interbreeding of farmed escapees with wild fishes is likely to inflict a negative fitness effect upon the native population.

In addition to domestication selection (Glover *et al.* 2004), aquaculture species typically undergo directional selection for a variety of commercially important traits, for example increased growth and late maturation (Gjedrem 2000; 2010; Thodesen & Gjedrem 2006). The hatchery environment is typically characterised by high densities, a lack of predation, and continuous feed availability. Farmed *Salmo salar* have exhibited changes in behavioural traits such as increased aggression, higher stress resistance and decreased predator awareness that are attributed to inadvertent selection resulting from the artificial hatchery environment (Einum & Fleming 1997; Fleming & Einum 1997; Houde *et al.* 2010a; 2010b; Solberg *et al.* 2013a; Debes & Hutchings 2014). Thus, direct and indirect selection has resulted in domesticated fishes that are adapted to their captive environment and that

typically display traits which may be maladaptive in the wild relative to their wild counterparts.

Since *S. salar* farming began in the late 1960s, domestication selection has been primarily directed at growth, with gains of up to 15% per generation seen in farmed *S. salar* (Gjedrem *et al.* 1991; Thodesen & Gjedrem 2006). Increased growth has been linked to an increased appetite and food conversion efficiency in farmed *S. salar* (Thodesen *et al.* 1999; Gjedrem 2000). Growth is mediated by the growth hormone (GH) in most vertebrates, including fish (Björnsson 1997). Studies have documented higher levels of GH (Fleming *et al.* 2002) and IGF-I (insulin-like growth factor I) (Solberg *et al.* 2012; although no changes were detected in Bicskei *et al.* 2014) in farmed *S. salar* compared to wild conspecifics, suggesting that selection for growth in farmed fishes stimulates shifts in endocrine control. Growth hormone influences appetite, feed conversion efficiency, foraging behaviour (through increased movement and risk taking), and may influence aggression (Neregård *et al.* 2008a; 2008b). Farmed *S. salar* exhibit differences relative to wild *S. salar* in all of the above behavioural traits (Fleming & Einum 1997; Thodesen *et al.* 1999; Houde *et al.* 2010a), supporting the endocrine findings of Fleming *et al*. (2002) and Solberg *et al*. (2012). Increased GH levels are also linked to a higher metabolism (Björnsson 1997). It has been suggested that higher levels of growth may incur a metabolic cost when resources are low or predation levels are high, such as in the wild (Sundt-Hansen *et al.* 2009). For example Sundt-Hansen *et al.* (2012) found that while GH-treated *S. salar* grew optimally under standard hatchery conditions (*ad libitum* feeding) their growth was negatively affected by the GH treatment under natural stream conditions.

When studied under hatchery conditions, growth differences of up to 2-3 fold exist between offspring of farmed and wild *S. salar* (Fleming & Einum 1997; Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b). In contrast, studies in the wild have shown that growth differences between farmed and wild *S. salar* are lower than in hatchery-reared *S. salar* (Fleming *et al.* 2000; Skaala *et al.* 2012). Thus, the question arises: what causes such differences in the relative growth rates of wild and farmed *S. salar*? Several potential explanations exist, including behavioural changes associated with higher growth in farmed *S. salar*, such as less efficient foraging behaviour, increased aggression and higher risk behaviour. Such behaviours will incur a higher metabolic cost, thus, while faster growth is often linked to higher fitness, such behavioural-mediated trade-offs may limit growth and survival of individuals with higher growth rates in the wild through reduced starvation

tolerance and increased predation risk (Martin-Smith *et al.* 2004; Biro *et al.* 2006). An especially pertinent factor influencing growth differences between farmed and wild fishes is variation in resource availability, specifically levels of food availability between the hatchery and the wild. Under standard hatchery conditions feed is readily available, and thus not limiting growth, while the frequency and nature of food in the wild is often more heterogeneous in time and space (Jonsson & Jonsson 2011a). It is possible that generations of direct and inadvertent domestication selection in farmed fishes will have resulted in a decreased ability to cope with the typically variable feed availability in the wild environment. Elucidating the factors influencing the ability of escaped farmed fishes in the wild to forage effectively crucially represents a key component of risk assessment.

In order to elucidate the potential mechanisms underlying the observed larger growth rate of farmed vs. wild fishes in the hatchery, contrary to trends detected in the wild, here the influence of varying levels of food availability on relative growth performance was examined. Growth of farmed, wild and F1 hybrid *S. salar* under three feeding regimes differing in availability and frequency of feed were examined under hatchery conditions. A gradient of feed availability were selected, ranging from the farmed environment (*ad libitum*) towards the wild environment (patchy and restricted).

4.2 Materials and methods

Contribution statement

This experiment was designed by Gary Carvalho, Kevin Glover, Martin Taylor and Monica Solberg. Initial experimental set-up was performed by Monica Solberg, Lise Dyrhovden, Ivar-Helge Matre and Alison Harvey. The sampling was carried out by Alison Harvey, Gareth Juleff and Monica Solberg. The statistical analysis and manuscript preparation was performed by Alison Harvey.

Family production

The farmed, hybrid and wild *S. salar* families used in this study were produced in November 2013 (week 46) at Matre Research station, Institute of Marine Research (IMR), Norway. *Salmo salar* originating from the commercial farmed Mowi strain, and wild *S. salar* caught in the River Etne (59°40'N, 5°56'E), were used to produce seven pure farmed, seven pure wild, and seven F1 hybrid families (Table S4.1). Mowi represents one of the oldest Norwegian domestic *S. salar* strains (Gjedrem *et al.* 1991) and has been selected for, among

other traits, increased growth rate, and is known to display significantly higher growth rates under standard hatchery conditions in comparison with the offspring of wild *S. salar* (Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b). However, in the wild, this farmed strain only displays slightly higher growth rates than the offspring of wild *S. salar* (Skaala *et al.* 2012). The three strains are from here on referred to as farmed, wild and hybrid groups.

The *S. salar* stock in the River Etne is the largest in Hordaland, Norway. Wild adult broodstock were collected in this river in the autumn of 2013 by angling, and transferred to the local hatchery and held until stripping of gametes. Fish scales were read on individuals to validate that they were indeed born in the wild and were not farmed escapees (Lund & Hansen 1991). The F1 hybrid *S. salar* were produced by crossing farmed females and wild males (Mowi ♀ x Etne ♂). Five of the seven hybrid families were maternal and paternal half-siblings with the farmed and wild families, respectively. One hybrid family was paternal half-siblings to one wild family and one hybrid family was maternal half-siblings to one farmed family.

Eyed eggs from families were sorted into hatchery trays representing the single-strain replicate treatments in week 5 of 2014. Each replicate treatment consisted of 20 eggs per family of each group, yielding 140 eggs in each of 18 tanks. Each replicate was start-fed and thereafter reared in 1.5 m^3 tanks at ambient water temperature (varying from 12.5 to 13°C during the experimental period). The treatments began when start feeding commenced in week 18, with fish fed on Skretting Nutra pellets (www.Skretting.com), which were size adjusted according to manufacturer's tables. The *S. salar* were kept on a 24 h photoperiod from transfer to tanks until experiment termination as per standard hatchery conditions, also known to reduce the development of precocious males (Good *et al.* 2015).

Experimental design

Salmo salar were reared in single-strain treatment tanks (two replicates/ treatment) with three contrasting feeding regimes (Table 4.1). The first treatment was regarded as the standard hatchery control, and involved feeding *S. salar* continually with automatic feeders 24 h a day, every day, with an excess ration. The second treatment consisted of providing *S. salar* with an excess ration for 4 h every day (thus 20 h without any feeding each day), referred to as the daily restricted treatment. The third treatment involved feeding an excess ration for 24 h on three alternative days in a week (Monday, Wednesday, and Friday), referred to as the triweekly treatment. The selected gradient of feed availability, ranging from

the farmed environment (*ad libitum*) towards the wild environment [patchy and restricted (Jonsson & Jonsson 2011a)] was designed in order to elucidate how growth differences between strains change with variable levels of food availability. Thus, treatments were chosen to represent a gradient in feeding opportunity from standard excess hatchery ration (treatment 1) to a more limited feed supply (treatment 3). Treatments are referred to as the control, daily restricted and triweekly treatments respectively.

Table 4.1: Experiment design. Average mass (M), standard deviations (S.D.) and mortality are presented for each group within each tank replicate and pooled treatment. The pooled mass and mortality were calculated as averages of the total mass and mortality in the replicates of each treatment.

Treatment	Group	Tank	Initial *n*	Sampled *n*	Mean M (g)	S.D. (±)	Pooled W	Mortality *n*	Pooled mortality (%)
Daily Restricted	Farm	1	20 eggs per family - 140 fish per tank	125	24.5	4.9	24.5	15	9.3
		2		129	24.5	4.9		11	
	Hybrid	3		117	19.4	5.7	19.4	23	10.4
		4		134	19.3	5.4		6	
	Wild	5		136	15.8	6.2	16.1	4	3.2
		6		135	16.5	5.4		5	
Control	Farm	7	20 eggs per family - 140 fish per tank	127	43.7	12.0	43.1	13	9.6
		8		126	42.5	10.6		14	
	Hybrid	9		131	34.4	8.8	36.4	9	8.6
		10		125	38.3	9.2		15	
	Wild	11		125	28.7	9.3	28.9	15	9.3
		12		129	29.0	9.0		11	
Triweekly	Farm	13	20 eggs per family - 140 fish per tank	127	36.4	8.7	36.1	13	6.4
		14		135	35.8	7.9		5	
	Hybrid	15		130	31.9	8.0	30.9	10	7.1
		16		130	29.9	7.1		10	
	Wild	17		134	22.4	8.5	22.6	6	4.3
		18		134	22.9	9.1		6	

The experiment was continued for 20 weeks, and terminated in week 37, 2014 *i.e. S. salar* were reared from egg to the parr stage. Upon termination, all *S. salar* in each tank replicate were euthanised with an overdose of Finquel® Vet anaesthetic (http://www.aqui-s.com, Årnes, Norway), and recordings of individual wet mass and fork length (L_F) were measured. A total of 2329 individuals were sampled.

Statistical analysis

Statistical analysis was carried out using R version 3.2.2, and all critical P-values were set to 0.05 unless otherwise stated (R Core Team 2015).

Mortality from week 5 (sorting into hatchery trays) to week 18 (commencement of experimental treatments) was low overall (<0.02%). Mortality for each tank was recorded during the experimental period. To investigate whether different feeding regimes or group

origin had any effect on survival, a generalized linear mixed effect model (GLMM) was fitted using the *glmer* function in the *lme4* package (Bates *et al.* 2014). The full model included the fixed covariates of group (G = representing the three groups; farmed, hybrid, and wild), treatment (T = representing the three feed treatments; control, daily restricted, and triweekly), and their interaction term (T_G). Tank was included in the model as a random intercept covariate (b_t):

$$\text{logit}(Y) = \beta_0 + \beta_1 T + \beta_2 G + \beta_3 T_G + b_t + \varepsilon \qquad (4.1)$$

where β_0 is the model intercept and ε is a random error term. The response variable, survival, was binary, and thus a binomial distribution was used, with the default logit link function. The random effect structure was investigated by plotting the 95% prediction intervals of the random effect using the *dotplot* function of the lattice package. If any of the tanks did not overlap zero, the effect was retained in the model. The *mixed* function from the afex package was used to investigate the significance of the fixed covariates (Singmann & Bolker 2014). The function calculates type 3-like *P*-values for each fixed covariate based on parametric bootstrapping (Singmann & Bolker 2014).

A linear mixed model (LME) was used to investigate the effect of group origin and feeding regime treatment on mass at termination. The response variable was logged mass at termination. The full model covariates were identical to the mortality model described above:

$$Y = \beta_0 + \beta_1 T + \beta_2 G + \beta_3 T_G + b_t + \varepsilon \text{ where } \varepsilon \sim N(0, \sigma 2) \qquad (4.2)$$

where β_0 is the model intercept and ε is the normally distributed error term. The LME model was fitted using *lmer* from the *lme4* package in R (Bates *et al.* 2014). The random effects structure was investigated as described above; similarly the *P*–values for the fixed effects were calculated as above while using the Kenward-Roger approximation for degrees of freedom.

Post-hoc multiple comparisons were carried out using the function *pairs* in the *lsmeans* package with a Tukey adjustment for multiple comparisons, which calculates the differences of least squares means for the factor covariates of the fixed part of the final model (Lenth 2016). The test computes all pair-wise comparisons of the interaction terms (Group x Treatment), and reports *P*-values and 95% confidence intervals for all comparisons (Lenth 2016).

Ethical statement

The experimental protocol (permit number 6447) was approved 23 March 2014, by the Norwegian Animal Research Authority (NARA). All welfare and use of experimental animals was performed in strict accordance with the Norwegian Animal Welfare Act. In addition all personnel involved in this experiment had undergone training approved by the Norwegian Food Safety Authority, which is mandatory for all personnel running experiments involving animals included in the Animal Welfare Act.

4.3 Results

Sampling & data

The experiment was terminated in week 37 of 2014, when 2329 *S. salar* were sampled from the 18 tanks. Five individuals were identified as outliers due to extreme condition factors caused by recording errors and removed from the dataset prior to statistical analysis, thus the final dataset consisted of 2324 *S. salar*.

Mortality

Overall, mortality within each treatment was low, ranging from 3.2 to 10.4 % (Table 4.1), typically within the range observed from start-feeding to first autumn stage. None of the fixed effects were found to be significant (Table 4.2), thus mortality did not differ between treatments or between strains. The random effect of tank replicate was found to be significant and thus controlled for by being retained in the final model.

Table 4.2: *P* values of the fixed effects of the GLMM model investigating survival. The Statistic represents a Chi-square value calculated as two- times the difference in likelihood between full and restricted model as specified by the *afex* package.

Effect	Statistic	*P* value
Treatment	1.47	0.57
Group	0.08	0.97
T x G	4.45	0.57

Growth

All *Salmo salar* (*i.e.*, farmed, hybrid and wild) grew better in the control treatment than in the two more restricted treatments, and growth within all groups was observed to be lowest in the daily restricted treatment (Table 4.3; Fig. 4.1). Farmed *S. salar* were larger than

both hybrid and wild *S. salar* at each treatment, and the hybrids displayed intermediate growth (Fig. 4.1).

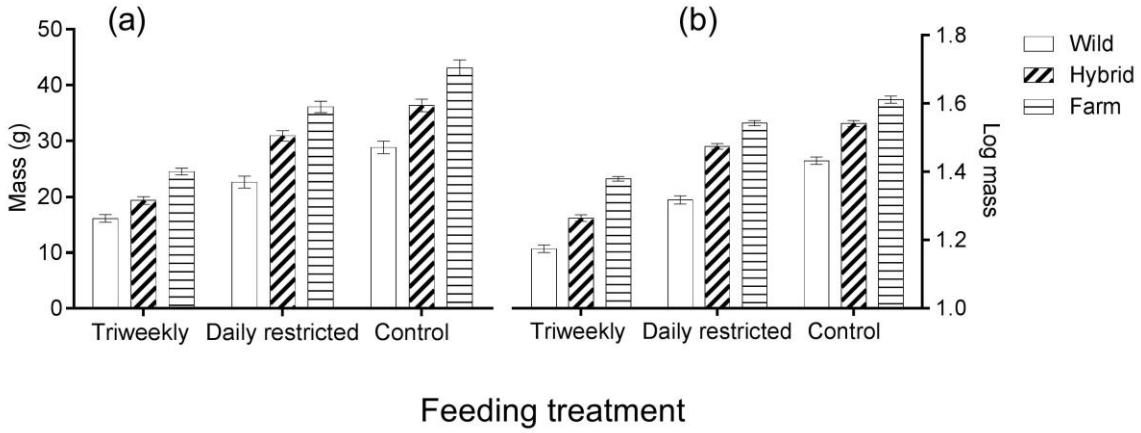

Figure 4.1: Average (a) mass and (b) $\log_L$ mass ± S. E of each group (farm, hybrid and wild Atlantic *Salmo salar*) across each feeding treatment (triweekly, daily restricted, control/*ad libitum*). Log mass was examined in the statistical analysis.

There was a marginally significant treatment-by-group interaction effect detected (*P*=0.05); however the relative growth differences between the groups across treatments were very similar (Table S4.2, Fig. 4.2). The relative growth differences between the wild and farmed *S. salar* were almost identical across treatments (1:1.5-1.6), as were the relative growth differences between hybrid and farmed *S. salar* (1:1.2-1.3) (Table S4.2, Fig. 4.2). Relative growth differences between the wild and hybrid *S. salar* increase incrementally from the daily restricted treatment (1:1.2) through the control treatment (1:1.3) to the triweekly treatment (1:1.4) (Fig 4.2), which is probably driving the marginally significant interaction of group and treatment in the LME model (*P*=0.05). Possible variation between tank replicates was taken into account in the initial model by including replicate as a random effect which was retained in the final model despite the model output suggesting it be dropped due to lack of effect.

Table 4.3: *P* values of the fixed effects of the LME model investigating growth. The F denotes the F statistic, Num Df denotes the numerator degrees of freedom and Den Df denotes the denominator degrees of freedom.

Effect	**F**	**Num Df**	**Den Df**	***P* value**
Treatment	129.39	2	9.12	<0.0001
Group	74.32	2	9.25	<0.0001
T x G	3.67	4	8.99	0.05

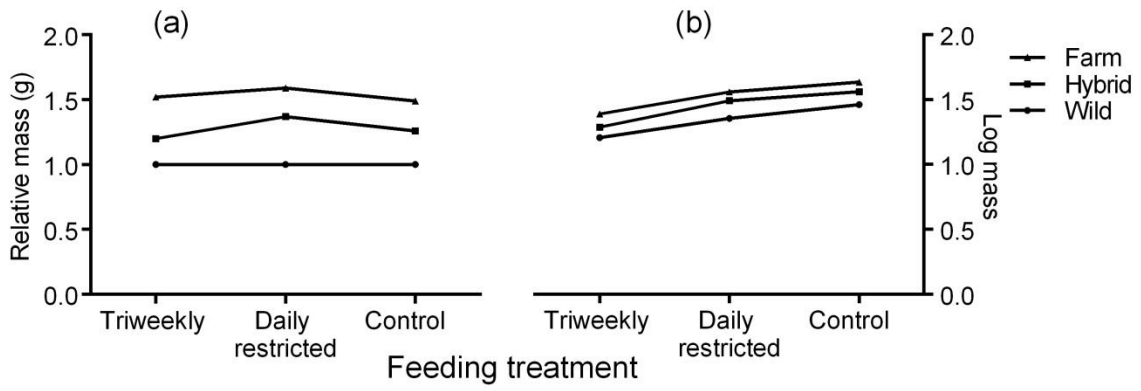

Figure 4.2: (a) Relative growth reaction norms for each group (farm, hybrid and wild Atlantic *Salmo salar*) and (b) their average log mass across the feeding treatments. In (a) the hybrid and farmed groups are compared to the wild group within each treatment (based upon their untransformed mass). The x-axis shows the feeding treatments (triweekly, daily restricted, control/*ad libitum*).

4.4 Discussion

The present study investigated the effect of feed variability on growth and survival of farmed, wild and F1 hybrid *S. salar* reared in single strain tanks. Understanding how farmed escapees interact with wild conspecifics is an important part of developing management and mitigation efforts for both conservationists and the aquaculture industry. In the hatchery, farmed *S. salar* typically outgrow wild *S. salar* markedly (Fleming & Einum 1997; Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b), while in the wild, corresponding growth differences are much lower (Fleming *et al.* 2002; Skaala *et al.* 2012). A striking difference between the farm and wild environments is the levels of food availability; constant *versus* varying in time and space (Jonsson & Jonsson 2011a). It is possible that plasticity in response to variable feed supply differs between farmed and wild fishes, which may potentially contribute to the contrast in growth differences observed between farmed and wild fishes in each environment. Here, although a marginally significant interaction was found between group and treatment, similar growth differences were observed between the farmed and wild *S. salar* across the feed availability gradient ranging from the farmed environment (*ad libitum*) to conditions more resembling the wild environment (patchy and restricted). Thus *S. salar* of both origins responded in a comparable manner relative to the varying levels of food availability, indicating a similar plasticity in response to feed availability. Mortality was low both within and among the treatments, indicating no effect of treatment or group origin on survival.

River environmental conditions, such as fluctuating natural food availability, can adversely affect the growth of fast growing fishes due to metabolic costs (Sundt-Hansen *et al.* 2012). In the present study growth of the farmed, hybrid and wild *S. salar* decreased along a food availability gradient ranging from the farmed environment to conditions more resembling the fluctuating levels in the wild. Lowest growth was observed in the daily restricted feeding regime -the most variable food availability. Growth was significantly different between the groups at all treatments, indicating an effect of feed availability on growth in all groups. Farmed *S. salar* were significantly larger than the wild *S. salar* in all treatments, and hybrid growth was intermediate between the farmed and wild *S. salar*. Despite differing growth rates, farmed and wild *S. salar* responded identically to the increasingly variable food supply, as shown by the similar relative growth differences and low mortality observed across the treatments. This indicates that more than 10 generations of directional selection with contentious access to feed has not resulted in farmed *S. salar* displaying reduced abilities to cope with fluctuating and/or restricted levels of feed by not being able to maintain their elevated growth rate as compared to wild *S. salar*. Morris *et al.* (2011) found that the response to compensatory growth (CG) in farmed, wild and hybrid (including backcrossed) *S. salar* was similar between the groups, although the mean control and CG growth rates were highest in the farmed group. This indicates that although selection has acted on growth, farmed *S. salar* have not lost their plastic ability to respond to a lack of food through compensation by increasing their growth rates when food becomes available (Morris *et al.* 2011).

The growth differences between farmed and wild *S. salar* observed in all treatments were, on average, less than previously documented in hatchery studies (Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b). It is still evident however that multiple generations of selection for growth in farmed *S. salar* have resulted in significant elevated growth relative to wild *S. salar*. Under typical hatchery conditions, where food supply is constant, generally uniform and plentiful, growth differences between farmed and wild *S. salar*, as much as 3- to 5-fold, have been observed (Solberg *et al.* 2013a; 2013b). Glover *et al.* (2009) investigated various trait differences between farmed, wild and F1 hybrid *S. salar* throughout the farming production cycle, including growth. For two experimental cohorts they found that at the freshwater stage the wild *S. salar* had mean weights of 1:1.6 and 1:2.4 relative to the farmed *S. salar*. However in nature, farmed and wild *S. salar* grow more similarly. For example, Skaala *et al.* (2012) found growth differences within three year classes of wild and farmed *S.*

salar in the wild to be just 1:1.07, 1:1.25 and 1:1.06 respectively. In an attempt to understand these growth differences, Solberg *et al.* (2013*b*) investigated the competitive balance between farmed, wild and hybrid *S. salar* by comparing growth in standard hatchery conditions, and restricted feed conditions in the hatchery and semi-natural environments. They found that the growth of farmed, hybrid and wild *S. salar* became more similar as their environmental conditions approached natural conditions. They hypothesised that the reduced growth differences observed in their study and in the wild (Skaala *et al.* 2012) could be due to size-selective mortality. The wild environment favours the survival of faster growing individuals which can out-compete smaller individuals for resources (negative size-selective mortality), while also selecting against larger risky individuals through mortality by predation (positive size-selective mortality). Positive size-selective mortality was, however, not tested directly in their study (Solberg *et al.* 2013b). Biro *et al.* (2006) demonstrated under natural conditions that domestic rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792), were able to grow faster than their wild conspecifics due to increased foraging behaviour, and that these larger *O. mykiss* were more susceptible to predation due to higher risk behaviour linked to foraging (Biro *et al.* 2006). Although studies indicate reduced predator awareness (Houde *et al.* 2010b) and potentially increased tolerance to predation stress (Fleming & Einum 1997; Debes & Hutchings 2014) in farmed relative to wild salmonids, no explicit evidence has been found for increased predator susceptibility in farmed *S. salar* (Skaala *et al.* 2014; Solberg *et al.* 2015). In the wild, faster growing farmed *S. salar* may also incur a metabolic cost through behavioural changes such as increased appetite (Thodesen *et al.* 1999) and foraging (Biro *et al.* 2006) which result in their expending more energy searching for food under low food availability conditions, leading to lower growth (Sundt-Hansen *et al.* 2009). The juxtaposition of these potential positive and negative size-selective forces may partly explain why growth differences seen in the wild are not as pronounced as in the hatchery environment (Solberg *et al.* 2013b).

Growth in the wild may also be influenced by other environmental factors, such as density and competition (Einum & Fleming 1997; Bohlin *et al.* 2002), and even natural stream conditions like substrate composition and flow rate (Jonsson & Jonsson 2011a). In comparative studies inter-strain competition between farmed, wild and hybrid groups could potentially influence the levels of relative growth differences observed. Thus, as the groups were reared in separate tanks, the lack of inter-strain competition in the present study may potentially explain the lower relative growth differences observed. A study examining the

relative growth differences of the same groups of farmed, hybrid and wild *S. salar* in both single strain and common garden experiments however found no difference in the relative growth differences across experimental designs (Solberg *et al.* 2013b). Therefore, it is concluded that the present experimental design is unlikely to drive the lower relative growth differences, and any potential tank effects were controlled for in the statistical model.

Based upon population genetic analyses, genetic changes in the population inhabiting the River Etne have been observed (Glover *et al.* 2012; 2013), and some level of admixture with farmed escapees has been demonstrated. It is therefore not possible to exclude the possibility that although the wild *S. salar* used in this study were indeed born in the wild (based upon scale reading), some individuals used as broodstock may represent some admixture with farmed escapees. This might explain why smaller growth differences were detected between the farmed and wild *S. salar* in this study, as compared to other studies of the same strains (Solberg *et al.* 2013a).

In the present study the hybrids displayed intermediate growth relative to both their farmed and wild conspecifics. There were slight differences in the slopes between each treatment for the hybrids, *versus* the farmed and the wild *S. salar*, that likely resulted in the marginally significant (P= 0.05) group by treatment interaction. Intermediate hybrid growth relative to their parental strains has been observed in similar studies under hatchery (Glover *et al.* 2009; Morris *et al.* 2011; Solberg *et al.* 2013a), semi-natural (Solberg *et al.* 2013b), and wild conditions (McGinnity *et al.* 1997). There was no evidence for hybrid vigour or outbreeding depression, whereby hybrids either perform better relative to their parents or display reduced fitness due to under-dominance, respectively. The hybrids in the present study were maternal half siblings to the farmed *S. salar*; therefore it is possible that maternal effects were influencing growth, although maternal effects are considered to be low at this life stage (Gilbey *et al.* 2005). Bicskei *et al.* (2014) examined gene transcription in farmed, F1 hybrid and wild *S. salar* at two early life stages, and found fewer significantly differentially expressed transcripts between farmed and hybrid individuals than between hybrid and wild individuals. Their hybrid crosses were generated from the farmed females, suggesting that maternal effects might account for this bias (Bicskei *et al.* 2014), highlighting the need for reciprocal hybrid crosses in comparative studies.

4.5 Conclusions

In summary, the results of the present study have demonstrated that the three feeding regimes implemented here did not influence the relative growth rate of farmed, hybrid and wild *S. salar* in the hatchery. Thus, while restricted to the hatchery, the present study provides evidence that variable food availability may not be the primary source governing the similar growth between farmed and wild *S. salar* in natural environments. Similarly, no evidence was found to indicate that more than 10 generations of adaption to the farmed environment, with continuous access to feed, has resulted in farmed *S. salar* exhibiting a reduced tolerance to limited or fluctuating levels of feed. Additional observations are required however that better simulate natural variation in food supply, which is typically not only variable in composition, but also varies markedly in time and space (Jonsson & Jonsson 2011a). It therefore remains a priority to elucidate further the nature of hybridisation and farm-wild interactions. Further studies in particular, exploring the key environmental differences between hatchery and wild environments (*e.g.*, predation, density) are evidently required, in conjunction with direct comparison of performance in respective conditions.

Acknowledgments

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Supplementary tables

Table S4.1: Family crosses for the experiment. The commercial farmed strain Mowi and the wild strain Etne were used to make seven pure wild, seven pure farmed and seven hybrid F1 groups. The hybrid families were made by crossing a female farmed *S. salar* with a wild male. Five of the seven hybrid families are half-siblings to five wild and five farmed families, and one family is maternal half siblings to one farmed family and one family is paternal half siblings to one wild family.

Family	Dam	Sire	Group
1	M1	M9	Farm
2	M1	E11	Hybrid
3	M2	M10	Farm
4	M2	E12	Hybrid
5	M3	M11	Farm
6	M3	E13	Hybrid
7	M4	M12	Farm
8	M4	E14	Hybrid
9	M5	M13	Farm
11	M6	M14	Farm
12	M6	E16	Hybrid
14	M7	E17	Hybrid
15	M8	M16	Farm
16	M8	E18	Hybrid
17	E1	E11	Wild
18	E2	E12	Wild
20	E4	E14	Wild
21	E5	E15	Wild
22	E6	E16	Wild
23	E7	E17	Wild
24	E8	E18	Wild

Table S4.2: Relative growth differences between each group within each treatment and Tukey adjusted *P*-values for the multiple pair-wise comparisons of groups within each treatment. The *P* -values are shown in the bottom left diagonal, and the significance level was set to 0.05, with non-significant *P*-values indicated in bold. Each group within a treatment was significantly different to each other group within that treatment. The relative growth differences between each group within each treatment are shown in bold in the top right section. The average mass of each group was compared to the average mass of the other groups by dividing the larger mass by the smaller mass (*i.e.* farm to wild), creating a relative growth difference ratio. Relative growth differences were not compared across treatments. Daily R corresponds to the daily restricted treatment and triweek corresponds to the triweekly treatment.

	DAILY R Farm	**DAILY R Hybrid**	**DAILY R Wild**	**CONTROL Farm**	**CONTROL Hybrid**	**CONTROL Wild**	**TRIWEEK Farm**	**TRIWEEK Hybrid**	**TRIWEEK Wild**
Mass (g)	24.51	19.345	16.125	43.09	36.355	28.845	36.09	30.91	22.64
DAILY R Farm	/	1: 1.3	1: 1.5						
DAILY R Hybrid	0.0005	/	1: 1.2						
DAILY R Wild	<0.001	0.0033	/						
CONTROL Farm	<0.001	<0.001	<0.001	/	1: 1.2	1: 1.5			
CONTROL Hybrid	<0.001	<0.001	<0.001	0.0181	/	1: 1.3			
CONTROL Wild	**0.0825**	<0.001	<0.001	<0.001	0.0007	/			
TRIWEEK Farm	<0.001	<0.001	<0.001	0.0189	**1**	0.0007	/	1: 1.2	1: 1.6
TRIWEEK Hybrid	0.0022	<0.001	<0.001	<0.001	0.0203	**0.2231**	0.0199	/	1: 1.4
TRIWEEK Wild	0.0366	**0.0786**	0.001	<0.001	<0.001	0.0006	<0.001	0.001	/

Chapter 5: Growth reaction norms of farmed, hybrid and wild Atlantic salmon: has domestication led to genetic divergence in diet preference?

Abstract

Current domestic strains of Atlantic salmon (*Salmo salar* L.) have been subjected to over ten generations of selection for fast growth, resulting in farmed salmon outgrowing wild salmon by large ratios under hatchery conditions. Selection for growth has been shown to influence various feed-related traits in farmed salmon, while domestication has caused changes in various other behavioural traits relative to wild conspecifics. It is possible that farmed salmon have become adapted to high energy commercial diets, which could influence growth differences observed between farmed and wild conspecifics under hatchery conditions, and even in the wild where growth differences are much less. The present study aimed to investigate growth and survival differences between farmed, wild and F1 hybrid salmon fed three contrasting diets under hatchery conditions. The diet treatments consisted of a commercially available pelleted salmon diet, a commercially available pelleted carp diet, and a diet consisting of varying amounts of invertebrates commonly found in Norwegian rivers (resembling a natural diet). Overall, farmed salmon outgrew hybrid and wild salmon in each treatment, and growth was significantly different between the groups, though all groups responded similarly relative to each other by displaying similar growth reaction norms across the treatments. Thus, similar plasticity towards differing diets was detected in salmon of all origins, and no indication of genetic-based adaptation to the shape or content of commercial diets was detected in the farmed salmon.

Submitted to *BMC Evlutionary Biology*

5.1 Introduction

Aquaculture is now the fastest growing food sector in the world, supplying over half of the world's fish protein (FAO 2014b). One of the most economically important aquaculture species is the Atlantic salmon (*Salmo salar* L.), an anadromous salmonid fish which is endemic to rivers on the west and east coasts of the Atlantic Ocean in the Northern hemisphere (Klemetsen *et al.* 2003). Atlantic salmon farming originated in Norway in the late 1960s, and in recent years the industry has grown worldwide to include commercial efforts in a number of countries, for example: Canada, Scotland (UK), and Chile (FAO 2014b). Current global production of Atlantic salmon exceeds 2 million tonnes, over half of which is produced in Norway alone (FAO 2014a).

Selective breeding programs began shortly after the first commercial farming efforts commenced in Norway, and current strains of salmon have undergone in excess of ten generations of directional selection for traits of commercial importance (Gjedrem 2000). The initial breeding goals for salmon aquaculture were to increase growth rate and delay sexual maturation, and that soon expanded to include disease resistance, flesh colour and body composition (Gjøen & Bentsen 1997). The genetic gain for growth in salmon has been estimated at 10-15% per generation (Gjedrem 2000), and selection has thus increased growth rates of farmed salmon by several-fold compared to wild conspecifics under hatchery conditions (Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b). It has also been demonstrated that selection for increased growth has indirectly increased appetite and feed conversion efficiency (FCE) (Thodesen *et al.* 1999; Handeland *et al.* 2003; Gjedrem 2010).

In intensive aquaculture systems, feed is continuously provided in the form of pellets, and is formulated to provide the fish with all of their species-specific nutritional requirements while maximising feed utilisation. In commercial salmon aquaculture one of the highest operating costs is feed, which can be as much as 60% of the cost of production (Gjedrem 2010). As the understanding of the nutritional requirements of farmed salmon has increased, commercial diets have been continuously refined to more closely meet energy and nutrient needs while striving to utilise more cost-effective ingredients (Li & Robinson 2015). Salmon are carnivorous, requiring diets that are high in protein and contain essential fatty acids (Lall & Dumas 2015). Traditionally these nutrients were obtained by including large amounts of fish meal and fish oil in salmonid diets; however the inclusion of marine sources of proteins and lipids in salmon diets is slowly declining in favour of plant substitutes (Ytrestøyl *et al.* 2015). Thus, the commercial salmon diet does not only deviate from the wild diet in terms of

shape, but also in terms of energy content and nutritional profile. The natural diets of wild fish can vary considerably in terms of type, shape, density of calories and nutrient composition. Wild salmon are opportunistic feeders, and they adapt their diet and feeding behaviour depending on their life stage and habitat (Jonsson & Jonsson 2011a). In freshwater habitats juvenile salmon typically feed on drift and benthic invertebrates, the availability of both will depend on the specific habitat characteristics such a flow rate and substrate (Jonsson & Jonsson 2011a).

Domestication involves adaptation to a captive environment, which is often very different to the natural environment typically experienced by wild conspecifics. These differences can lead to both phenotypic and behavioural changes between domesticated and wild individuals (Weber & Fausch 2003; Huntingford *et al.* 2012), and domestication-linked genetic changes may occur within a single generation (Christie *et al.* 2016). The changes are a result of direct and indirect responses to artificial selection and relaxed natural selection, and the low mortality associated with the domestic environment resulting in phenotypes persisting where they would not have persisted in the wild (Ruzzante 1994; Weber & Fausch 2003; Huntingford 2004). In addition to a moderately increased FCE linked to significantly higher growth rates, farmed salmon also exhibit changes relative to wild salmon for other feeding related traits such as increased appetite (Thodesen *et al.* 1999), growth hormone (GH) (Fleming *et al.* 2002) and insulin-like growth factor (IGF-I) (Solberg *et al.* 2012; but see Bicskei *et al.* 2014). It is possible that generations of selection for fast growing fish have resulted in farmed salmon that are adapted to the shape and high calorie content of salmon pellets. In the wild fish actively seek out feed, and typically vary their diet in order to obtain the essential nutrients required for growth (Jonsson & Jonsson 2011a). Therefore, adaptation to commercial salmon pellets may partly explain why there are such large growth differences observed between farmed and wild salmon under farming conditions (Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b) with considerably less differences observed under natural conditions (Fleming *et al.* 2000; Skaala *et al.* 2012; Reed *et al.* 2015).

Exploring whether indirect selection for feeding related traits has influenced growth and survival in domestic and wild conspecifics will advance our knowledge of the consequences of escapees and interbreeding, including hybridisation between farmed and wild conspecifics. Therefore, we investigated the growth and survival of farmed, wild and F1 hybrid Atlantic salmon offspring fed three contrasting diets within the hatchery using a common garden experimental design. The overall aim was to investigate whether over ten

generations of selective breeding in farmed salmon has resulted in the indirect selection for adaptation to commercial salmon diets in terms of nutritional content and shape, thus explaining why farmed salmon are able to outgrow wild salmon by larger ratios in the hatchery and not in the wild. Specifically, we hypothesised that farmed salmon would be able to maintain or increase their large relative growth difference over the wild salmon when fed with the diets more closely resembling the commercial salmon diet and not when fed a diet resembling natural diets.

5.2 Materials and methods

Contribution statement

This experiment was designed by Kevin Glover and Monica Solberg. Initial experimental set-up was performed by Monica Solberg, Lise Dyrhovden and Ivar-Helge Matre. The sampling was carried out by Lise Dyrhovden, Ivar-Helge Matre, Monica Solberg and Eva Troianou. Samples were genotyped by Eva Troianou. Preliminary data analysis was performed by Eva Troianou and Monica Solberg. The statistical analysis and manuscript preparation was performed by Alison Harvey.

Experimental crosses

The farmed, wild and F1 hybrid families were produced in November 2013 (week 46) at the Matre Research station, Institute of Marine Research (IMR), Norway. Atlantic salmon originating from the commercial Mowi strain and wild Atlantic salmon caught in the river Etne (59°40'N, 5°56'E), were used to produce five pure farmed, five pure wild, and five F1 hybrid families (Table S5.I).

The Mowi strain is the oldest Norwegian domestic salmon strain (Gjedrem *et al.* 1991). The strain has been primarily selected for, among other traits, increased growth rate and has undergone over ten generations of selective breeding. As a consequence, offspring of Mowi farmed salmon display significantly higher growth rates under standard hatchery conditions in comparisons with the offspring of wild salmon (Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b). However, in the wild, this farmed strain only displays slightly higher growth rates than wild conspecifics (Skaala *et al.* 2012).

The salmon stock in the River Etne, located in south-west Norway, is the largest population within its fjord system Hardangerfjorden; the fourth longest fjord in the world and second longest in Norway. Wild adult broodstock were collected in the river in the autumn of

2013 by angling, transferred to the local hatchery and held until the stripping of gametes. Growth patterns on fish scales were read on individuals in order to ensure that they were indeed born in the wild and were not farmed escapees (Lund & Hansen 1991).

The F1 hybrid fish were produced by crossing farmed females and wild males (Mowi ♀ x Etne ♂). The five hybrid families are thus maternal and paternal half-siblings with the farmed and wild families, respectively. All 15 families were incubated at ambient water temperature in single-family units until the eyed-egg stage.

Table 5.1: Overview of experimental design indicating the ratios of families within each group and the final number of fish sampled from each replicate.

Treatment	Control		Carp		Natural	
	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 5
Initial number per tank	15 Families: 5 farmed 5 F1 hybrid 5 wild 30 eggs per family n=450	15 Families: 5 farmed 5 F1 hybrid 5 wild 30 eggs per family n=450	15 Families: 5 farmed 5 F1 hybrid 5 wild 30 eggs per family n=450	15 Families: 5 farmed 5 F1 hybrid 5 wild 30 eggs per family n=450	15 Families: 5 farmed 5 F1 hybrid 5 wild 30 eggs per family n=450	15 Families: 5 farmed 5 F1 hybrid 5 wild 30 eggs per family n=450
Sampled	n = 422	n = 423	n = 290	n = 328	n = 215	n = 306

Experimental design & rearing conditions

Eyed eggs from families were sorted into hatchery trays representing the replicate treatments in week 5 of 2014. Each replicate treatment consisted of 30 eggs per family of each group, yielding 450 eggs in each of six replicates (two per treatment). One control replicate contained 451 eggs, as at the time of sorting one family was accidentally allocated one extra egg. In week 18 the hatched and ready-to-start feeding fry were transferred to six identical tanks (1.5m^3, ambient water temperature ranging from 4.5 to 14.6 °C with an average of 8.6 °C). The diet treatments were initiated when start feeding commenced in week 18 of 2014.

The control treatment consisted of a diet of commercial pelleted salmon feed, Skretting Nutra, which has a high protein and lipid content, with a low carbohydrate content. The carp treatment consisted of a commercial pelleted carp diet, Skretting Coarse Fish, which has a high level of carbohydrates and a lower protein and lipid content than the control diet.

The natural treatment was composed of a combination of different frozen organisms which are typically present in the rivers of Norway; namely, a mix of freshwater copepods Cyclopidae *Cyclops*, water fleas Daphniidae *Daphnia* and insect larvaes; black mosquito larvae Culicidae and glassworms, *i.e.*, transparent larvae of the phantom midge Chaoboridae *Chaoborus*. The three treatments are from here on referred to as the control, carp and natural treatments. Pellet sizes for the control and carp diets were adjusted according to the manufacturer's feed table for the commercial salmon feed as the fish grew throughout the experiment. In order to get similar sized pellets for the control and carp diet, carp pellets were crushed and sieved (500 μm, 700 μM and 1mm filter). Insects in the natural treatment were weighed and thawed before they were fed to the natural treatment. The percentage of each organism within the natural diet treatment varied manually throughout the experiment in order to compensate for the growth of the fish, with smaller insects given in higher amounts at the start. All treatments received the same caloric value each day, and feed was provided in excess for all treatments. The fish were fed for 12 hours on full ration (5 % of the fish dry weight /day) in order to eliminate competition effects. Non-eaten insects were removed from the natural treatments daily, before a new daily feeding cycle was initiated. The fish were kept on a 24 hour photoperiod from transfer to tanks until experiment termination. During the experimental period there was a non-biological mortality incident in one of the natural treatment replicates. Due to a clogging of the drains by the excess feed given, the water level was elevated allowing fish to jump out of the tank. Potential variation in growth and survival between replicated tanks were later on statistically controlled for. Furthermore, both relative survival and growth at the family level was observed to be stable between replicates in this treatment, indicating that this mortality event did not unduly influence the results of this study. For an overview of the experimental design see Table 5.1. See Figure 5.1 for a simple representation of the average contents of each diet as supplied by the manufacturers (Skretting for the pelleted diets and Ruto Frozen Fish Food for the frozen organisms) and Table S5.2 for detailed nutritional contents of each diet.

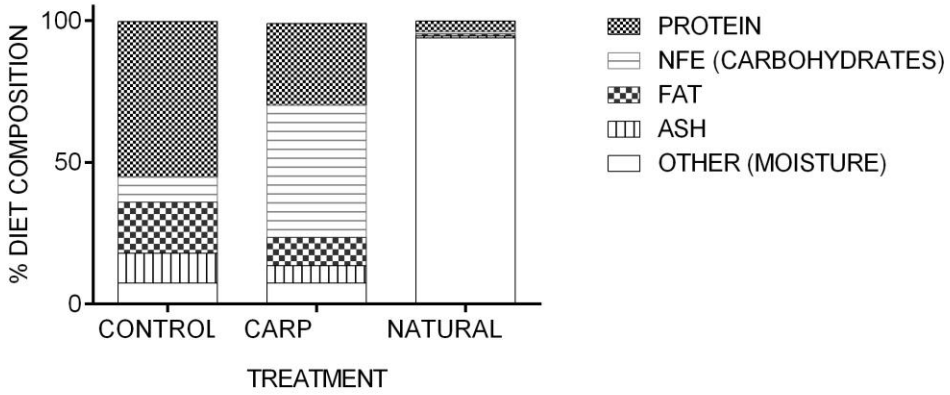

Figure 5.1: Stacked graph showing the average proportions of the main nutritional contents of each diet treatment (control, carp, natural). A more detailed description of the diet components is presented in Supplementary Table S5.2.

Sampling, genotyping & family assignment

The experiment was terminated in week 36 of 2014, when fish in all tanks were euthanised following standard guidelines with an overdose of Finquel® Vet anaesthetic (ScanVacc, Årnes, Norway). The fish were measured for wet weight and fork length, and a fin clip was taken from each and stored in individually labelled tubes filled with 100% ethanol for DNA analysis. A total of 1984 individuals were sampled (Table 5.2).

DNA-based parentage testing was used to identify the sampled fish back to family of origin. DNA was extracted in 96-well plates using the HotSHOT genomic DNA preparation method as recommended by manufacturers (Biotechniques, 2000). Five microsatellite markers, MHC1 (Grimholt *et al.* 2002), SSsp3016 (Genbank # AY372820), SsOsl85 (Slettan *et al.* 1995), Ssa197 (O'Reilly *et al.* 1996), and SsaF43 (Sanchez *et al.* 1996) were amplified in one PCR multiplex (See Supplementary Table S5.6 for PCR conditions). PCR products were resolved on an ABI Applied Biosystems 3730 Genetic Analyser and sized using a 500LIZ standard (Applied Biosystems). Genemapper Version 5.0 was used to score alleles manually. Individuals were then assigned back to family using the Family Analysis Program (FAP) (v3.6) (Taggart 2007), an exclusion-based assignment program that has been routinely used for the purpose of parentage assignment in other comparative studies of salmonids within this lab (Glover *et al.* 2004; Solberg *et al.* 2013a).

Table 5.2: Average weights and mortality of farmed, wild and hybrid Atlantic salmon within each replicate and each treatment

Treatment	Group	Tank	Initial n	Final n	Mortality		W (g)			Pooled W (g)				Pooled Mortality
					n	%	Mean	Median	SD	N	Mean	Median	SD	%
Control	Farm	1	150	141	9	6%	30.26	31.00	6.13	280	29.70	31.00	6.62	6.67
		2	150	139	11	7%	29.12	31.00	7.03					
	Hybrid	1	150	144	6	4%	25.20	25.00	6.09	288	23.95	23.90	5.40	4.00
		2	150	144	6	4%	22.71	23.00	4.27					
	Wild	1	150	137	13	9%	15.62	16.00	5.79	276	15.55	15.00	5.53	8.00
		2	150	139	11	7%	15.49	15.00	5.26					
Carp	Farm	3	150	85	65	43%	8.53	8.30	4.31	183	10.10	10.20	4.90	39.00
		4	150	98	52	35%	11.54	11.80	4.94					
	Hybrid	3	150	111	39	26%	6.56	5.90	3.24	237	6.87	5.90	3.56	21.00
		4	150	126	24	16%	7.15	6.20	3.80					
	Wild	3	150	94	56	37%	4.85	4.20	2.46	197	4.80	4.20	2.36	34.33
		4	150	103	47	31%	4.76	4.00	2.26					
Natural	Farm	5	150	81	69	46%	5.11	5.10	2.17	192	4.71	4.70	2.15	36.00
		6	150	111	39	26%	4.42	4.40	2.09					
	Hybrid	5	150	72	78	52%	4.30	4.05	2.12	182	3.89	3.60	1.90	39.33
		6	150	110	30	20%	3.62	3.20	1.74					
	Wild	5	150	62	88	59%	2.91	2.60	1.48	145	2.93	2.60	1.40	51.67
		6	150	83	72	48%	2.94	2.60	1.37					

Statistical analysis

Statistical analysis was carried out using R version 3.2.1 (R Core Team 2015) with all critical p-values set to 0.05 unless otherwise stated.

Growth

A linear mixed model (LME) was used to investigate the effect of genetic background (group), egg size and diet on body weight at termination. The response variable was the continuous variable of log-transformed (log_{10}) wet weight at termination. The full model included the fixed factor covariates of genetic group and treatment and the fixed continuous covariate of log-transformed (log_{10}) and centred egg size, plus all two-way interactions between the fixed covariates. Differences in variance patterns between the replicate treatment tanks were controlled for by including replicate nested within treatment in the model as a random intercept effect with 6 levels. Differences in variance patterns between families across the treatments were controlled for by including family as a random intercept effect (15 levels) with differing slopes for the effect of treatment.

The LME model was fitted using the *lmer* function from the *lme4* package in R (Bates *et al.* 2014). Model selection of the full models was performed by the use of the *lmerTest* package, which allows for automatic model selection using the *step* function (Kuznetsova *et al.* 2014). This function eliminates insignificant random effects before eliminating insignificant fixed effects using backwards selection to yield the final model. The p-values for the random effects are calculated using likelihood ratio tests where the significance level was set at 0.1, while the p-values for the fixed covariates are calculated based upon Satterthwaite's approximations, and the significance level was set to 0.05 (Kuznetsova *et al.* 2014). The F-statistics and degrees of freedom for the fixed effects are calculated based upon Satterthwaite's approximations (Kuznetsova *et al.* 2014). The full and final models, as given by the step function output, are presented in Table 5.3. Pair-wise comparisons between treatments and between groups were performed by the use of the *glht* function in the *multcomp* package (Hothorn *et al.* 2008) using the final model (Table S5.3). The relative growth differences comparing the average weight of farmed to wild and hybrid to wild fish are presented for each treatment in Table 5.4.

Table 5.3: Model selection of the linear mixed effect model used to investigate the influence of treatment, group and egg size upon body weight at termination.

Model	N	Response Variable	Random effects				Fixed effects					
			Variable	Chi.sq	Chi.df	P	Variable	Sum.sq	Num.df	Den.df	F	P
	1972	Log Weight	T:r	0.54	1	0.46	T x G	0.068	4	11.30	0.52	0.72
			T/G:f	**85.06**	**5**	**<1e-07**	G x E	0.094	2	9.22	1.46	0.28
							T x E	0.25	2	13.01	3.74	0.052
							T	**41.76**	**2**	**13.80**	**645.12**	**<1e07**
							G	**6.85**	**2**	**11.56**	**105.35**	**<1e07**
							E	**0.34**	**1**	**11.92**	**10.29**	**0.0076**

Significance levels of random and fixed effects included in the full LME model investigating variation in log body weight at termination. N; number of individuals. Log weight; log10 (wet weight+1) at termination. Random effects: T:r; replicate (r) nested within treatment (T) (random intercept). T/G:f; familiy (f) nested within group (G), across treatments (T) (random intercept and slope). Chi.sq; the value of the Chi square statistics. Chi Df; the degrees of freedom for the test. P; P-value of the likelihood ratio test for the random effect. Fixed effects: T, diet treatment (control, carp, natural). G; genetic group (farmed, wild, hybrid). E; mean family (log10) centred egg diameter. Two-way interactions terms included in the full model: T x G, T x E and G x E. Sum.Sq; sum of squares. Num Df, numerator degrees of freedom. Den Df; denominator degrees of freedom based on Sattherwaithe's approximations. F; F-value. The variables in bold were retained in the final model.

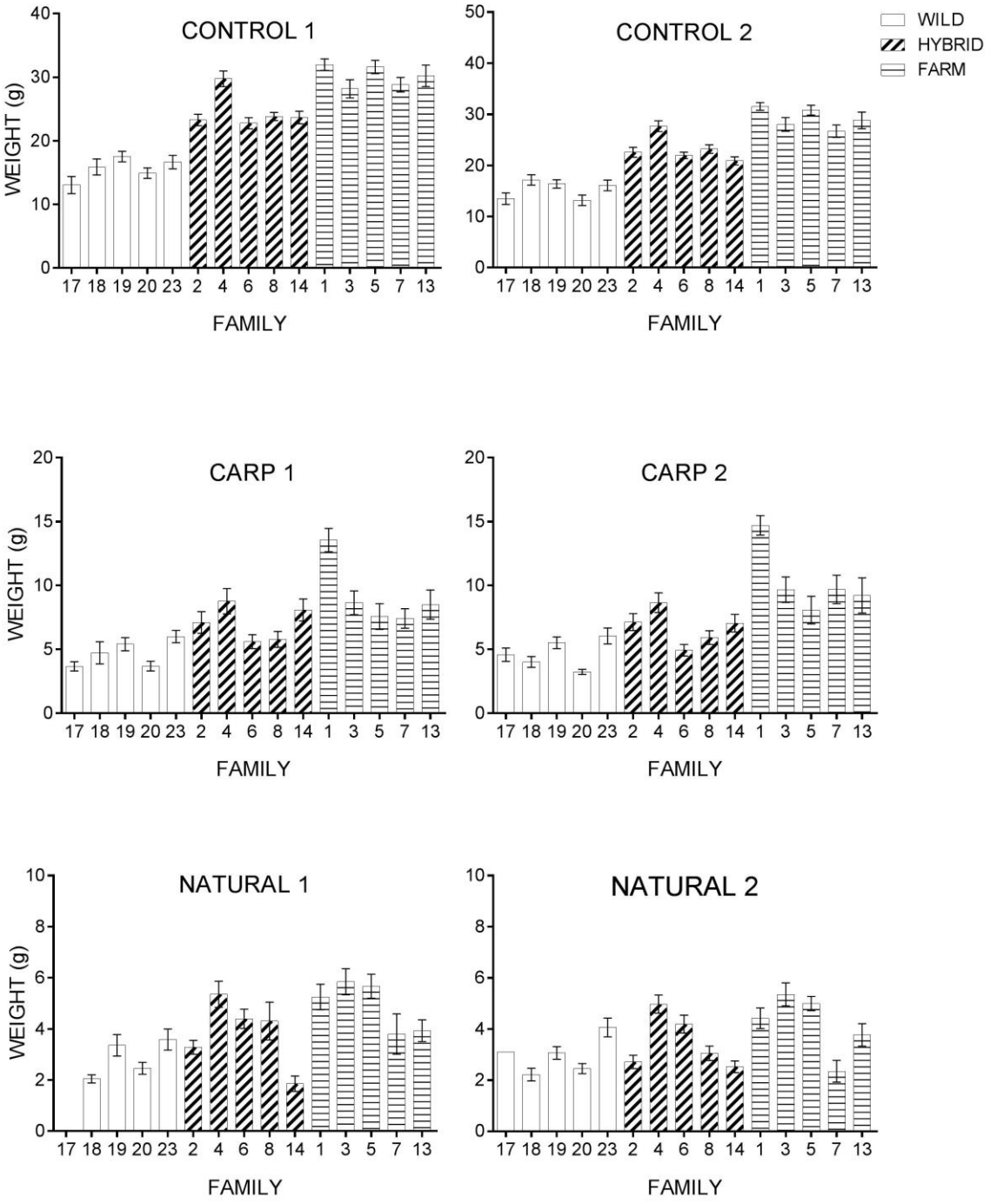

Figure 5.2: Average weight (grams) and standard error of each family within the farmed, wild and F1 hybrid groups in the replicates of each treatment (control, carp, and natural). Farmed fish were significantly larger than hybrid and wild fish across all treatments, and family variation in growth was visible among the treatments.

Table 5.4: Relative weight differences between farmed, wild and hybrid salmon within each treatment.

Treatment	Group	Weight (g)	Relative difference	
			to Wild	to Hybrid
Control	Farm	29.70	1.9	1.2
	Hybrid	23.95	1.5	-
	Wild	15.55	-	-
Carp	Farm	10.14	2.1	1.5
	Hybrid	6.87	1.4	-
	Wild	4.80	-	-
Natural	Farm	4.71	1.6	1.2
	Hybrid	3.89	1.3	-
	Wild	2.93	-	-

The relative growth differences were calculated by dividing the average weight (in grams) of the farmed fish by the wild and hybrid fish respectively, and the average weight of the hybrid fish by the wild fish within each treatment.

Survival

A generalised linear mixed model (GLMM) was used to investigate whether genetic background (group), egg size or diet affected survival. The response variable, survival, was binary, and thus the binomial distribution was used with the default logit link function and was fitted using the Laplace approximation. The full model covariates were identical to the growth model described above. Differences in variance patterns between the replicate treatment tanks were controlled for by including replicate as a random intercept effect. Differences in variance patterns between families across the treatments was controlled for by including family as a random intercept effect with differing slopes for the effect of treatment.

The GLM model was fitted using the *glmer* function from the *lme4* package (Bates *et al.* 2014).The random effect structure was investigated by fitting the full model with only one random effect at a time and plotting the 95% prediction intervals of the random effect using the *dotplot* function in the *lattice* package (Deepayan 2008). If all the prediction intervals of the random effect overlapped zero then this effect was removed from the final model. Backward selection using a likelihood ratio test (LRT) was performed on a full fixed effect model comparing two random effect structures (Table S5.4), *i.e.* a random intercept model for family versus a random intercept and slope model for family. The fixed effect structure of the final model was determined by backward selection using the *drop1* function based on AIC values (Bolker *et al.* 2009) (Table 5.5). The number of fish from each family within each treatment is shown in Figure 5.3. Pair-wise comparisons between treatments and between groups were performed as for growth above (Hothorn *et al.* 2008) using the final model

(Table S5.5). Figure 5.4 shows the phenotypic reaction norms for growth and survival at the family level and the relative weight and survival across each treatment.

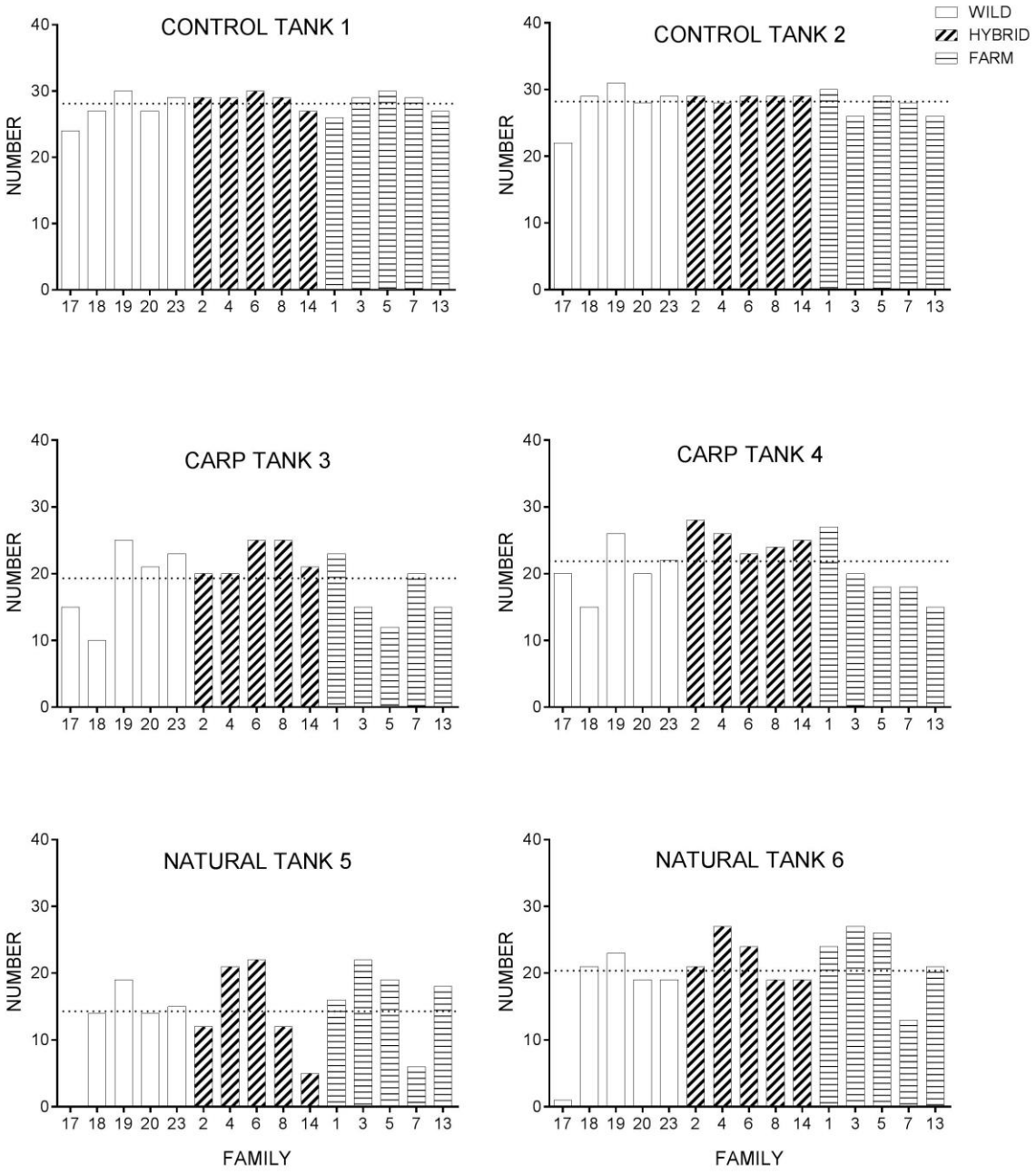

Figure 5.3: Number of fish surviving from each of the 15 families within the replicates of each treatment (control, carp, and natural). Dotted horizontal lines represent the expected number of fish per family in each replicate based on average mortality.

Table 5.5: Model selection of the fixed effects of the generalised linear mixed model investigating mortality.

		Fixed effects							
N	Response	T x G	G x E	T x E	Treatment	Group	Egg size	AIC	ΔAIC
2696	Survival	x	x	x	x	x	x	2540.1	2
		x		x	x	x	x	2539.7	2.45
				x	x	x	x	2540.5	1.64
					x	**x**	**x**	**2542.1**	**0**
					x	x		2555.4	13.3
					x		x	2554.3	12.23
						x	x	2552.4	10.3

T x G; Treatment by group interaction. G x E; Group by egg size interaction. T x E; Treatment by egg size interaction. AIC; Akaike information criterion. Δ AIC; difference in AIC value. Models which differed by less than 2 AIC were interpretted as equally good, with the simplest best fitting model chosen. The final fixed effect structure is shown in bold.

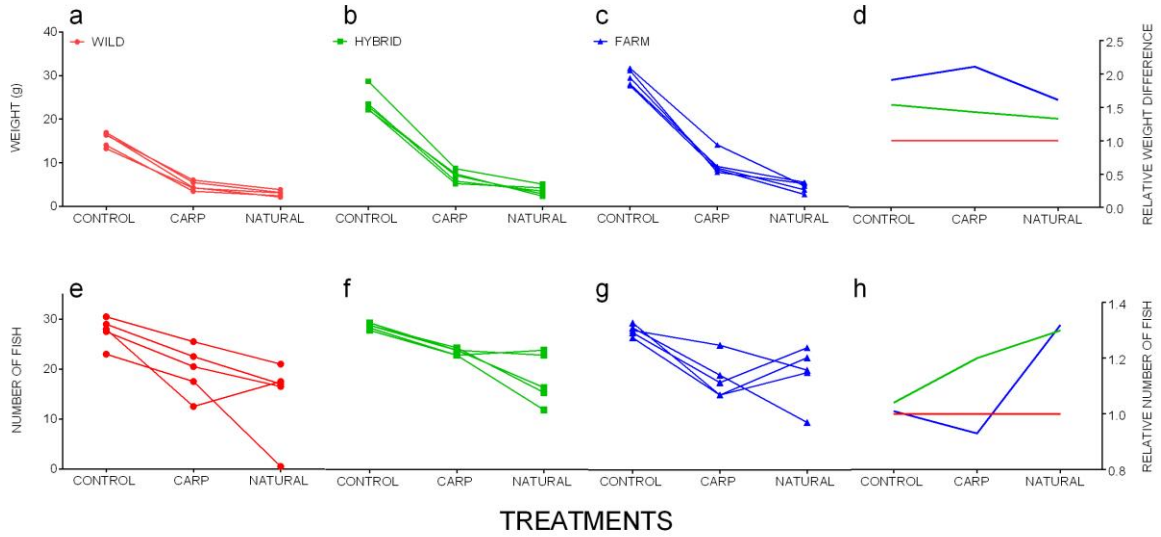

Figure 5.4: Phenotypic reaction norms for growth (a-d) and survival (e-h) across the treatments. (a-c) The phenotypic growth reaction norms for each group at the family level using untransformed weight in grams and (d) average weight relative to the wild group where the hybrid and farmed groups are compared to the wild group within each treatment. (e-g) The survival reaction norms for each group at the family level between the treatments and (h) the relative survival reaction norms for each group where farmed and hybrid fish are compared to the wild fish within each treatment. Treatments (control, carp, natural) are indicated on the x-axis.

Ethical statement

The experimental protocol (permit number 6546) was approved by the Norwegian Animal Research Authority (NARA). All welfare and use of experimental animals was performed in strict accordance with the Norwegian Animal Welfare Act. In addition all personnel involved in this experiment had undergone training approved by the Norwegian Food Safety Authority, which is mandatory for all personnel running experiments involving animals included in the Animal Welfare Act.

5.3 Results

Sampling & data

The experiment was terminated after 19 weeks in week 36 of 2014 when all 1984 surviving fish were sampled. Six individuals could not be assigned unambiguously back to one family, and were removed from the dataset prior to analysis. A further six individuals were removed from the dataset after being identified as outliers due to extreme condition factors, indicating sampling errors. Thus the dataset consisted of 1972 individuals.

Growth

Overall, growth was several times higher in the control treatment in comparison with the carp and natural treatments: 23.10 g in the control treatment, 7.18 g in the carp treatment and 3.92 g in the natural diet treatment. Thus diet had a highly significant effect on growth of all groups despite the fact that the total amount of energy available to the fish in each treatment was identical (Table 5.3, Figure 5.2).

Across the treatments farmed fish grew significantly larger than the hybrid fish, which were in turn larger than the wild fish (Figure 5.2, Table S5.3). The relative difference in weight between the farmed, hybrid and wild salmon (Table 5.4) did not vary significantly between the three treatments, and a significant interaction between treatment and group was not detected (Table 5.3, Figure 5.4). Thus salmon of all genetic groups responded to the diet treatments in a similar plastic manner, resulting in similar growth reaction norms across the treatments (Figure 5.4).

The effect of the interaction between egg size and treatment was marginally non-significant, and the effect of egg size alone was negatively correlated to weight. The latter was due to the generally larger egg sizes of the wild families used in the present study coupled

with their lower growth compared to the farmed and hybrid families. Removing the effect of egg size upon final weight in the selected LME model did not influence the results of the analysis (data not presented here). There was some visible weight variation between families within the three genetic groups, and variation between families differed furthermore between treatments (Figure 5.4). For instance, family 1 of farmed origin exhibited exceptional growth in the carp diet treatment. In order to control for these trends the selected LME model included family nested within group as a random intercept effect with differing slopes for the effect of treatment.

Survival

Observed survival in the control, carp and natural diet treatments was 93.78%, 68.56% and 57.67%, respectively. Mortality was thus lowest in the control diet treatment, and was significantly different to both the carp and natural diets (Figure 5.3, Table S5.5). Mortality did not differ significantly between the carp and natural diet treatments, although on average mortality was higher in the natural diet treatment (due to variation in survival between replicated tanks in this treatment) (Table 5.2, Table S5.5). Thus diet had a significant effect on mortality (Table 5.5).

There was a significant effect of group on mortality (Figure 5.3, Table 5.5). Across all treatments, hybrid and farmed fish displayed significantly different mortality to each other, while wild fish displayed similar mortality to both groups (Table S5.5). Within treatments hybrids displayed the lowest average mortality within the control and carp diet treatments, while the farmed fish displayed the lowest average mortality in the natural diet treatment (Table 5.1, Figure 5.3). Egg size had a significant positive effect on mortality (data not presented here).

Mortality levels differed between some of the replicated treatments tanks (Figure 5.3), thus the random effect of replicate nested within treatment was retained in the final model in order to control for this variation. Similarly, there was an under-representation of some families, e.g., wild family 17, within the genetic groups within some of the treatments (Figure 5.3) and visible variation between families within the three genetic groups, and between treatments (Figure 5.4). In order to control for this the final GLM model included family as a random intercept effect with differing slopes for the effect of treatment.

5.4 Discussion

In order to investigate whether multiple generations of selective breeding has indirectly led to farmed salmon adapting to the nutritional content and shape of commercial salmon pellets, and furthermore, if this can explain why farmed salmon maintain a high growth rate relative to wild salmon in the hatchery contra to the wild; we investigated growth and survival of farmed, wild and F1 hybrid fish fed three contrasting diets under common garden hatchery conditions. Fry were fed either a commercial salmon diet, a diet resembling the commercial diet in shape but not in nutritional content (a commercial carp diet) or a more natural diet containing salmon prey (insects) which are typically present in the rivers of Norway. Salmon of all groups grew best on the commercial salmon diet, and all groups had the lowest growth on the natural diet. There was no interaction between diet and group for growth, indicating that the groups all responded identically relative to each other on the different diets. Thus similar plasticity towards the differing diets as well as similar reaction norms were detected in salmon of all origins. Similarly, all groups survived the best on the commercial salmon diet, and there was no interaction effect of diet and group for survival.

Growth

Growth was significantly different between the treatments, being highest in the control diet, intermediate in the carp diet, and lowest in the natural diet treatment. The relatively large difference in overall growth (68% growth decrease) between the control and carp treatment was not unexpected, despite the fact that the percentage calorie difference (MJ/kg) between the two diets was only ~15%. The carp diet contained roughly 4.5 times as much carbohydrate, a third less protein and half as much lipid than the salmon diet. The ability of fish to utilise carbohydrates varies between species and carbohydrate complexities, and salmon are less effective at it than some other fish species (Wilson 1994; Hemre *et al.* 2002). Salmon diets typically contain low levels of carbohydrates as salmon do not require high levels of carbohydrates in their diets, unlike warm water species such as carp, although the inclusion of low amounts of carbohydrates can facilitate the utilisation of other nutrients (Hemre *et al.* 2002). Farmed salmon get most of their energetic requirements from the high dietary levels of lipids and proteins (Hardy 1998). Thus, it is likely that the lower growth observed in the carp treatment relative to the control diet was a result of the mismatch in the dietary levels of specific nutrients resulting in all fish not being able to fully utilise or digest the food efficiently. Studies have shown that a high level of dietary carbohydrate negatively affects feed utilisation and growth in several fish species, including Atlantic salmon (Hemre

et al. 1995), European sea bass (*Dicentrarchus labrax* L.) (Pérez-Jiménez *et al.* 1997), and Wuchang bream (*Megalobrama amblycephala,* Yih 1955) (Zhou *et al.* 2013).

Domestic selection for growth has affected various feeding related traits including appetite and FCE (Thodesen *et al.* 1999; Handeland *et al.* 2003). Thodesen *et al* (1999) found that farmed salmon consumed more food and utilised their food more efficiently than wild conspecifics under controlled conditions, and attributed this to genetic changes in domesticated fish through direct selection for growth. Similarly, Handeland *et al* (2003) found that growth and FCE was higher in farmed salmon smolts compared to wild smolts under controlled conditions. In the present study, neither feed utilization nor FCE was investigated; therefore adaption to nutritional content of commercial diets was indirectly tested by comparing growth of farmed and wild salmon when fed nutritionally contrasting commercial pelleted diets and a diet consisting of natural prey. Farmed fish did not exhibit a higher appetite for pellets relative to their wild conspecifics; similarly wild fish did not find the pelleted diets more unpalatable than farmed fish due to their shape. Furthermore, salmon of all groups responded to the carp treatment in a similar manner, by displaying similar growth reaction norms relative to each other. If farmed fish have adapted to the shape and nutritional content of commercial pellets, we would expect the relative growth between farmed and wild fish in the carp diet to be higher than observed, as compared to the control treatment. Either farmed fish would have consumed more pellets or the wild fish would have consumed less pellets, even with a nutritional content to which none of the strains could possibly have been adapted. The present study therefore found no evidence that farmed fish have become adapted to the shape of commercial pellets, and fish of all groups utilised the carp diet poorly.

Growth of salmon is generally found to be less under natural than domestic conditions, and this is thought to be linked to the metabolic costs associated with actively seeking prey, defending territories, predator avoidance, and the abundance of food and energy in river systems. As the present study took place within a hatchery with no predation, food was not limiting nor did fish have to actively seek prey, it is unlikely that the lower overall growth in the natural diet treatment is attributable to any of the above. While efforts were made to ensure that the natural diet contained a similar calorie content as the other diets, it is possible that fish were unable to obtain and utilise the correct balance of nutrients in order to maximise growth. Simply, that fish were unable to consume enough food to match the calorie content of the two formulated diets. As above, the farmed, hybrid and wild salmon displayed similar reaction norms for growth between the control treatment and the natural diet treatment. If

farmed salmon have become adapted to the nutritional content of commercial diets, one would expected the relative growth differences between farmed and wild salmon to be significantly lower when feed a natural diets as compared to a commercial diet. Therefore we found no evidence that farmed fish are unable to maintain their relative growth advantage with a natural diet.

Farmed salmon escaping into the wild may not initially be accustomed to actively seeking and selecting prey due to differences in environmental experiences relative to wild salmon. Release experiments have demonstrated that farmed salmon previously reared on pellets were less likely to actively feed than their wild conspecifics in a natural environment, and were more likely to ingest prey of lower nutritional value (Orlov *et al.* 2006). In general, after a period of acclimation farmed fish display similar feeding behaviour as their wild conspecifics (Huntingford *et al.* 2012), although this often depends on the life stage (Olsen & Skilbrei 2010). However, experiments conducted in the wild from the egg stage reveal that the diets of the offspring of farmed and wild salmon do overlap (Fleming *et al.* 2000; Skaala *et al.* 2012), therefore farmed fish are able to feed in the wild. In the present study the natural diet was composed of dead organisms; therefore it is possible that the natural diet was too accessible to the fish, and using a live diet where the fish had to chase the prey itself, may have elicited a different response between the salmon groups. Live prey was not used as we would not be able to disentangle if a possible reduction in growth difference between farmed and wild salmon would be due to farmed salmon being adapted to the commercial diet, or due to farmed salmon not being able to catch live prey.

Overall, farmed salmon outgrew hybrid and wild salmon in each treatment, and growth was significantly different between the groups. The relative growth difference between wild and farmed fish was highest in the carp treatment (1: 2.1), lowest in the natural diet (1:1.6) and intermediate in the control treatment (1:1.9). This was as expected, although these differences did not elicit a statistically significant diet by group interaction. Large within-group variation was detected at the family level, and true difference between the groups may thus potentially have been masked. For instance, family 1 (farmed) exhibited high growth in the carp treatment relative to other families (Figure 5.2), although as mentioned previously, any family variation was controlled for in the final model. Using a higher number of families per group might have reduced the per family impact upon the group's mean expression, resulting in the growth ratio trend mention above being significant, Thus, we cannot rule out that farmed salmon may be adapted to the commercial diet, even though we

were not able to detected this in this study. We can however, based upon the results of this study, tentatively suggest that this is contributes to the deviating growth differences detected between farmed and wild salmon in the hatchery and in the wild.

Although the absolute growth differences observed between the farmed, hybrid and wild salmon experimental groups in the present study are lower than previously observed under hatchery conditions (Solberg *et al.* 2013a; 2013b), it is clear that multiple generations of selection has resulted in farmed salmon which outgrow their wild conspecifics, although this effect is not as pronounced in the wild. In the present study, the hybrids originated from maternal farmed and paternal wild crosses, therefore hybrid growth may be influenced by maternal effects (Mousseau & Fox 1998). However, hybrids in the present study displayed somewhat intermediate growth, similar to findings of other comparative studies (Einum & Fleming 1997; Solberg *et al.* 2013a; Harvey *et al.* 2016), illustrating that additive inheritance is responsible for the majority of the variation of this trait.

Survival

The natural mechanism of feeding is understood to be innate in all fishes, however studies show that fish which have been reared in captivity and fed only commercial diets display a low survival in the wild once they are released or escape as they are not initially able to efficiently switch from pelleted feed to natural feed (Soto *et al.* 2001; Olsen & Skilbrei 2010; Abrantes *et al.* 2011). Comparative survival studies in the wild found that the freshwater survival of farmed fish was low compared to wild conspecifics, and that hybrids generally displayed intermediate survival (McGinnity *et al.* 1997; 2003). Skaala *et al* (2012) observed that offspring of farmed fish planted out as eggs in a natural river system had a significantly reduced survival relative to their hybrid and wild conspecifics. Similarly, Fleming *et al* (2000) found that offspring of farmed fish had lower early stage survival than wild conspecifics in the wild, although at a later stage (parr to smolt) there was no difference in survival. Lower survival in farmed salmon may be the result of inefficient feed behaviour (Orlov *et al.* 2006; Olsen & Skilbrei 2010) and behavioural differences, such as increased aggression or decreased predator awareness (Einum & Fleming 1997; Houde *et al.* 2010b), which may also expose fish of farmed backgrounds to more predation than their wild conspecifics. Farmed fish may also have become adapted to the shape and nutritional content of commercial salmon diets, contributing to their low survival in the wild. If farmed salmon had lost their ability to digest natural feed it would be expected that they would suffer the

highest mortality in the natural treatment, however farmed salmon displayed the lowest average mortality in the natural treatment (Table 5.2, Figure 5.4), and across all treatments there was no difference in mortality between farmed and wild fish (Table S5.5). Therefore, there was no evidence to suggest that farmed fish have become adapted to the shape and nutritional content of commercial salmon diets to the extent it influences the survival of their offspring in the wild. Indeed, as mentioned previously, studies have demonstrated that the diet composition of farmed salmon in the wild tends to overlap with those of wild salmon (Fleming *et al.* 2000; Skaala *et al.* 2012).

It is possible that the higher mortality within the natural and carp diet treatments relative to the control treatment is due to all fish being unable to efficiently utilise the diets or consume enough calories as discussed above. While there was no overall difference in mortality between farmed and wild groups across all treatments (Table S5.5), within the natural diet treatment wild fish had the highest average mortality (Table 5.2, Figure 5.4). Sundt-Hansen *et al* (2015) found that offspring of farmed salmon displaced and out-competed offspring of wild salmon in a stream environment, resulting in a lower survival of wild conspecifics. In the present study food was presented in excess in each treatment in order to eliminate resource competition, however, it is possible that farmed and hybrid fish in the natural treatment may have had a competitive size advantage over the wild salmon. Potentially the acceptability of the non-live prey may have influenced the palatability of the natural diet for the wild fish. There was a significant difference in overall mortality detected between hybrid and farmed fish, which could be the result of hybrid fish exhibiting particularly high survival in the carp treatment relative to their farmed and wild conspecifics. It is unknown why there was such a large difference in survival relative to their parental groups in the carp treatment.

Egg size was significant and positively correlated with survival, suggesting that a larger egg size was beneficial for survival under these conditions. Studies indicate that egg size has a positive effect on survival in salmonids (Einum & Fleming 1999; Einum & Fleming 2000), and may contribute to the offspring of farmed salmon displaying higher than expected survival in the natural habitat when the eggs of farmed fish are larger than those of the competing wild fish (Skaala *et al.* 2012). In two of the treatments in the present study wild fish survived the worst on average (Table 5.2, Figure 5.3), despite having larger egg sizes than the farmed and the hybrid salmon, which indicates that the wild exhibited an even lower than expected survival.

5.6 Conclusion

The present study contributes to our understanding of the potential genetic and ecological interactions between farmed and wild salmonids, including their underlying mechanisms. Understanding the impacts of growth differences between farmed and wild fish, and their hybrid interactions is important for conservation and management of wild fish, and for the sustainable development of the aquaculture industry. The present study was unable to find evidence that the elevated growth differences observed between farmed and wild salmon in the hatchery is a result of farmed fish being adapted to commercial salmon diets, *i.e.*, either nutritional content or shape. Similarly, we were unable to find evidence that farmed salmon perform less well on an ad lib diet containing organisms which are typically present in the wild, relative to wild salmon. Our study took place in a hatchery environment, did not include live prey, nor took predation or other environmental parameters which may influence growth and survival into account. Therefore we encourage further studies under wild or semi-natural conditions in order to elucidate why farmed salmon do not outgrow wild salmon extensively in the natural environment.

Acknowledgements

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Supplementary tables

Table S5.1: Experimental crosses.

Family	Female	Group	Male	Group	Family type
1	M1	Mowi farm	M9	Mowi farm	Farm
2	M1	Mowi farm	E11	Etne wild	Hybrid
3	M2	Mowi farm	M10	Mowi farm	Farm
4	M2	Mowi farm	E12	Etne wild	Hybrid
5	M3	Mowi farm	M11	Mowi farm	Farm
6	M3	Mowi farm	E13	Etne wild	Hybrid
7	M4	Mowi farm	M12	Mowi farm	Farm
8	M4	Mowi farm	E14	Etne wild	Hybrid
13	M7	Mowi farm	M15	Mowi farm	Farm
14	M7	Mowi farm	E17	Etne wild	Hybrid
17	E1	Etne wild	E11	Etne wild	Wild
18	E2	Etne wild	E12	Etne wild	Wild
19	E3	Etne wild	E13	Etne wild	Wild
20	E4	Etne wild	E14	Etne wild	Wild
23	E7	Etne wild	E17	Etne wild	Wild

Three different populations were used to make three experimental groups: 5 farmed families of the Mowi commercial strain; 5 F1 hybrid families; and 5 wild families of the Etne strain. F1 hybrid families were created by crossing a farmed female with a wild male and each hybrid family is thus a maternal and paternal half-sib to a respective farmed and wild family.

Table S5.2: Approximate nutritional content of each diet.

			Natural ingredients			
	Salmon pellets	Carp pellets	Cyclops	Daphnia	Black mosquito larvae	Glassworm (white mosquito larvae)
Protein	55	29	3.5	2.4	5	5
Fat	18	10	0.4	0.7	1	1
NFE (Carbs)	8.7	46.5	0.4	0.3	0.9	0.9
Ash	10.5	5	0.1	0.7	0.8	0.8
Other (Moisture)	0.5	7.5	95.3	96.3	92	92
Energy content	21.6 MJ/kg	18.6 MJ/kg	0.78 MJ/kg	0.70 MJ/kg	1.31 MJ/kg	1.31 MJ/kg

The nutritional content of the commercial pelleted diets were obtained from the manufacturer (Skretting), and the nutritional content of the invertebrate ingredients of the natural diet was calculated manually.

Table S5.3: Multiple comparisons for overall growth for both groups and treatments using a Tukey adjustment for multiple comparisons. SE; standard error.

Comparisons	Estimate	SE	Z value	P value
Control - Carp	0.55	0.021	26.41	<1e-10
Natural - Carp	-0.28	0.035	-7.89	<1e-10
Natural - Control	-0.83	0.030	-27.18	<1e-10
Hybrid - Farm	-0.079	0.021	-3.83	0.00036
Wild - Farm	-0.33	0.023	-14.32	<1e-04
Wild - Hybrid	-0.25	0.023	-10.56	<1e-04

Table S5.4: Model selection of the random effect of the generalized linear mixed effect model used to investigate survival.

		Random effects										
N	Response	T:t	F	T:F	Df	AIC	BIC	logLik	Deviance	Chisq	Chi Df	P
2696	Survival	x	x		16	2558.6	2653	-1263.3	2526.6			
		x	x	x	21	2540.1	2664	-1249	2498.1	28.53	5	29e-05

T:t; replicate nested within treatments (random intercept). F; family (random intercept). T:F; family across treatments (random intercept and slope). Df; Degrees of freedom. AIC; Akaike information criterion. BIC; Bayesian information criterion; logLik; loglikelihood value. Chisq; Chi square value. Chi Df; Chi square degrees of freedom. P; p-value.

Table S5.5: Multiple comparisons for overall mortality for both groups and treatments using a Tukey adjustment for multiple comparisons. SE; standard error.

Comparisons	Estimate	SE	Z value	P value
Control - Carp	2.35	0.50	4.66	<1e-04
Natural - Carp	0.40	0.46	0.86	0.67
Natural - Control	-1.95	0.51	-3.84	0.00033
Hybrid - Farm	0.89	0.33	2.69	0.019
Wild - Farm	0.074	0.34	0.22	0.97
Wild - Hybrid	-0.81	0.36	-2.29	0.057

Table S5.6: PCR conditions for the microsatellite multiplex used to assign individuals back to family.

	Temperature (°C)	Time	
Denaturation	94	4 min	
Denaturation	94	50 s	Repeat x 26
Annealing	55	50 s	
Extension	72	80 s	
Final extension	72	10 min	
Storage	4	unlimited	

Chapter 6: General discussion

Salmon families of farmed, wild and F1 hybrid populations were grown under different environmental conditions, namely contrasting water temperatures, densities and rearing conditions, food availabilities and diets. The growth and survival differences between the groups were investigated in order to elucidate the fitness consequences of hybridisation including an assessment of whether farmed salmon have become adapted to specific hatchery conditions. In all experiments farmed fish outgrew wild fish, with hybrids displaying mostly intermediate growth. In Chapter 2, a significant genotype-by-environment (G x E) interaction was observed, indicating that population-specific differences in growth-rate are present between the multiple farmed, wild and F1 hybrid populations across different temperatures. In all other chapters, G x E interactions for growth were either non-significant (Chapter 3 or Chapter 5), or moderately significant (Chapter 4). Mortality was low and similar among all groups in Chapter 2, Chapter 3 and Chapter 4. A significant group effect was found for mortality in Chapter 5, although there was no significant difference in mortality between farmed and wild fish across the treatments, and wild fish on average exhibited the lowest survival across the treatments.

6.1 Growth differences and adaptations to the farming environment

Current strains of farmed Atlantic salmon have been subjected to over ten generations of domestication, including directional selection for a range of commercially important production traits such as high growth rates (Gjedrem 2010). As discussed previously, several comparative studies have demonstrated that farmed salmon outgrow wild conspecifics by large ratios under hatchery conditions (Glover *et al.* 2009; Solberg *et al.* 2013a; 2013b). The present thesis was able to document higher growth in farmed salmon relative to their wild and hybrid conspecifics, albeit at lower ratios than previously seen (Solberg *et al.* 2013a; 2013b), in all experiments. The higher growth differences observed under hatchery conditions are likely to be the result of farmed salmon becoming adapted to the highly regulated farming (hatchery) environment. Concurrently, adaptation to the farm environment may also explain why offspring of farmed salmon do not perform as well in the wild as in the hatchery.

Domesticated species are known to become adapted to their rearing environment (Christie *et al.* 2012; 2016), most notably exhibiting a reduced sensitivity to environmental change or exhibiting inappropriate behaviour in the natural environment (Price 1999). For example, Hill and Robertson (1998) found that domesticated pheasants (*Phasianus colchicus*

L.) were more susceptible to predation and exhibited lower territorial behaviours than wild pheasants. Similarly, Andersson *et al* (2001) found that domesticated poultry (*Gallus gallus domesticus* L.) were less able to adapt their foraging strategy to a more variable environment, with wild-type birds (*Gallus gallus* L.) being more willing to increase their energy expenditure to gain food. Domesticated Japanese Masu salmon (*Oncorhynchus masou* B.) tended to stay close to the water surface when feeding, opposite to wild-derived Masu salmon that remained near the bottom of the stream tank (Reinhardt 2001). While surface feeding is an optimum feeding strategy under hatchery conditions, in the wild such behaviour may expose the domesticated fish to a higher risk of predation than wild conspecifics (Reinhardt 2001).

In Atlantic salmon farming, water temperature in hatchery environments is typically regulated and often elevated during start feeding in order to promote growth and smoltification (Fjelldal *et al.* 2009). Thus, farmed fish may have become adapted to growth at higher temperatures and may not perform well in more variable natural water temperatures or at very low temperatures. No evidence was found for a reduced low temperature tolerance in farmed salmon relative to wild salmon when reared at extremely low water temperatures (Solberg *et al.* 2016). Similarly, the present thesis was unable to find evidence for a lack of low temperature tolerance in farmed fish (Chapter 2) as all groups displayed a lack of growth in the lowest temperature treatment. Therefore, an adaptation to higher, regulated temperatures is probably not causing the large divergence in growth differences observed among farmed and wild fish between the hatchery and wild environments.

Another potential explanation for the divergence in growth observed between farmed and wild conspecifics in hatchery and wild conditions could be adaptation to high densities. Typically, domestic fish experience much higher densities under hatchery conditions than they would in the wild. Increasing density in a rearing environment can cause decreased growth through a variety of mechanisms. Growth is decreased through competition (Imre *et al.* 2005), crowding stress (Montero *et al.* 1999), and reduced feeding as a result of the competition and stress effects (Holm *et al.* 1990). For example, Solberg *et al* (2013a) found that, although growth of all groups was decreased in the stressed treatment, farmed salmon were able to maintain their growth to a greater degree compared to their wild conspecifics under stressed conditions relative to controlled conditions; thus indicating a relaxed response to stressful conditions (Solberg *et al.* 2013a). It is possible that farmed fish are less stressed under high density rearing conditions than wild fish, allowing them to maintain their growth

advantage under such conditions. In the present thesis however, there was no indication that farmed fish had become adapted to growth at higher densities as the relative growth differences were not significantly different across the treatments (Chapter 3). Although it is possible that the densities used in the present thesis were not sufficient to elicit an adaptive response, fish from all groups displayed lower growth in the higher density treatments demonstrating the treatments employed elicited a strong response in fish of all genetic origins (Chapter 3). In the semi-natural environment, growth of all groups was depressed relative to the control treatment, although these treatments had the lowest overall densities. Salmonids are territorial and tend to establish social hierarchies in the natural environment, whereby the larger, more dominant fish have more access to resources than smaller fish (Jørgensen *et al.* 1993). However fish can exhibit behavioural plasticity depending on their circumstances and may switch from territorial behaviour to schooling when the metabolic effort of defending a territory becomes too high (Sundstrom *et al.* 2003; Brännäs *et al.* 2004). There were no territorial or dominance effects observed in the semi-natural treatments and it is more likely that the difference in degree days between the semi-natural and indoor treatments resulted in the low growth observed in the semi-natural treatments.

Domesticated animals often exhibit differences in feeding related traits relative to their wild counterparts. Studies have shown that foraging behaviours in fish have been altered by domestication (Orlov *et al.* 2006), however this may not be permanent (Olsen & Skilbrei 2010; Huntingford *et al.* 2012). Selection for increased growth in salmon has been linked to increased appetite (Thodesen *et al.* 1999) and FCE (Handeland *et al.* 2003) detected in farmed salmon, as well as shifts in endocrine control (Fleming *et al.* 2002; Solberg *et al.* 2012). Farmed fish may also have higher metabolism than wild fish due to the endocrine changes from selection mentioned previously (Björnsson 1997). Selection for increased growth has also been linked to behavioural changes in domesticated fish, such as increased aggression in the hatchery (Einum & Fleming 1997; Fleming & Einum 1997) and decreased predator awareness (Houde *et al.* 2010b). High growth levels and the associated behavioural changes may incur a high metabolic cost when resources are low. Therefore, while faster growth is often linked to higher fitness, behavioural-mediated trade-offs could reduce the growth and survival of individuals with higher growth rates in the wild through reduced starvation tolerance and increased predation risk (Martin-Smith *et al.* 2004; Biro *et al.* 2006; Sundt-Hansen *et al.* 2009). Alternatively, farmed fish may be able to utilise commercial pelleted feed better than wild salmon (Thodesen *et al.* 1999) or may have become adapted to

the shape or nutritional content of commercial pellets, resulting in the larger growth differences seen under hatchery conditions. By examining growth and survival at different feed availabilities, here, there was no evidence that farmed salmon had become adapted to constant feed availability (Chapter 4). Similarly, no evidence was observed for farmed fish being adapted to the shape and nutritional content of commercial salmon pellets (Chapter 5). It appears that farmed salmon have thus not lost their plasticity for growth in response to varying food levels or diets.

Our work was unable to find evidence that the divergence in growth differences between the hatchery and farm environment is the result of farmed fish being adapted to any of the above environmental parameters individually. Thus farmed Atlantic salmon have not lost their plasticity for growth under these environmental conditions. It is possible however that the difference in growth observed between farmed and wild Atlantic salmon in the hatchery versus the wild environment may be a combination of several factors, including predation, competition and those tested individually here. Furthermore, all experiments in the present thesis were limited to a controlled environment, with no predation and where the fish were fed to excess. Therefore extrapolating the conclusions to wild environments should be done with caution.

6.2 Hybrid growth and maternal effects

In all experiments, we found that the growth of the hybrids was intermediate relative to their parent groups, *i.e.* additive. Under an additive model of inheritance the average offspring phenotype will approximate the average phenotype of the contributing parental populations. As mentioned previously, additive genetic (intermediate) growth of F1 hybrids has been observed in several comparative studies of Atlantic salmon in various environments, including controlled conditions (Glover *et al.* 2009; Fraser *et al.* 2010; Solberg *et al.* 2013a), semi-natural environments (Solberg *et al.* 2013b), and in the wild (Einum & Fleming 1997). Similarly, intermediate expression of various traits, including growth and behavioural traits; have been observed in hybrids of other fish species (Tymchuk & Devlin 2005; Tymchuk *et al.* 2006). If the parent populations typically experience different environments, hybrids may display outbreeding depression due to a disruption of local adaptation through a breakdown of co-adapted gene complexes or a loss of additive epistasis, and this usually does not manifest until after recombination, *i.e.* in F2 or backcrosses individuals. For example,

Tymchuk *et al* (2007) investigated the effects of hybridisation and introgression on survival of rainbow trout in semi-natural environments with either food competition or predation using three generations of hybrids. They found that in a competitive environment all the hybrids displayed additive gene action, whereas predation led to outbreeding depression in the second generation backcrossed hybrids (Tymchuk *et al.* 2007). Therefore the consequences of hybridisation may also be influenced by environment (Tymchuk *et al.* 2007). Often the effects of hybridisation and interbreeding on fitness are difficult to predict, especially when heritability patterns are non-additive in the hybrid offspring phenotypes (Debes & Hutchings 2014). The effects of hybridisation may also differ depending on the native parent population (Einum & Fleming 1997). It was found that crossing a farmed strain with one wild population resulted in hybrids displaying predictable additive behaviour, while crossing the same farmed population to another wild population resulted in hybrids displaying hybrid vigour (Einum & Fleming 1997). In Chapter 3, the hybrids grew significantly different to their wild and farmed parental strains; however there was an observable non-significant trend of hybrid growth being more similar to their farmed parents, *i.e.* non-additive. Bicskei *et al* (2014) found evidence for either intermediate or maternally dominant heritability patterns in the differentially expressed transcripts of hybrid fish. Their hybrid crosses were generated using farmed females, suggesting that maternal effects may explain this bias (Bicskei *et al.* 2014). The hybrids involved in Chapter 3 were also created using farmed female parents, and the non-significant trend of similarity to the farmed fish may thus be a result of maternal effects, such as egg size. Therefore the use of reciprocal crosses in comparative studies is encouraged, in order to account for maternal effects. Similarly, the use of F2 hybrids and backcrosses is also encouraged, in order to elucidate the consequences of interbreeding beyond the F1 generation. With additive genetic variation between farmed and wild conspecifics, the phenotypic effects of low-level interbreeding on wild populations may be diluted over multiple generations (McGinnity *et al.* 2003; Tymchuk & Devlin 2005; Harbicht *et al.* 2014). However, large-scale or frequent escape events and subsequent interbreeding may cause changes in baseline genotypes of wild populations (Tymchuk & Devlin 2005) and trait distributions may not be completely restored after multigenerational backcrossing (Fraser *et al.* 2010). Therefore the consequences of multiple generations of hybridisations on population fitness remain unclear.

Egg size was significantly associated with growth in all experiments apart from those in Chapter 4, either individually or with treatment as an interaction. In Chapter 5 and Chapter

3 egg size was found to be negatively correlated with weight, due to the larger average egg sizes of the wild families coupled with their lower growth compared to the hybrid and farm families. Egg size in experiments in Chapter 2 was significantly associated with growth at the low temperature treatment, most likely due to slow development and growth. Similarly, egg size was significantly associated with growth in the semi-natural treatments and the high density hatchery treatment of Chapter 3, where the fish were smaller on average due either to the higher densities experienced or the shorter number of degree days than the hatchery tanks. It is to be expected that egg size was associated with growth in the treatments where the fish were smaller, as egg size is known to influence early growth in fish (Dunham 2004). Egg size is often positively associated with fitness (Skaala *et al.* 2012), although it is a maternal effect that decreases with time (Mousseau & Fox 1998). It was also found that families with large eggs tended to be larger at emergence, and that the advantage of this size difference over siblings from smaller eggs was only detected in certain environments (Einum & Fleming 1999).

6.3 Survival

Survival in the hatchery environment is usually high due to the carefully controlled environments conditions (notably highly accessible feed and lack of predation), whereas salmonids in the wild experience high mortality as a result of natural selective forces like predation and competition. In the present study survival was either not tested (Chapter 2), there were no detected effects on survival (Chapter 4), there was no difference in survival between the groups but a significant treatment effect (Chapter 3), or both the group origin and treatment significantly affected survival (Chapter 5). Experiments conducted in the wild have demonstrated that the offspring of farmed salmon display a significantly lower survival than the offspring of wild conspecifics, and that hybrids display intermediate (additive genetic variation) survival (McGinnity *et al.* 1997; McGinnity *et al.* 2003), although this may not always be the case. For example, Skaala *et al* (2012) found that hybrid salmon families survived better than expected (non-additively) compared to farmed families under natural river conditions. In their study the farmed females used in the hybrid crosses had large eggs, and the authors attribute the elevated survival of the hybrid families to the maternal effect of the large egg size coupled with the advantage of having a wild parent contribution. Lower relative survival of farmed fish in the wild was found in other comparative studies (Skaala *et al.* 2012), although Fleming *et al* (2000) found that this was confined to early stage survival.

The various behavioural and genetic differences discussed previously that can arise in farmed fish due to domestication may influence their survival and that of their offspring in the wild. Escapees may initially be constrained in switching from pelleted food to live prey, potentially leading to starvation (Orlov *et al.* 2006; Olsen & Skilbrei 2010), although generally diets of farmed and wild salmon in the wild are found to overlap (Fleming *et al.* 2000; Skaala *et al.* 2012). Farmed salmon often exhibit behavioural differences (Einum & Fleming 1997; Houde *et al.* 2010b) relative to wild salmon. Hormonal changes associated with selection for growth in farmed salmon (Fleming *et al.* 2002; Solberg *et al.* 2012) are also associated with various feeding related traits which are found to be divergent in farmed salmon, including higher metabolism (Björnsson 1997), increased foraging behaviour and FCE (Neregård *et al.* 2008a; 2008b), and increased appetite (Thodesen *et al.* 1999). Changes in such traits could result in lower survival in the wild when resources are low or predation is high (Biro *et al.* 2006). We found no evidence that contrasting levels of food availability unduly influenced the survival of salmon of all groups (Chapter 4). Similarly, we found no evidence that the survival of farmed salmon was compromised when fed a diet of natural prey (Chapter 5). Although group origin was significantly associated with survival in Chapter 5, the average survival of farmed individuals was highest in the natural diet treatment, and there was no difference in overall survival between farmed and wild fish detected. In Chapter 3 there was no difference in survival within the hatchery treatments, however survival was significantly lower for all groups in the semi-natural treatments. Within the semi-natural treatments the lowest survival was observed in the low density replicates. Generally, in the wild mortality is positively related to density (Jørgensen *et al.* 1993), and it is unclear why the low density semi-natural replicates experienced the highest mortalities. In natural conditions salmon are territorial (Post *et al.* 1999; Imre *et al.* 2005) and this could have density dependent effect on survival. As population density increases within a natural stream environment, those fish, which cannot secure access to food, may either disperse or die (Post *et al.* 1999; Imre *et al.* 2005). In a study investigating the growth and survival of farmed, wild and F1 hybrid salmon families along an environmental gradient from a hatchery to a semi-natural environment with restricted feed the smaller wild salmon survived less well than larger hybrid and farmed salmon, indicating negative size-selective mortality (Solberg *et al.* 2013b). Similarly, it was found that under semi-natural conditions with competition larger domesticated rainbow trout and their hybrid offspring had a competitive advantage and survived better than wild conspecifics (Tymchuk *et al.* 2007). However when predators were introduced a negative correlation between growth and survival was observed, potentially

attributed to the faster growing domesticated trout being more susceptible to predation (Tymchuk *et al.* 2007).

Therefore, there is the potential for resource availability, behaviour and predation to interact to influence survival of farmed fish and their offspring in the wild. Since all experiments conducted in the present study took place in the hatchery or under semi-natural conditions with no food competition or predation, it is possible that the experimental conditions were simply unable to elicit a survival response in all groups.

6.4 Population-specific growth differences

Successful hybridisation and the subsequent introgression of farmed fish into wild populations will depend on a number of factors, including the level of escape and the local effective population size (Weir & Grant 2005; Baskett *et al.* 2013). Smaller local populations will be more at risk from introgression than large local populations, although large populations may also be negatively affected through increasing resource competition and predation risk (Weir & Grant 2005). Although large scale escape events will affect local populations more quickly (Hindar *et al.* 2006), low levels of constant escapes can cause the continued lowering of wild population fitness over time (Baskett *et al.* 2013). Interbreeding success will also depend on the life stage of the escapee and the degree of maladaptation of the escapees (Baskett & Waples 2012; 2013). A spatio-temporal study conducted using historical and contemporary samples of fish from 21 wild Atlantic salmon populations in Norway revealed significant temporal genetic changes had occurred in six of the 21 rivers; suggesting that, overall, introgression from farmed escapes was population-dependent and potentially linked to local population density (Glover *et al.* 2012). In a similar study based on 20 populations in Norway, Glover *et al* (2013) estimated that the introgression levels from farmed escapes varied from 2% to 47% per population. These authors concluded that the density of the natural population represented a major effect moderating introgression, a suggestion supported later on by modelling data (Heino *et al.* 2015). Other studies have also documented changes in the genetic structure of wild populations due to farmed escapes (Skaala *et al.* 2006; Bourret *et al.* 2011) or restocking (Sušnik *et al.* 2004) and furthermore that these changes are population-dependent (Hansen *et al.* 2009; Ozerov *et al.* 2016).

Here, population-specific differences for growth were found between farmed and wild salmon under different water temperatures (Chapter 2). Population level differences have been observed between farmed and wild conspecifics and their hybrids or backcrosses for a

variety of life-history traits, including compensatory growth (Morris *et al.* 2011), early development (Darwish & Hutchings 2009) and gene expression levels (Normandeau *et al.* 2009). Thus, different salmon populations will be affected by interbreeding to varying degrees dependent on their genetic architecture (Normandeau *et al.* 2009). Such evidence, including that observed here, highlights the merits of adopting a more spatially complex approach to risk management of local wild populations. This is especially true for species where populations are likely to be locally adapted to their native environmental conditions. The application of management strategies such as the portfolio effect may thus benefit wild fish populations. The idea that inter-population diversity encourages the stability of population complexes has been demonstrated in studies of the Californian Chinook salmon (*Oncorhynchus tshawytscha* L.) and the Alaskan sockeye salmon (*O. nerka* L.), whereby aggregates of both populations were able to sustain productivity despite changes in environmental conditions and anthropogenic activity (Hilborn *et al.* 2003; Schindler *et al.* 2010; Carlson & Satterwaithe 2011).

While a moderately significant effect of group by treatment (food availability) was found in Chapter 4, the growth reaction norms and relative growth differences between the groups across the treatments indicate that this effect is probably the result of the slight differences in growth observed in the hybrids relative to the other groups. In Chapter 4, fish were reared in single strain tanks, eliminating the need for genotyping and family information. Therefore it is also possible that the marginal significant effect is the result of unobservable family variation in growth, which was not taken into account in the random effects of the model. In all other chapters there were no significant G x E interactions detected.

6.5 Implications and future research

The present study demonstrated a significant population level effect on growth under varied temperatures. Moreover, it demonstrated that farmed fish have not lost their plasticity for growth or survival in response to contrasting food availability, diets and densities under hatchery conditions. Thus, while incremental, the results from this thesis demonstrate that these factors, taken individually, are probably not responsible for the divergence in growth differences found between hatchery and wild conditions. Studies which include multiple environments, including natural conditions, are encouraged in order to further validate the present findings. Common garden studies are a robust method of detecting population-level

variation and plasticity among populations and further studies of this nature are encouraged with a wider range of populations and families.

In all experiments, both farmed and hybrid fish were able to outgrow wild conspecifics, with implications for the outcome of competitive interactions between wild and domesticated fish in the wild. While growth differences in the wild are often observed to be less than reported for hatchery studies, offspring of farmed salmon may displace wild conspecifics and compete for resources, ultimately affecting the productivity of wild populations. It was estimated that the lifetime success of farmed salmon was 2% of that of wild salmon in an Irish river system (McGinnity *et al.* 2003), while Fleming *et al* (2000) estimated that the lifetime reproductive success of farmed individuals to wild fish was 16% in a Norwegian river. Thus if farmed and hybrid salmon exhibit higher growth rates than wild fish, are displacing and outcompeting wild fish in nature, but are not successfully completing their life cycle, the overall productivity and fitness of the wild population will be reduced (McGinnity *et al.* 1997; 2003; Fleming *et al.* 2000). In wild populations with low effective population sizes or those which experience repeated escape events, the effect of this could eventually be population extinction (McGinnity *et al.* 1997).

The reproductive success of farm origin salmon is often found to be inferior to wild fish (Fleming *et al.* 1996; 2000), and has been shown to be sex-biased towards the males, who exhibit inappropriate mating behaviours (Fleming *et al.* 2000). Therefore the most likely route of interbreeding would be between escaped females and wild males (Fleming *et al.* 2000), resulting in hybrid offspring. It is thus important to understand the potential effects of hybridisation on individual fitness and wild population productivity. Investigating the consequences of hybridisation are important for the further management and conservation of wild stocks, not just for salmonids, but for other species where aquaculture practises occur within their native range. Therefore further studies which assess the fitness of several generations of hybrids in varied environmental conditions are encouraged.

The typically low levels of population structure found in marine species (Hauser & Carvalho 2008; Milano *et al.* 2014), coupled with differences in life-history and current level of genetic knowledge make it difficult to draw parallels between the consequences and risks associated with interbreeding of these fishes and salmonids. Using Atlantic salmon as a model species for marine aquaculture impacts has its limitations, although several aspects of the present thesis can be adapted to marine species. A finer spatial scale of understanding of

population structure would be important in order to tailor risk management to population-specific conservation. Several studies now demonstrate that population structuring in several key marine species is much higher than previously expected (Nielsen *et al.* 2004; Mariani *et al.* 2005). Although breeding programs are not as temporally advanced as in the salmon farming industry, the effects of domestication on marine species have been documented (Karaiskou *et al.* 2009) and potential consequences of genetic interactions between farmed escaped marine fish and their wild conspecifics can begin to be identified. One shortcoming of the current marine finfish aquaculture system in Europe is the lack of enforced reporting of escapes, and the lack of transparency regarding escapees in the industry (Glover *et al.* 2010; Arechavala-Lopez *et al.* 2013). It should be encouraged that farmers and conservationists alike are allowed to share their knowledge and experience as this will help with the prediction and mitigation of farm-wild interactions.

In general, there are several management approaches which could mitigate the effects of escaped fish on wild populations. Improvement of cages to withstand adverse weather conditions would prevent loss of fish during storms. In Norway, sites, cages and various other technical aspects of the farm need to adhere to certain technical standards, with research focused on improving the robustness of farming equipment (Jensen *et al.* 2010). Changes to managerial or organisational aspects of fish farms which decrease human error may also prevent fish escapes (Thorvaldsen *et al.* 2015). Rearing fish in closed (recirculating) aquaculture systems, such as land-based systems, could effectively prevent any escaped fish from successfully reaching open water. Recirculating aquaculture systems are becoming more common for several commercially important aquaculture species, although the high set-up and operational costs are still a major challenge (Dalsgaard *et al.* 2013). Genetic containment is possible through the use of sterile farmed fish. For example, the Atlantic salmon farming industry is currently exploring the use of sterile (triploid) farmed fish to help to mitigate the negative effects of escapees on wild salmon (Piferrer *et al.* 2009), and it has been recently documented that triploid salmon escapees display approximately a 10-fold decreased likelihood of swimming to freshwater post escape (Glover *et al.* 2016), which would solve genetic interactions as well as reduce the potential for ecological interactions between escapees and wild fish in rivers.

What is clear is that escaped farmed fish have negative ecological and genetic effects on wild fish populations, and further research elucidating the effects of interbreeding and

hybridisation in various environments is encouraged in order to be able to mitigate the negative consequences of escapees.

6.6 Conclusions

- Population-specific differences in growth of farmed, wild and F1 hybrid strains under contrasting water temperatures highlight the need for a more spatially-resolved approach to risk management of escapees (Chapter 2).
- No evidence was found to suggest that farmed salmon have become adapted to the domestic environment with regards to water temperature (Chapter 2), rearing densities (Chapter 3), food availability (Chapter 4) or diets (Chapter 5).
- First generation (F1) hybrids typically displayed intermediate growth and survival in all experiments.

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Appendices

Appendix 1 – Primer information

Table S1.1: Details of the microsatellite multiplexes used to assign the individuals back to family in Chapter 2 (Multiplex 3), Chapter 3 (All), and Chapter 5 (Multiplex 3).

Multiplex	Primers	Dye	Allele Size	No. Alleles	Direction	Sequences	References
1	SSsp2210	6FAM	124-176	14	F	AAG TAT TCA TGC ACA CAC ATT CAC TGC	Paterson et al. 2004
					R	CAA GAC CCT TTT TCC AAT GGG ATT C	
	SSspG7	PET	119-207	22	F	CTT GGT CCC GTT CTT ACG ACA ACC	Patterson et al. 2004
					R	TGC ACG CTG CTT GGT CCT TG	
	SsaD144	NED	102-254	37	F	TTG TGA AGG GGC TGA CTA AC	King et.al 2005
					R	TCA ATT GTT GGG TGC ACA TAG	
	Ssa202	6FAM	230-298	18	F	CTT GGA ATA TCT AGA ATA TGG C	O'Reilly et al. 1996
					R	GTT CAT GTG TTA ATG TTG CGT G	
	Sp2201	PET	227-367	33	F	TTA GAT GGT GGG ATA CTG GGA GGC	Patersson et al. 2004
					R	CGG GAG CCC CAT AAC CCT ACT AAT AAC	
	SsaD157	NED	271-411	35	F	ATC GAA ATG GAA CTT TTG AAT G	King et.al 2005
					R	GCT TAG GGC TGA GAG AGG AAT AC	
2	Ssa289	PET	112-134	10	F	CTT TAC AAA TAG ACA GAC T	McConnell et al. 1995
					R	GTC ATA CAG TCA CTA TCA TC	
	Ssa14	NED	134-146	6	F	CCT TTT GAC AGA TTT AGG ATT TC	McConnell et al. 1995
					R	CAA ACC AAA CAT ACC TAA AGC C	
	Ssa171	NED	197-255	26	F	TTA TTA TCC AAA GGG GTC AAA A	O'Reilly et al. 1996
					R	GAG GTC GCT GGG GTT TAC TAT	
	Sp2216	6FAM	190-270	21	F	GGC CCA GAC AGA TAA ACA AAC ACG C	Paterson et al. 2004
					R	GCC AAC AGC AGC ATC TAC ACC CAG	
	Sp1605	PET	216-268	22	F	CGT AAT GGA AGT CAG TGG ACT GG	Paterson et al. 2004

					R	CTG ATT TAG CTT TTT AGT GCC CAA TGC	
3	**SSsp3016**	NED	62-150	23	F	GAC AGG GCT AAG TCA GGT CA	*Genbank no. AY372820*
					R	GAT TCT TAT ATA CTC TTA TCC CCA T	
	SsaF43	6FAM	99-139	18	F	AGC GGC ATA ACG TGC TGT GT	Sanchez et al. 1996
					R	GAG TCA CTC AAA GTG AGG CC	
	SSa197	PET	140-272	31	F	TGG CAG GGA TTT GAC ATA AC	O'Reilly et al. 1996
					R	GGG TTG AGT AGG GAG GCT TG	
	MHC1	VIC	114-166	22	F	AGG AAG GTG CTG AAG AGG AAC	
					R	CAA TTA CCA CAA GCC CGC TC	
	MHC2	VIC	210-380	14	F	GAT GGC AAA GAG GAA AGT GAG	
					R	TTG TTA TGC TCT ACC TCT GAA	
	SsOSL85	6FAM	175-222	26	F	TGT GGA TTT TTG TAT TAT GTT A	Slettan et. al. 1995
					R	ATA CAT TTC CTC CTC ATT CAG T	

Appendix 2 – Published papers