Thermal reaction norms for larval Atlantic cod (*Gadus morhua*)

Exploring population differences on a micro-geographic scale

by

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Master thesis in Marine Biology (MSc)

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If one truly loves nature
one finds beauty everywhere.

Vincent van Gogh, 1874

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October 2016 Elisabeth H. Juliussen

ABSTRACT

It is important both from a management and conservation point of view to uncover the potential of a species to cope with climate warming, and whether this potential differ among populations. In this thesis I used Atlantic cod (*Gadus morhua*), a harvested marine fish, as a model species to investigate population differences in thermal responses of larval growth and survival on a very fine spatial scale (<30 km). I undertook a common-garden experiment on cod from the inner and outer part of the Risør fjord system, in the southern part of Norway. Larvae were randomly sampled and reared at three experimental temperatures (6°C, 9.5°C and 13°C) for 28 days. As parent cod from both locations were reared together, I found offspring with a pure inner or outer heritage, and offspring with a mixed heritage. Reaction norms constructed for larval growth revealed a significant interaction effect between population and temperature. However, this interaction effect was not significant for the reaction norms of the inner fjord population and the outer fjord population. Here, I found only a marginally significant effect of population. For larval survival, I found no effect of population, only an additive effect of temperature. Both growth performance and survival was lowest at the highest temperature for the inner and outer fjord cod. Improved knowledge about the spatial scale on which a species is structured can help define management units on a level that preserves genetic diversity and promotes recovery of overfished stocks. My study suggests that fjord cod responses to temperature change are similar at a scale of 30 km or less. Additional studies from other systems could clarify whether this is a general trend.

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1. INTRODUCTION

1.1 Climate change

Global climate is getting warmer. The last three decades is, with high confidence, the warmest 30-year period seen during the last 800 years. The surface of the ocean and land combined have on average warmed by 0.85 °C from 1880 to 2012, and the upper 75 metres of the ocean have warmed 0,11°C per decade since 1971 - and temperatures are still rising (IPCC, 2014). In the North Sea, there has been an increase in mean annual sea surface temperatures (SST) of 0.5 °C since the mid 1980s (Mackenzie and Schiedek, 2007). Modelled temperature trends for the North Sea vary among different climate change scenarios (Clark *et al.*, 2003; Sheppard, 2004; Dye *et al.*, 2013). Following the most conservative scenario, HadCM3B1, the region is expected to experience an annual increase in SST of at least 0.005 °C (Clark *et al.*, 2003). Changes in sea temperatures are also associated with changes in ocean chemistry, circulation, sea level and precipitation patterns that might impact salinity in coastal ecosystems (Harley *et al.*, 2006; Sherman *et al.*, 2009). In their fifth assessment report (AR5), the Intergovernmental Panel on Climate Change (IPCC) acknowledged with confidence that human activities have influenced the global climate. Since the late 1800s, human emissions of greenhouse gases have increased, and during the last decade emissions have reached a peak. Greenhouse gases in the atmosphere are now higher than they have been for 800,000 years.

Anthropogenic climate change affects species in various ways. Either directly by altering their physiology, phenology or distribution range, or indirectly via changes in the food chain (Hughes, 2000; Portner and Peck, 2010). Climate change may also influence whole communities, as seen in the recent regime shift in the North Sea (Beaugrand, 2004). Ectotherms, who rely on external heat sources, are considered to be especially sensitive to changes in temperature (Paaijmans *et al.*, 2013). Temperature controls a number of fundamental organismal processes, and thereby have a direct influence on an organism's developmental rate and survival (O'Connor *et al.*, 2007). Because many marine organisms already live close to their thermal tolerances limits, further increases in temperature are likely to negatively affect the performance and survival. For instance, increasing temperatures can limit oxygen uptake, and cause a drop in the aerobic scope (Portner and Knust, 2007). Understanding mechanisms and potential for rapid responses to anticipated climate change thus represents a major challenge in marine science. In particular, there is a need to quantify the role of evolutionary adaptations and within-generation plastic changes in phenotypes (Crozier and Hutchings, 2014).

1.2 Plasticity and reaction norms

In a changing environment, organisms can either adapt through evolution, adjust plastically, move to a more benign environment or go extinct. Phenotypic plasticity is recognized as a primary mechanism of rapid response to environmental change (Canale and Henry, 2010), and describes the ability of a genotype to vary in phenotype along an environmental gradient – i.e. the proportion of the phenotypic variation explained by the environment (Stearns, 1992). Plasticity can differ among genotypes within a population and among populations, and is commonly expressed as a reaction norm – a linear or nonlinear function that describes the relationship between a phenotypic trait and the environment (Hutchings, 2011). If the reaction norms of different genotypes are parallel, the effect of genotype and environment is said to be additive (G+E) (Figure 1A). In this case different genotypes respond to a changing environment in a similar manner (Stearns, 1992; Halliburton, 2004). In other cases, reaction norms can have different slopes, meaning that genotypes will respond differently to a changing environment (Figure 1B) (Stearns, 1992). Such genotype-by-environment interactions can reflect evolutionary adaptations to environmental variation. In general, reaction norms are considered phenotypic traits that may be heritable and thus capable of evolving in response to selection (Hutchings, 2011). In other words, plastic responses to within-generation environmental changes are expected to shift the phenotype across the reaction norm, while longer-term evolutionary changes are expected to shift the position and/ or shape of the underlying reaction norm.

A population can be defined as a group of individuals belonging to the same species inhabiting a geographically continuous area, where the degree of intermixing with other groups of the same species inhabiting adjacent areas may vary (Halliburton, 2004). Importantly, reaction norms can be used to predict how a population might respond to environmental change (Hutchings *et al.*, 2007). Variation in reaction norms among populations has been documented in terrestrial (Bronikowski, 2000; Sørensen *et al.*, 2001; Liefting and Ellers, 2008; Richter-Boix *et al.*, 2010) as well as aquatic (Yamahira *et al.*, 2007; Jensen *et al.*, 2008; Piché *et al.*, 2008; Thomassen *et al.*, 2011; Gale *et al.*, 2013) species, also on very fine spatial scales (Thomassen *et al.*, 2011). As it can be difficult to disentangle the effect of environmental variables and genotype in the wild, reaction norms are often studied in common garden laboratory experiments (Conover *et al.*, 2006; Oomen and Hutchings, 2015a). Here, individuals from different populations or genetic groups are reared under equal environmental conditions. By controlling for the environment, observed differences in phenotypic traits between populations or groups will likely reflect genetic differences. Reaction norms can be constructed for experimental groups by rearing them under multiple environmental conditions. The slopes of the reaction norms will indicate whether there is an interaction effect between genotype and environment on the expressed phenotypes (i.e. genetic differences in plasticity).

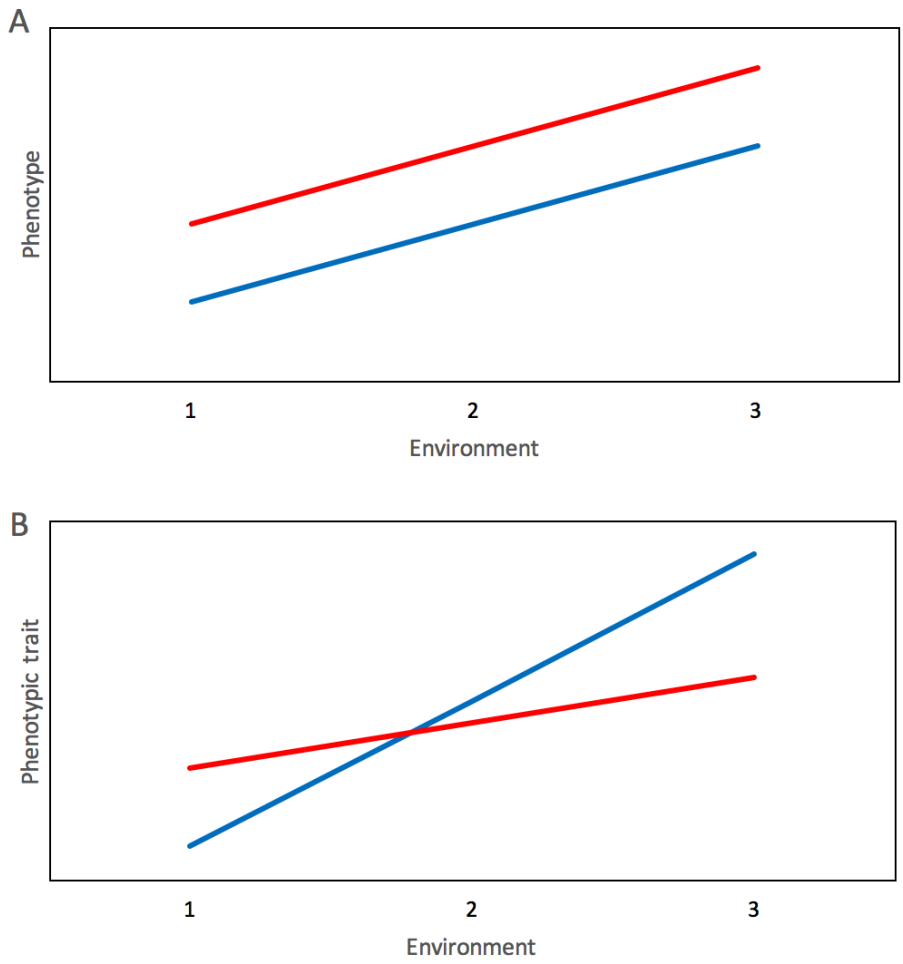

Figure 1. *Reaction norms (phenotypic trait expressed across three different environments) for two genotypes; genotype 1 (red) and genotype 2 (blue). Higher trait value is assumed to increases fitness, and higher fitness is assumed to be selected for. A: Genotype one will be selected for in all three environments. The reaction norms are parallel, and the effect of the environment is additive (G+E). B: Crossing reaction norms will have a different phenotypic ranking on either side of the crossing point (Stearns, 1992). Genotype one will thus be selected for in environment one, and genotype two will be selected for in environment three in. In environment two both genotypes will be selected for. The reaction norms cross, which indicates an interaction effect between genotype and environment (GxE). Background theory for making this figure is Stearns (1992).*

1.3 Mechanisms influencing population structure in marine organisms

Many marine species have evolved pelagic life stages with high dispersal potential. For marine fish, passive transport of eggs and larvae, and migratory capabilities of adults offer great opportunities for homogenizing gene flow (Waples, 1998). Genetic studies face a challenge in that small levels of gene flow might conceal genetic evidence for population structure within species (Waples, 1998). Still, genetic studies have detected genetic population structure within many marine species (Conover *et al.*, 2006; Hauser and Carvalho, 2008).

At large scales, geneflow is reduced due to geographical distance (Mork *et al.*, 1985), presence of contrasting current systems (Gonzalez *et al.*, 2015) or bathymetry (Catarino *et al.*, 2015). There is also evidence for population structure on smaller spatial scales (Knutsen *et al.*, 2003; Árnason, 2004; Knutsen *et al.*, 2013). Several mechanisms can be responsible for generating such small-scale genetic differences (Hemmer-Hansen *et al.*, 2007). For Atlantic cod (*Gadus morhua*; hereby referred to as cod), we find structure on several geographic scales (Hardie *et al.*, 2006; Hutchings *et al.*, 2007; Jorde *et al.*, 2007; Bradbury *et al.*, 2010; Andre *et al.*, 2016). For coastal cod along the Norwegian coast south of 62°, natal homing (Andre *et al.*, 2016), sedentary behaviour of adults, local topography and retention of eggs and larvae by currents (Jorde *et al.*, 2007; Knutsen *et al.*, 2007; Ciannelli *et al.*, 2010) are important structuring mechanisms on small spatial scales. In addition to producing genetic structure, spatial variation in environmental variables may allow for local adaptation among populations of marine species on very fine scales. Such local adaptations in fish biology could well involve plasticity (Oomen and Hutchings, 2015a), although there is limited research on genetic variability in reaction norms of marine fishes (Yamahira and Conover, 2002; Hutchings *et al.*, 2007; Olsen *et al.*, 2008; Wijekoon *et al.*, 2009; McCairns and Bernatchez, 2010; Baumann and Conover, 2011; Donelson and Munday, 2012; Côté *et al.*, 2014; Oomen and Hutchings, 2016).

In order to manage a sustainable fishery, it is important to know on what spatial scale fish populations are structured and adapted to their local environments (Lindegren *et al.*, 2013). Many marine fish populations in the Northwest Atlantic have collapsed in recent decades, and show only slow rates of recovery (Hutchings and Reynolds, 2004). For cod, intense fishing since the onset of industrial fishing in the 1900s led to the collapse of many stocks (Hutchings and Myers, 1994; Bartolino *et al.*, 2012). There are also strong indicators of fishing being a major factor in shaping fish life histories (Olsen *et al.*, 2004a; Jørgensen *et al.*, 2008). Prolonged overexploitation negatively affects the resilience of the exploited populations, thereby making recovery less probable (Neubauer *et al.*, 2013). Also, failing to acknowledge the existence of fine-scale population structure within a species might lead to overexploitation of sub-populations and loss of genetic diversity (Sterner, 2007). In the face of environmental disturbances, a high degree of genetic diversity could be a key factor in securing the species' future existence. Long-term changes in climate can strengthen the link between climate and recruitment success (Linderholm *et al.*, 2014), for instance by affecting the seasonal timing and amount of plankton (Beaugrand *et al.*, 2003). Fishing can, by altering the composition of the spawning stock, further increase the negative impact of climate change on fish populations (Perry *et al.*, 2010).

In this thesis I use cod as a model species to investigate population differences in phenotypic plasticity on very fine spatial scales. Population differences in plasticity has previously been

documented in cod on relatively large (600-800 km) (Hutchings *et al.*, 2007; Oomen and Hutchings, 2016) and intermediate (~200 km) spatial scales, but not on small spatial scales. I undertook a common-garden experiment on cod from the Risør fjord system, south Norway. Thermal reaction norms were constructed for larval growth and survival across a range of temperatures, including both native and atypical environments. If genotype-by-environment interactions are present, I would expect the slope of the reaction norms to vary among populations. This would mean that the two populations differ genetically in their plastic response to temperature changes. Alternatively, the populations can have genetic differences in reaction norms, without having different plastic responses. In this case, the reaction norms would be parallel, and the populations would display the same plastic response to temperature, however with different means.

2. MATERIALS AND METHODS

2.1 Study system

2.1.1. Atlantic cod

Cod is a marine teleost fish native to the North Atlantic. For centuries, it has been an important food for humans (Kurlansky, 2011). Cod is found from shallow coastal waters down to several hundred meters depth at the continental slopes, thriving at temperatures from 2-10 °C (Muus and Nielsen, 1998). Cod can grow as old as 30 years or more (Árnason, 2004), but due to high fishing pressure old individuals are now rare (Jørgensen *et al.*, 2008; Opdal, 2010). The global cod stock is comprised of many distinct populations, where different populations display clear differences in life histories (Blanchard *et al.*, 2005; Olsen *et al.*, 2008). Age and size at maturation, as well as seasonal timing of spawning, varies considerably both within and among populations (Blanchard *et al.*, 2005). For instance, female cod on Georges Bank (Canada) reach 50% maturity at 2.1 years of age and 43.3 cm length, while cod around Iceland reach 50% maturity at 6.6 years and 75.6 cm (ICES, 2005). Cod also have several migratory life history strategies. While most of the coastal cod are considered rather sedentary, some of the offshore stocks can perform large annual spawning migrations (Robichaud and Rose, 2004; Jørgensen *et al.*, 2008).

In Norwegian waters three main stocks of cod has been defined by the authorities; Norwegian coastal cod south of 62°, Norwegian coastal cod north of 62° and the Northeast Arctic cod, where the latter is known for its long-distance migrations between key feeding areas in the Barents Sea and its spawning areas along the northern Norwegian coast (Rollefsen, 1934; Holt and Jørgensen, 2014). In this thesis I focus on coastal cod from Skagerrak, southern Norway. Skagerrak coastal cod are considered highly stationary (Espeland *et al.*, 2007; Rogers *et al.*, 2014), apparently structured into local sub-populations that may extend only 30 km or less (Jorde *et al.*, 2007; Knutsen *et al.*, 2011). Natal homing of adults (Andre *et al.*, 2016), as well as local topography and circulatory patterns retaining pelagic eggs and larvae, are proposed as important mechanisms structuring the populations (Jorde *et al.*, 2007; Knutsen *et al.*, 2007; Ciannelli *et al.*, 2010). Offshore North Sea cod has been shown to influence coastal cod populations (Knutsen *et al.*, 2004; Stenseth *et al.*, 2006). The degree of influence is not well documented, but juvenile cod in exposed coastal areas are genetically very similar to the North Sea cod (Knutsen *et al.*, 2011; Sodeland *et al.*, 2016). The populations used in this thesis are cod from the fjord system around Risør (Figure 2), where cod from the inner and outer part of the fjord are demographically separated and probably represent two separate populations (Knutsen *et al.*, 2011). However, there is a possibility that the two populations are influenced by North Sea cod, and that the proportion of cod with North Sea origin is higher in the outer part of the fjord system.

Skagerrak coastal cod mature at an age of two to six years (Godø and Moksness, 1987; Olsen *et al.*, 2004b), and typically spawn from February to April (Espeland *et al.*, 2007). Young cod dominate the spawning stock, and individuals older than eight years are rarely seen (Gjøsæter *et al.*, 1996; Gjøsæter and Danielssen, 2011). Cod are batch spawners, and egg number and length of the spawning period increases with female age and size (Kjesbu *et al.*, 1996). Mating is not completely random; there is evidence of mate choice and mate competition during the spawning period (Hutchings *et al.*, 1999; Rowe *et al.*, 2007). Dominant behaviour and larger size of males is positively correlated to fertilization success (Hutchings *et al.*, 1999), and mating with a larger individual enhances reproductive success for both males and females (Rowe *et al.*, 2007).

Norwegian coastal cod spawn in fjords and coastal basins. The pelagic eggs hatch after two to four weeks, and the larvae spend an additional three to five months in the water column, before they settle at the bottom in relatively shallow water (Muus and Nielsen, 1998). Eelgrass meadows are important nursery areas for coastal cod, and the young fish rarely go down to deeper water (Bakketeig *et al.*, 2015). Pelagic cod larvae feed on smaller zooplankton, while after settlement the cod typically shift from eating marine invertebrates and crustaceans to a more piscivorous diet (Daan, 1973; Hop *et al.*, 1992; Munk, 1997).

In general, fishes have an indeterminate growth pattern, and both environmental differences (e.g. food and temperature) and genetic differences can cause growth rate to vary among populations (Weatherley and Gills, 1987). Skagerrak coastal cod juveniles typically grow around 9-11 cm during their first summer after settlement, depending on year and locality (Rogers *et al.*, 2011). Fine-scale spatial variation in growth is also evident at older life stages (Olsen *et al.*, 2004b; Kuparinen *et al.*, 2015). Age 0 juveniles tend to be smaller (grow slower) in years with warm summers, when surface waters in this region may reach 18-20 °C (Rogers *et al.*, 2011). Skagerrak coastal cod juveniles also tend to be more abundant during periods of cold temperatures, such as the 1960s and 1970s (Barceló *et al.*, 2015). Recent telemetry studies also show that cod seek to deeper cool waters as sea surface temperature rises during summer, with the effect being stronger for larger cod (Freitas *et al.*, 2015).

2.1.2 Study area

Norway has a heterogeneous coast with fjords, inlets, islets, skerries and variable depths. Such heterogeneity influences temperature, ocean current patterns and several other parameters, thus providing great opportunities for species to adapt to local conditions. In this experiment I investigated cod from the fjord system around Risør in the southern part of Norway (Figure 2). The Risør fjord system covers an area of 20 km^2, and is shaped like a 'U'. The outer part of the fjord (Østerfjorden) is dominated by skerries, and to a large extent

exposed to the Skagerrak and the North Sea. The inner part of the fjord (Sørfjorden) is a more sheltered area, with numerous sills with depths from 20-40 m (Gjøsæter *et al.*, 1996; Knutsen *et al.*, 2007; Ciannelli *et al.*, 2010).

From February to May 2014, IMR Flødevigen had temperature loggers deposited in the fjord. Originally there were a total of 12 loggers distributed at three depths (5, 10 and 15 m) in four locations; two in Østerfjorden and two in Sørfjorden (see Figure 2). As some loggers were lost and some damaged, we were only able to collect temperature data from two depths at one location in Sørfjorden and from three depths at one location in Østerfjorden. In February, at the time when spawning initiated in the lab, the mean temperature at 5 metres was 2.95°C (SD±1.21) in Sørfjorden and 2.78°C (SD±0.84) in Østerfjorden. Throughout the spawning season, temperatures rose from 2°C to above 8°C at both locations. However, temperatures in Østerfjorden appear to be more stable and with a smoother increase than temperatures in Sørfjorden (Figure A1, Appendix A).

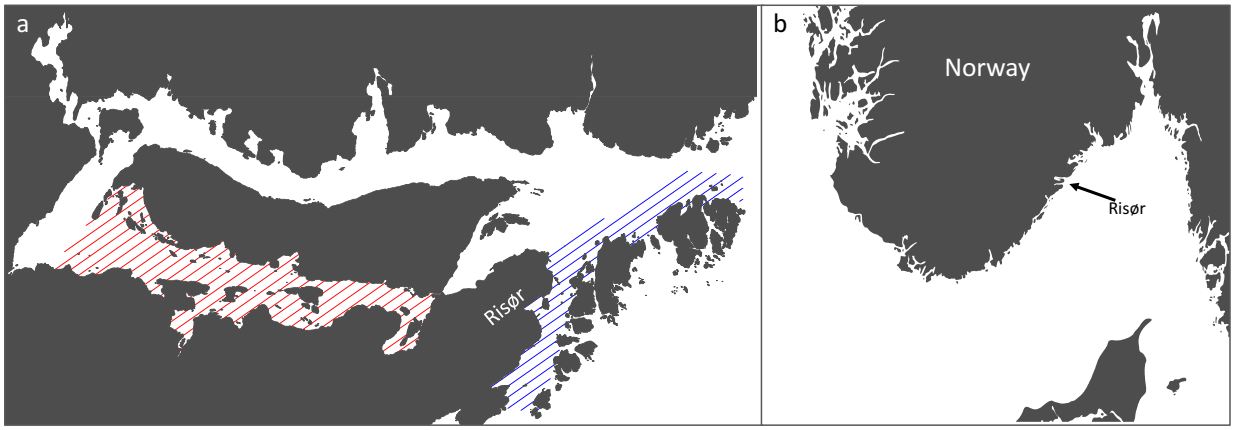

Figure 2. *a) Atlantic cod were sampled from two locations in the fjord system near Risør; the inner part of the fjord, Sørfjorden (marked with red vertical stripes) and the outer part of the fjord, Østfjorden (marked with blue vertical stripes). B) A map overviewing the southern part of Norway and the Skagerrak sea, with an arrow pointing towards the location of the study fjord.*

2.2 Common-garden experiment

2.2.1 Sampling

A common-garden experiment was conducted in spring 2014 with cod from the Risør area. Cod were caught using non-baited fyke nets (mesh size: 20 mm) from November 2013 to early January 2014. Individuals larger than 65 cm and smaller than 40 cm were not included in the experiment, to avoid cannibalism (Daan, 1973; Bogstad *et al.*, 1994) and to ensure that the fish were mature. This left a total of 73 potentially mature cod for the experiment: 21 females and 16 males from the inner part of the system (Sørfjorden cod, hereby referred to as inner cod) and 24 females and 12 males from the outer part (Østerfjorden cod, hereby referred

to as outer cod) (Figure 2). Sampling of these adult cod is also described by Kuparinen *et al.* (2015), analysing spatial variation in growth of the parent fish used in the experiment.

Upon capture, cod were tagged individually, measured for length to the nearest centimetre and weighed to the nearest gram. Mean length of the inner cod was 51 cm (range: 45-58 cm) and the mean weight was 1506 g (range: 751-2093 g). The mean length of the outer cod was 53 cm (range: 45-64 cm), and the mean weight was 1839 g (range: 1038-2918 g). Analyses of otolith growth patterns indicated that the cod ranged in age from 4 to 8 years, and that outer cod tended to be larger at a given age than inner cod (Kuparinen *et al.*, 2015). Tissue samples were taken from the caudal fin and stored in ethanol for later use in genetic analyses. Cod were individually marked using T-bar anchor tags (Hallprint Pty. Ltd., South Australia) and a standard tag applicator. Immediately after capture the cod were transported to the research facility of the Institute of Marine Research Flødevigen in Arendal. Adults were held together in a 45 m^3 spawning basin and allowed to spawn undisturbed. Daily observations of egg production (from a collecting box at one end of the basin) showed that the fish started spawning in February and continued spawning till mid-April. The spawning basin and the surrounding environment reflected ambient temperature and photoperiod.

2.2.2 Experimental set up and larvae sampling

Eggs for the experiment were collected on the peak day (a day in the middle of the spawning season with greatest number of eggs) of spawning, day 45, aiming for adequate sample sizes of eggs from both inner and outer cod (as the seasonal timing of spawning is known to vary among populations of cod (Blanchard *et al.*, 2005), and the inner and outer Risør cod likely represent two separate populations (Knutsen *et al.*, 2011). As cod have pelagic eggs, a mesh collector situated at the surface outflow of the spawning basin was used to collect the eggs. The eggs were held in a 900 L water tank at 6°C until hatch, when the newly hatched larvae were randomly sampled and transferred to 40 L experimental tanks (Figure 3). There were nine tanks in total with 2000 larvae in each. The larvae were reared at three different temperatures (target temperatures of 6°C, 9.5°C and 13°C) with three replicate tanks per temperature. Oxygen levels and temperatures were measured each day. The average (±SD) temperatures were 13.3 ± 0.3°C, 9.7 ± 0.2°C, 6.3 ± 0.2°C in the high, intermediate and low temperature tanks, respectively. The larvae were fed rotifers (*Brachionus plicatilis*) in excess (4500 prey/L three times daily) (Hutchings *et al.*, 2007). After 28 experimental days, the experiment was terminated.

Forty larvae were sampled randomly from each experimental tank at experimental day 2 and day 28 (n=720). To get the larvae evenly distributed in the tank, and avoid sampling bias due to population-originated differences in behavior, a plastic rod was used to gently stir up the water prior to sampling.

Figure 3. *Picture from the lab where the experiment was conducted. Egg tank in the front, where Atlantic cod eggs were kept until hatch, and experimental tanks in the back.*

2.3 Molecular laboratory work

Microsatellite loci (Table 1) were used to score the genotype of both adults and larvae. As these markers display many alleles and are presumed to be neutral, they are well suited for parental assignment. Previous genetic studies on cod have been successful in assigning offspring to population using five to seven microsatellite loci (Hutchings *et al.*, 2007; Oomen and Hutchings, 2015b; Oomen and Hutchings, 2016). Here I used eight loci for the parental assignment.

DNA was extracted from both whole larvae and fin clips from the adults. All extractions were done using E-Z 96 DNA/RNA plate kits from Omega Bio-Tek (USA), following the manufacturers protocol for tissue samples (a plate is a faster way to do DNA extractions, as one plate offers the opportunity to extract DNA from 96 samples simultaneously). One well on every plate was used as a negative control, to be able to exclude possible errors due to contamination and wrong plate orientation. Primer sets for eight loci were used to amplify microsatellites, and PCR amplification was done in two multiplexes. The multiplexes were modified from Delghandi *et.al.* (2003) (Multiplex 1) and Dahle *et al*. (2006) and Glover et al. (2010) (Multiplex 2). See Table 1 for details.

The PCR protocol for the first multiplex was 1.50 mM buffer, 0.30 mM dNTP, 0.80 U QiagenTaq pol, 0.12 µM GMO19, 0.32 µM TCH11, 0.04 µM GMO8, 0.20 µM GMO35 (all primers one forward and one reverse) and distilled H_2O. The second multiplex protocol for PCR was 1.50 mM buffer, 0.3 mM dNTP, 0.80 U QiagenTaq pol, 0.12 µM GMO34, 0.23 µM GMO132, 0.18 µM GMO2, 0.35 µM TCH13 (forward and reverse) and distilled H_2O. The total volume of each PCR was 10 µl, of which 1 µl was DNA extract of unknown concentration.

The PCR cycling conditions for both multiplexes were the same, consisting of an initial 5-minute denaturation at 95°C followed by 30 cycles of denaturation (30 seconds at 90°), annealing (90 seconds at 56°C), and extension (60 seconds at 72°C). A final step of 10 minutes extension completed the amplification. PCR products were kept at 4°C until further analysis.

The PCR fragments were visualized and separated by length with an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems), and the genotypes were scored using Genemapper software (version 4.0; Life Technologies). Individuals with 3 or more loci lacking alleles or with unclear genotypes were run a second and a third time, and all three runs were compared when scoring those individuals. The cod were independently genotyped by two (larvae) or three (adults) people. In Genemapper, default analysis settings were applied; minimum peak height 50 and size standard GS500LIZ.

2.4 Statistical analysis

2.4.1 Microsatellite loci and parentage analysis

I estimated heterozygosity of each locus (H_O=observed heterozygosity within samples, H_e=expected heterozygosity total loci (Nei and Chesser, 1983)) using Genetic Data Analysis software (GDA) (Lewis and Zaykin, 2012). F_{IS}-values were estimated in GENEPOP v.4.2 (Rousset, 2008), and I tested for deviations from Hardy-Weinberg genotype proportions (Weir and Cockerham, 1984) using the exact probability test on the software GENEPOP on the web v.4.2. The level of significance was set to 0.05, however, as we expect false positive tests, we corrected for multiple tests using the false discovery rate approach (FDR) (Benjamini and Hochberg, 1995). Microsatellite loci estimates and tests were run on adult genotypes only, as the adult cod are more representative for genotype proportions in the wild populations.

Parents were assigned to offspring using CERVUS v3.0 (Kalinowski *et al.*, 2007). Allele frequencies in the populations were calculated with the adult cod as a reference. To get an

estimate of genotyping error, 78 larvae were amplified twice, genotyped and the genotypes were compared. This gave an error rate of 0.006536. Assignment was run with both the estimated error rate and a global error rate of 0.01. Proportion of loci typed was 0.9944, and the minimum number of typed loci was four. For all other parameters, default settings were used.

Larvae with both parents caught in the inner fjord are considered to belong to the inner cod population. Larvae with both parents caught in the outer fjord are considered to belong to the outer cod population. Larvae with a mixed heritage are referred to as hybrids, or the hybrid population.

2.4.2 Spawning success

The effects of length, sex and locality on the probability of being assigned as a parent were explored using a generalized linear model with, with length, sex, locality and their interaction as explanatory variables and a binomial distribution (equation 1). Model was selected following the AIC criteria, and stepwise removal of the least significant variable.

Eq.1 P (parent) = locality + sex + length + (locality x length) + (locality x sex) + (sex x length)

2.4.3 Growth reaction norms

All sampled larvae were put on a glass slide containing RNA later and immediately photographed using a stereoscope and a Leica DFC 425 C camera. The photographs were used to measure the larval standard length in pixels (i.e. the backline of the larva from the tip of the tail to the neck and straight to the upper jaw, (Figure 4) (Kahn *et al.*, 2004)), using the software ImageJ (Abràmhoff *et al.*, 2004). A 1 mm scale bar photographed at the same time as the larva was used to convert pixels to mm. A total of four larvae were excluded from the analyses due to missing or poor quality photographs. As larvae were photographed in different positions, larval curvature (0=not curved, 1=slightly curved and 2=very curved) and larval position (0=side, 1=back and 2=stomach) was defined.

To check the precision of my measurements, I measured a subset of larva (three tanks with a total of 120 larva) twice. A linear regression using the first measurement as predictor variable

and the second measurement as response variable indicated very small measurement errors (slope = 1.0054, $R^2 = 0.99$).

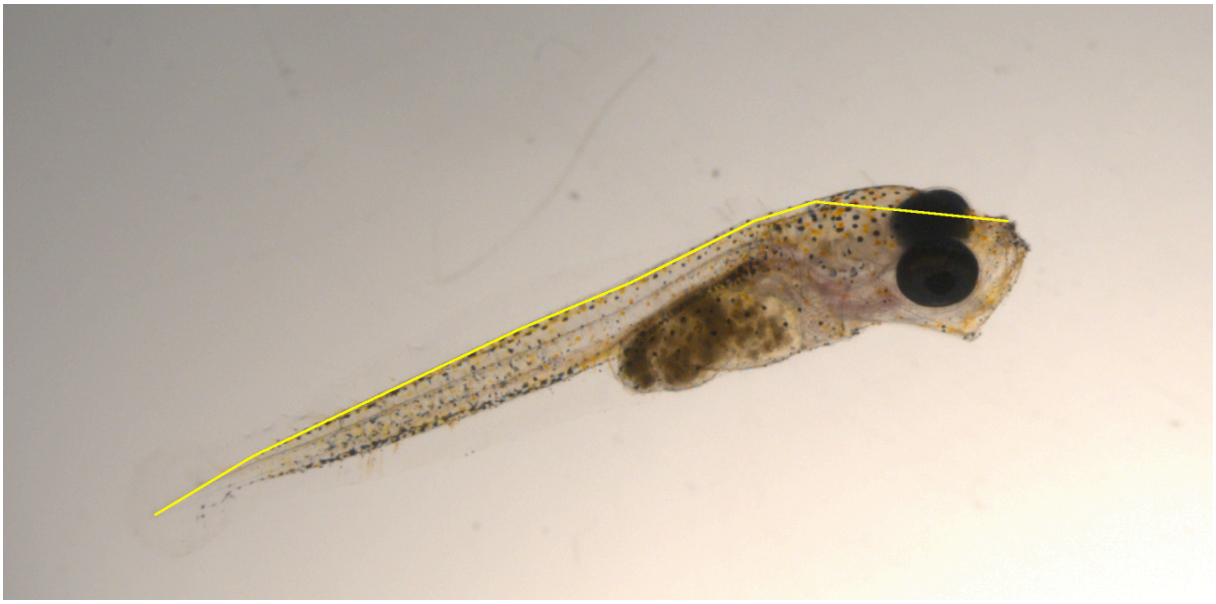

Figure 4. *All larvae were photographed, and the photographs were used to estimate length. Visualized by the yellow line, larval length was measured from the tip of the tail following the backline of the larvae to the neck, and then straight to the jaw. This photo shows a larva from the high (13°C) temperature tank at the end of the experiment.*

All statistical analysis on growth reaction norms and survival were conducted in R v.3.2.3 (R Development Core Team, 2015).

A linear model was applied to test for population differences in length at the beginning of the experiment, using length at day 2 as response variable. However, the samples from day 2 only contained 7 larvae from the outer cod population, compared to 107 larvae from the inner population, and 242 hybrids. Therefore, all larvae from all tanks were analysed together, assuming that tank-effects on length at day 2 are not important.

The distribution of lengths at day 28 does not deviate substantially from normal, and was assessed visually using a residual plot (Figure B1, Appendix B) and a quantile-quantile (Q-Q) plot (Figure B2, Appendix B). Variance is slightly deviating, but with no emergent pattern, and appears fairly homogenous across temperatures and between populations (Figure B3, Appendix B).

Growth was defined as larval length at the end of the experiment (day 28), not accounting for potential length-differences at the beginning of the experiment. Population differences in thermal growth reaction norms were explored using a mixed effects linear model (Schielzeth and Nakagawa, 2013) with population, temperature and their interaction as fixed effects, and

tank as a random effect nested within temperature. Larval position and curvature were added as covariates in the model.

Eq. 2 length = population + temperature + pop x temp + curvature + position + tank(temperature)

First, differences in growth between tanks was evaluated by comparing the full model (Equation 2) to a model without a random tank effect, using a restricted maximum likelihood approach (REML). Second, the fixed effects were evaluated by comparing equation 1 to simpler models by sequentially removing position, curvature and the pop x temp interaction effect, using a maximum likelihood approach (ML). Model selection was based on the AIC criteria, where the model with the lowest AIC value was used for inference (Burnham & Anderson, 1998).

A significant interaction between population and temperature indicates a significant difference in the slopes of the reaction norms, and suggests that there are genetic differences between populations. P-values equal to or less than 0.05 are considered significant, and p-values $0.05 \leq 0.1$ are considered marginally significant.

2.4.4 Survival

Survival was quantified using data from both day 2 and day 28, as the proportion of larvae alive at the end of the experiment (day 28). Population differences in survival reaction norms were explored using a generalized linear model with population, temperature and their interaction as fixed effects, and a quasi-binomial distribution. Chi square tests were used to select the best model, using a forward stepwise approach and a p-value of 0.05 as a cut-off point for including variables.

Eq. 3 survival = population + temperature + pop x temp

Survival reaction norms were constructed for inner fjord offspring and hybrid offspring only. Outer fjord offspring were very sparse in the samples from day 2 (see above). Therefore, survival could not be reliably estimated for this group. As an alternative approach, we estimated relative survival as the percentage of larvae from each population in each tank at day 28. Effects of temperature and population were explored using a generalized linear model and Chi square tests as described above. If all populations survived equally well across temperatures, we would expect their percentages to be similar.

No serious deviations from normality was detected for either models, assed through quantile-quantile plots of model residuals (Appendix B, Figure B4 and B5).

3. RESULTS

3.1 Genetic diversity of parental cod

I successfully scored eight loci, with the number of alleles spanning from 7 (TCH13) to 24 (GMO19) (Table 1). Genetic variability, expressed as observed heterozygosity, ranged from 0.595 at locus GMO34 to 0.946 at locus TCH11 for the inner population, and from 0.667 at locus GMO34 to 1.000 at locus GMO8 for the outer population. For both populations, some loci seemed to have more heterozygotes than expected from Hardy-Weinberg (F_{IS}-values Table 1). However, only GMO19 (one out of 16 estimates) for the inner population deviated significantly from Hardy-Weinberg equilibrium before correcting for multiple tests using FDR (Table 2). After correcting for multiple testing, none of the loci deviated from Hardy-Weinberg equilibrium.

Table 1. Summary statistics and base pare (bp) range for the microsatellite loci used for parentage analysis of 73 Atlantic cod sampled from the inner and outer Risørfjord. Ho is observed heterozygosity, He is estimates of expected heterozygosity and ($N_{(a)}$) number of alleles. F_{IS} values are estimates of deviation from Hardy-Weinberg equilibrium, where negative values indicate a surplus of heterozygotes.

Locus	$N_{(a)}$	Range (bp)	Reference	$He_{(Outer)}$	$Ho_{(Outer)}$	$F_{IS\ (Outer)}$	$He_{(Inner)}$	$Ho_{(Inner)}$	$F_{IS\ (Inner)}$
GMO8[1]	23	110-205	Miller et al. 2000	0.953	1.000	-0.050	0.881	0.865	0.018
GMO19[1]	24	120-220	Miller et al. 2000	0.930	0.833	0.106	0.919	0.784	0.149
GMO35[1]	12	110-145	Miller et al. 2000	0.826	0.750	0.093	0.827	0.865	-0.046
TCH11[1]	20	121-193	O'Reilly et al. 2000	0.917	0.806	0.123	0.937	0.946	-0.009
GMO2[2]	14	102-138	Brooker et al. 1994	0.887	0.833	0.061	0.838	0.784	0.065
GMO34[2]	8	80-120	Miller et al. 2000	0.659	0.667	-0.012	0.665	0.595	0.108
GMO132[2]	30	100-186	Brooker et al. 1994	0.923	0.944	-0.024	0.883	0.892	-0.011
TCH13[2]	7	74-86	O'Reilly et al. 2000	0.919	0.889	0.033	0.924	0.919	0.006

[1] Multiplex 1 (Delghandi et al. 2003)
[2] Multiplex 2 (Dahle et al. 2006; Glover et al. 2010)

Table 2. I tested for deviations from Hardy-Weinberg equilibrium for the inner and outer population separately, on microsatellite loci listed in table LOCUS. Only GMO19 for the inner population displayed a significant deviation from Hardy-Weinberg equilibrium.

Locus	$P\text{-value}_{(Outer)}$	$P\text{-value}_{(Inner)}$
GMO35	0.124	0.331
GMO19	0.163	0.038
GMO8	0.966	0.281
TCH11	0.196	0.444
TCH13	0.504	0.352
GMO34	0.900	0.301
GMO2	0.509	0.074
GMO132	0.876	0.177

3.2 Parental assignment

A total of 720 cod larvae were sampled, genotyped and used for parentage assignment. Six of these were not included in further analysis, due either to poor picture quality or problems with DNA extraction. This leaves a total of 714 larvae to be included in the analyses of growth and survival. Microsatellite genotypes were obtained for 356 individuals from the start of the experiment (day 2) and 358 from the end of the experiment (day 28). The proportion of loci typed was 0.9944, and the minimum number of typed loci was four. All the 714 larvae were successfully assigned to parents using a maximum of one allele mismatch between parents and offspring (Table 3). The assignments were identical whether the assignment was run with a global error rate of 0.01 or with the calculated error rate (0.006536). On day 2, a total of 107 larvae were assigned to pure inner population parents, while 7 larvae were assigned to pure outer population parents and 242 larvae were assigned as hybrids between the inner and outer population. On day 28, 99 larvae were assigned to the inner population, 31 were assigned to the outer population and 228 assigned to the hybrid population (Table 3).

Table 3. Number of cod larvae assigned to the inner populations, outer population and both populations (hybrids) at the start of the experiment (D2) and end of experiment (D28), from replicate tanks representing three different temperature treatment levels.

Population and temperature	D2	D28
Inner		
6°C	36	41
9.5°C	35	35
13°C	36	23
Outer		
6°C	1	15
9.5°C	3	11
13°C	3	5
Hybrid		
6°C	82	64
9.5°C	80	73
13°C	80	91

Of the 73 potential parents kept in the spawning basin, a total of 23 individuals (32%) were assigned as parents to larval offspring in this experiment: five females and ten males from the inner fjord, and four females and four males from the outer fjord (Table 4). I tested if the probability of being assigned as parent differed depending on various explanatory variables. A simple GLM-model with sex as the only explanatory variable was supported over more complex models that also included fish length and population (and two-way interaction effects) as explanatory variables (ΔAIC=3.5). This suggests that the probability of being assigned as parent was similar between populations and did not depend on fish length (Table 5). The probability of being assigned as parent to offspring did however differ among sexes, with males having a higher probability of being assigned (Parameter estimate: 1.386, SE:

0.5308, P: 0.009). Of the potential spawners, 50% (n=14) of the males were assigned as parents, in contrast to 20% (n=9) of the females.

A total of 53% (n=8) of the successful parents from the inner population produced hybrid offspring. In comparison, a total of 88% (n=7) of the successful parents from the outer population produced hybrid offspring. Most of the parents that produced hybrid offspring, also produced pure inner or outer population offspring (47% and 63% for the inner and outer population respectively) (Table 4).

Table 4. Individual (ID) characteristics of spawner cod assigned as parents to offspring in the experiment, showing the sampling population (Inner and outer Risør fjord), total body length upon capture (in mm), sex (F: female, M: male) and total weight (g), as well as the number of offspring assigned to each of the parents (hybrid offspring: one parent from each of the two populations, pure offspring: both parents from the same population).

ID	Population	Length upon capture	Sex	Weight	Hybrid offspring	Pure offspring
F03	Inner	540	F	1825	49	110
F15	Inner	490	F	-	0	77
F25	Inner	510	F	1565	129	7
F31	Inner	496	F	1182	13	11
F34	Inner	520	F	1410	12	1
F06	Inner	490	M	1105	0	47
F13	Inner	460	M	1220	5	0
F14	Inner	560	M	2093	0	72
F19	Inner	460	M	-	33	13
F20	Inner	500	M	1261	82	2
F21	Inner	510	M	-	146	18
F29	Inner	490	M	-	0	7
F30	Inner	510	M	1423	0	30
F33	Inner	540	M	1723	0	13
F35	Inner	460	M	1214	0	4
RIC5060	Outer	470	F	1248	148	34
RIC5068	Outer	510	F	1811	4	0
RIC5071	Outer	560	F	1643	82	1
RIC5078	Outer	550	F	1822	33	3
RIC5064	Outer	570	M	1623	47	1
RIC5076	Outer	570	M	1976	2	35
RIC5087	Outer	510	M	1548	0	2
RIC5100	Outer	640	M	2364	154	0

Table 5. Mean length and standard deviation (SD) for the cod used in the experiment, split into groups based on population, sex (F: female, M: male) and reproductive success (yes: assigned progeny, no: no progeny assigned). The number of cod within each group is also given (n).

Population	Sex	Assigned parentage	n	Mean length (mm)	SD
Inner	F	yes	5	511	20
Inner	F	no	16	506	44
Inner	M	yes	10	498	34
Inner	M	no	6	525	25
Outer	F	yes	4	523	41
Outer	F	no	20	537	46
Outer	M	yes	4	573	53
Outer	M	no	8	489	26
Hybrid	F	yes	8	520	30
Hybrid	M	yes	7	530	66

3.3 Growth reaction norms

Larval length at the start of the experiment (day 2) differed among populations ($F_{2, 353}$=6.79, p=0.0013), with the hybrids (4.61 ± 0.02 mm; mean ± SE) being smaller than the larvae from the inner (4.70 ± 0.02 mm) and outer (4.68 ± 0.07) population. Not accounting for these initial differences in size, growth reaction norms were subsequently constructed for larval length at day 28.

Table 6. AIC based model selection for constructing growth reaction norms in Atlantic cod. First, model A and B were compared to assess whether or not tank should be included as a random effect nested within temperature. Second, the importance of population (pop), temperature (temp), larval curvature (curv) and larval position (pos) as fixed effects where evaluated through model A1-A4. The response variable was larval length at the end of the experiment (day 28).

Model name	Model structure	AIC	ΔAIC
A	pop + temp + (pop x temp) + curv + pos + tank(temp)	748.8	0
B	pop + temp + (pop x temp) + curv + pos	757.1	8.3
A1	pop + temp + (pop x temp) + curv + pos + tank(temp) [ML]	723.2	3.7
A2	pop + temp + (pop x temp) + curv + tank(temp) [ML]	719.5	0
A3	pop + temp + (pop x temp) + tank(temp) [ML]	734.8	15.3
A4	pop + temp + tank(temp) [ML]	724.0	4.5

Model selection based on AIC supported an interaction effect between population and temperature on larval length at day 28 (Table C1, Appendix C) (Table 6). Also, there was support for including larval curvature as a covariate and tank as a random effect nested within temperature (Table 6). Adding larval position as a fixed effect in the model increased the AIC values (Table 6), suggesting that position explain little of the variation in the data. Removing

curvature as a fixed effect also increased the AIC value, indicating that this variable explains some of the variation in the data, and should be included in the model.

The only (marginally) significant effect of temperature on length within each population, was change in length from the intermediate (9.5°C) to the high (13°C) temperature for the hybrid population (Table 7). Comparing the length of the populations at each temperature, the only significant difference was found at the highest temperature (Table 8). Here, the hybrid population is significantly larger than the outer population, and marginally significantly larger than the inner population. The inner and outer population are almost significantly different, with the inner population being larger than the outer population (Table 8).

The outer population and the hybrid population differ significantly in reaction norm slopes (Table 9). Differences in reaction norms slopes of the inner population and the hybrid population are marginally significant (Table 9). The reaction norms of the inner and outer population do not display a significant difference in slopes (Table 9). The reaction norm slopes for the inner and outer populations are negative, while the slope for the hybrid population is positive (i.e. population differences in growth plasticity) (Figure 5) (Table 9).

The amount of variance explained by tank within each temperature treatment and by tank nested under temperature is given in table 10.

Table 7. Effects of temperature on the change in length from the low (6°C) to the intermediate (9.5°C) temperature and on the change in length from the intermediate to the high (13°C) temperature for the inner, outer and hybrid population cod larvae, following the best model. Parameter estimate, standard error (SE) and p-value are given for each population at each contrast. Point of contrast is stated in the column header.

	Change in length from 6°C to 9.5°C			Change in length from 9.5°C to 13°C		
Population	P. estimate	SE	P-value	P. estimate	SE	P-value
Inner	0.3	0.22	0.22	-0.25	0.23	0.32
Outer	0.18	0.3	0.58	-0.78	0.38	0.08
Hybrid	0.32	0.19	0.15	0.10	0.19	0.6

Table 8. Comparisons between populations (column header) for length at each temperature (row header). Estimates are extracted from the best growth model for larval cod length at the end of the experiment. Parameter estimates (PE), standard error (SE) and p-value are given for each comparison. Point of contrast is stated in the column header.

	From Inner to Outer			From Inner to Hybrid			From Outer to Hybrid		
Temperature	PE	SE	P-value	PE	SE	P-value	PE	SE	P-value
6°C	0.05	0.20	0.80	-0.09	0.13	0.46	-0.14	0.19	0.45
9.5°C	-0.07	0.22	0.76	-0.07	0.13	0.60	-0.001	0.21	0.99
13°C	-0.60	0.32	0.06	0.29	0.15	0.05	0.89	0.29	0.003

Table 9. Differences in growth reaction norms for cod assed by pairwise population contrasts. The estimates represent the change in length from the intermediate (9.5°C) to the high (13°C) temperature. Estimates, with standard error in parenthesis, are shown to the lower left of the table. P-values are shown in the upper right. Point of contrast for the estimates is the row header.

	Inner	Outer	Hybrid
Inner		0.17	0.07
Outer	0.53 (0.39)		0.01
Hybrid	-0.36 (0.20)	-0.89 (0.36)	

Table 10. Amount of variance explained by tank nested under temperature, and by tank alone. Unexplained variance is given as residuals.

Model term	Variance	Standard deviation
Temp: tank	0.0081	0.0902
Tank	0.0294	0.1716
Residual	0.4038	0.6354

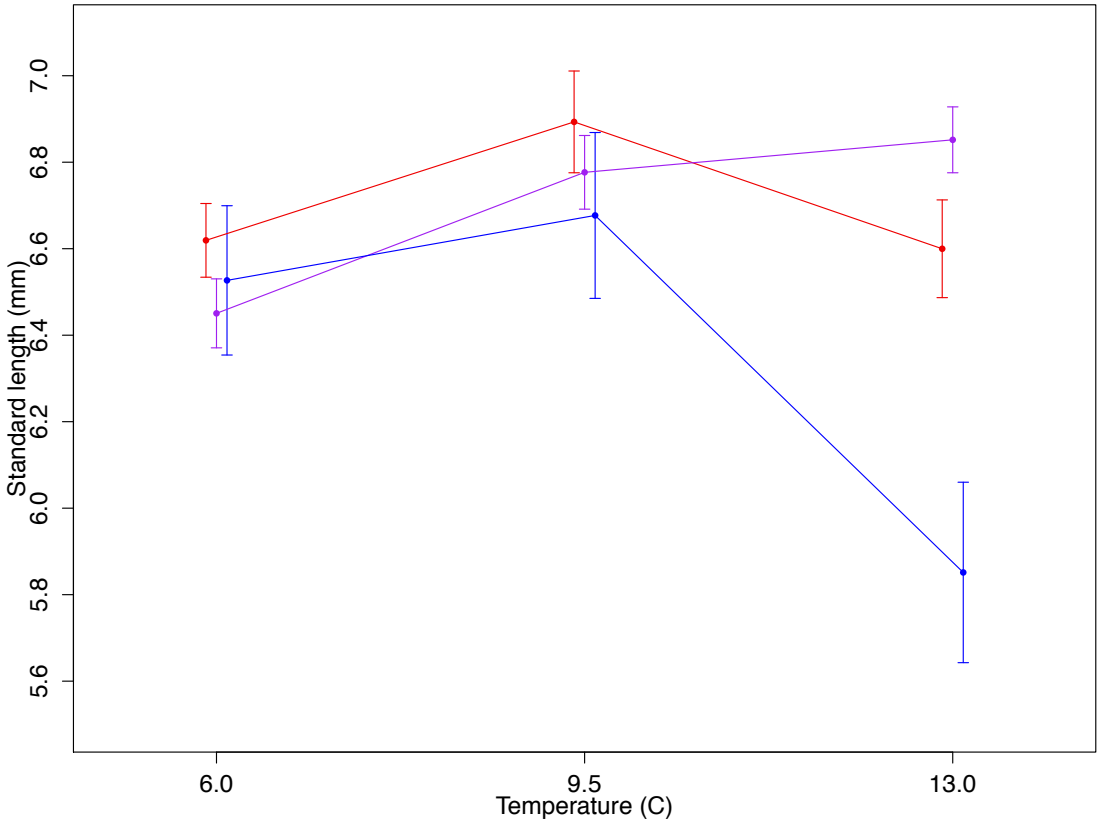

Figure 5. *Growth reaction norms constructed for cod larval length at the end of the experiment (day 28), across three temperatures (6°C, 9.5°C and 13°C). The reaction norm of the inner population is visualized by the red line, the reaction norm for the outer population is visualized by the blue line and the reaction norm for the hybrid population is visualized by the purple line. The standard error for each population at each temperature is shown as vertical lines.*

3.4 Survival

Survival of the cod larvae was defined as the proportion of larvae alive at the end of the experiment (day 28) and quantified using data from both day 2 and day 28. Model selection based on p-values from Chi square tests supported a temperature effect on larval survival (i.e. overall survival plasticity), but not a population effect or interaction term between these two explanatory variables (Table 11). This corresponds to the visual impression from Figure 6, where the reaction norms for the two populations seem similar and parallel. For both the inner and hybrid population, survival was lower than 0.3 across all temperatures (Figure 6). Survival was significantly lowest at the highest temperature (Table 12).

Table 11. Chi square tests for selecting a GLM model used for constructing survival reaction norms in Atlantic cod larvae across temperatures for two populations (inner and outer Risør fjord). The response variable was the proportion of cod larvae surviving in each tank at the end of the experiment (day 28). The best model is supported by a significant p-value (Pr(Chi)). Degrees of freedom, deviance, residual degrees of freedom and residual deviance is given.

Model	df	Deviance	Residual df	Residual dev	Pr (Chi)
null	-	-	17	1.825	-
temperature	2	1.356	15	0.469	<0.001
population	1	0.028	16	1.797	0.597
temp + pop	1	0.000	14	0.469	0.930
temp x pop	3	0.084	12	0.386	0.448

Table 12. Parameter estimates, with associated standard errors (SE), from the selected model describing cod larvae survival at different temperatures. The intermediate temperature (9.5°C) was set as reference level in the model.

Contrast	Parameter estimate	SE	P-value
Intercept	0.26	0.03	<0.001
6°C	-0.06	0.04	0.175
13°C	-0.22	0.03	<0.001

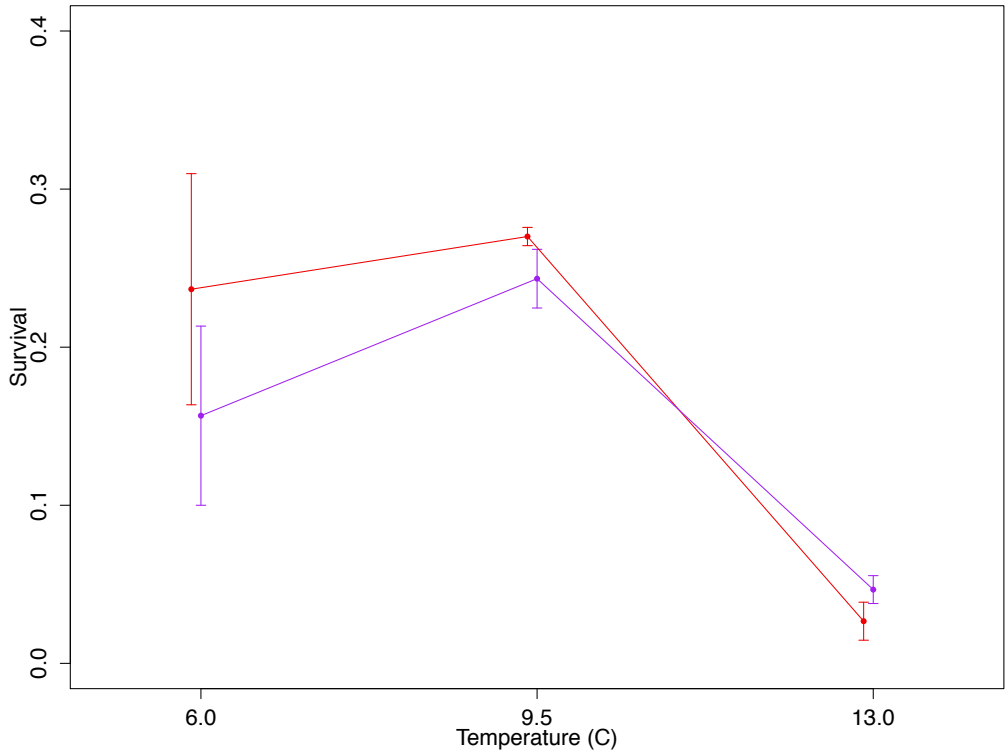

Figure 6. *Survival reaction norms constructed for larval Atlantic cod across three experimental temperatures (6°C, 9.5°C and 13°C), with survival quantified at day 28 as the number of larvae alive per tank relative to the number alive at day 2. Population plasticity in survival is visualized by the red line for the inner population, and by the purple line for the hybrid population. Vertical lines represent standard error for each population at each temperature.*

Table 13. Model selection for the alternative approach to larval survival. The best model was the one including the interaction effect, evaluated by a significant p-value (Pr(Chi)). Degrees of freedom, deviance, residual degrees of freedom and residual deviance is given.

Model	df	Deviance	Residual df	Residual dev	Pr (Chi)
null	26	-	-	7.33	-
temperature	24	0.00	2	7.33	0.999
population	24	6.48	2	0.85	<0.001
temp + pop	22	0.04	2	0.82	0.622
temp x pop	18	0.64	6	0.22	<0.001

Survival could not be reliably estimated for the outer population, since very few larvae were sampled at the beginning of the experiment. As an alternative approach to compare survival plasticity among populations, I simply estimated relative survival as the percentage of larvae from each population in each tank at the end of the experiment. Model selection based on p-values from Chi square tests supported an interaction effect between population and temperature on relative survival (Table 13), indicative of population differences in survival plasticity across temperatures. The reaction norm slope of the hybrid larvae from the intermediate (9.5°C) to the high (13°C) temperature is significantly different than the reaction norm slopes of the inner and outer population (Table 14). Relative to each other, it appears

that the hybrid population survived better at the high temperature, while the inner and outer population survived better at the low temperature (Figure 7).

Table 14. Parameter estimates, with associated standard errors (SE), from the selected model describing relative survival of cod larvae at different temperatures. The intermediate temperature (9.5°C) and the hybrid population was set as reference levels in the model.

Model term	Parameter estimate	SE	P-value
Intercept	0.617	0.03	<0.001
Inner	-0.323	0.04	<0.001
Outer	-0.523	0.04	<0.001
Temp 6°C	-0.083	0.04	0.0744
Temp 13°C	0.143	0.04	0.0025
Inner 6°C	0.137	0.06	0.0367
Outer 6°C	0.120	0.05	0.0337
Inner 13°C	-0.240	0.06	0.0004
Outer 13°C	-0.183	0.05	0.0011

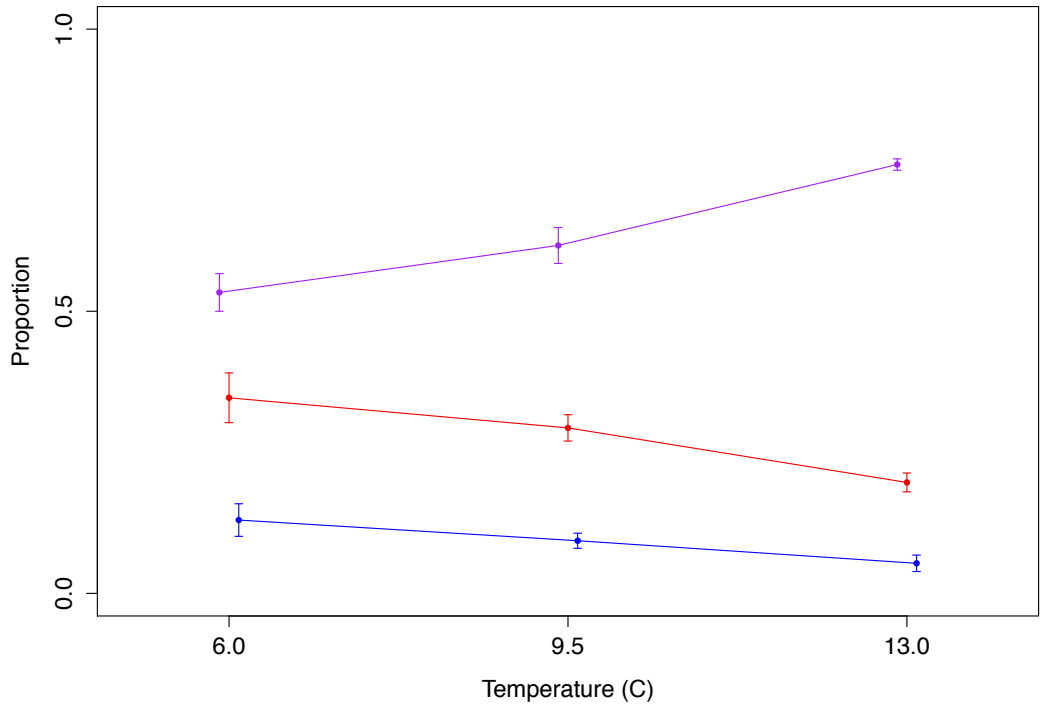

Figure 7. *Survival reaction norms for cod larvae from the inner (red), outer (blue) and hybrid (purple) population, expressed as the percentage of larvae alive from each population in each tank at the end of the experiment. Standard error is represented by vertical lines at each temperature point for each population*

4. DISCUSSION

Understanding how natural populations respond to climate change is challenging (Merilä and Hendry, 2014), but important from a management and conservation perspective. In Atlantic cod, plastic responses to changes in temperature and genetic differences in plasticity among populations have been explored (Wijekoon *et al.*, 2009) and detected at both large (Hutchings *et al.*, 2007; Oomen and Hutchings, 2016) (600-800 km) and intermediate spatial scales (Oomen and Hutchings, 2015b) (~200 km). In this thesis I found support for genotype-by-environment interactions on a finer spatial scale (<30 km) than previously detected in this species. Reaction norms constructed for larval growth in offspring from two neighbouring populations of cod (including hybrid larvae with a mixed population heritage) revealed a significant interaction effect between temperature and population. Interestingly, larvae from the hybrid population appeared to grow faster as temperature increased from 6°C to 13°C. In contrast, larvae with both parents from either the inner fjord population or the outer fjord population both experienced the highest growth at the intermediate temperature (9.5°C). Considering reaction norms constructed for larval survival, I found only an additive effect of temperature, and no population effect. Both the inner fjord and hybrid populations had the lowest survival at the highest temperature (13°C). The outer population were not included in these analysis, due to poor sample size at the beginning of the experiment. To compare survival for all three populations, I also used a more indirect approach and analysed the percentage of surviving larvae belonging to each population in each tank at each temperature at the end of the experiment. Here, I found a significant interaction effect between temperature and population, suggesting that the hybrid population performed better than the inner and outer fjord population at the highest temperature. Below I discuss these findings in more detail.

I detected a significant population x temperature interaction effect on larval cod growth, which indicates that there are genetic differences in plasticity. A closer examination of the model revealed a significant difference in the reaction norms of the hybrid population and the outer fjord population, and a marginally significant difference in the reaction norms of the inner fjord and the hybrid population. This implies that the hybrid population has a different ability to respond to thermal changes than both the inner and outer population. While the inner and outer population exhibits the highest growth at the intermediate temperature with a decline in growth to the highest temperature, the hybrid population display an increase in growth with increasing temperatures, resulting in crossing reaction norms. However, no significant difference in reaction norms was found between the inner and outer population. Here, only an additive effect of population was detected, suggesting that there are genetic differences in growth (but not growth plasticity) between the populations. These findings are interesting because there is evidence for population genetic structure in cod between inner and outer areas of several fjords, both in Norway (Knutsen *et al.*, 2011; Sodeland *et al.*, 2016) and

in the Swedish Gullmar fjord (Øresland and André, 2008). Using a genomic approach, Sodeland *et al.* (2016) found support for adaptive divergence in cod from inner and outer fjord areas. Population specific plastic responses in larval traits is not well explored for cod, but it has been documented at large (Hutchings *et al.*, 2007; Oomen and Hutchings, 2016) and intermediate (Oomen and Hutchings, 2015b) spatial scales for Canadian cod. For Norwegian coastal cod, differences in maturation patterns has been observed for many populations along the Norwegian Skagerrak coast (Olsen *et al.*, 2008), at a scale comparable to those of Oomen and Hutchings (2015b). Although my study did not detect a difference in thermal growth reaction norms between the inner and outer fjord population, field studies conducted in the same coastal region have shown that cod from the outer areas grow faster than cod from the inner areas (Lekve *et al.*, 2006; Kuparinen *et al.*, 2015). My experiment suggests that this spatial variation in growth could be mainly environmentally driven, and explained by different populations having similar reaction norms only that they realize different parts of it. Growth differences observed among the two locations for juvenile (Lekve *et al.*, 2006) and adult cod (Kuparinen *et al.*, 2015) could also represent adaptations to local environmental conditions other than temperature.

If I look at the overall larval growth response to temperature (only including larvae with parents from the same population), the results of my study is in slight contrast to those of Hutchings *et al.* (2007) and Oomen & Hutchings (2015b; 2016). I found larval growth to be decreasing from the intermediate (9.5°C) to the high temperature (13°C). In comparison, Hutchings *et al.* (2007) found larval growth to be increasing with increasing temperatures, and Oomen & Hutchings (2015b; 2016) found larval growth to be increasing or unaffected by temperature. The different growth response detected in my study compared to the Canadian cod studies could be due to the fact that the highest temperature in both the Hutchings et al. (2007) study and the two Oomen & Hutchings (2015b; 2016) studies was 11°C, while in my study the highest temperature was 13°C. Also, since I only measured length at three temperatures (not including the highest temperature used in the Canadian studies), I do not know at what specific temperature growth started to decrease. The difference in growth response to temperature could also be explained by larval cod in Canada being adapted to a different temperature regime than larval cod along the Norwegian Skagerrak coast. For my study, these findings indicate that the growth optimum for larval cod in the Risør fjord system is somewhere between 6°C and 13°C. However, it is important to note that optimal temperatures for fish larval growth can be higher in experimental environments than in the wild (Buckley *et al.*, 2004). In nature, growth is likely associated with a number of trade-offs, such as vulnerability to predation (Lankford *et al.*, 2001) and swimming performance (Billerbeck *et al.*, 2001). Also, larval growth in the wild can be negatively affected food availability through climate-induced alterations of the plankton community (Beaugrand *et al.*, 2003). The hybrid population in my study, did however display a growth pattern similar to those detected by Hutchings *et al.* (2007) and Oomen & Hutchings (2016), with growth

increasing with temperature. This could reflect a better ability of the hybrids to cope with rising temperatures, or it could simply be an artefact observed under experimental conditions.

Using larval length at the end of the experiment (day 28) as a proxy for growth assumes that the larvae from different populations were similar in size at the beginning of the experiment (day 2). However, I did find some initial differences in larval length. These differences could be due to maternal effects, where female environment (Hurst *et al.*, 2012) and phenotype (Kjesbu, 1989) influences the size of the offspring. Cod are batch spawners and one female will produce multiple batches of eggs throughout the spawning season (Kjesbu, 1989). Egg size has been shown to be bigger at the beginning of the spawning season (for a given female), and larger females shown to produce larger batches and bigger eggs than smaller females (Kjesbu, 1989). Older cod may also initiate spawning at a later point in the spawning season (Hutchings and Myers, 1993). For many reasons therefore, spawning time can differ among cod populations (Blanchard *et al.*, 2005). By sampling only in the middle of the overall spawning season, I could, in theory, have sampled at the end of one populations spawning period, and at the beginning of the other populations spawning period. In my samples, neither fish length nor population of origin had any significant effect on the probability of being assigned as parent in my study. Note that Roney (2016) sampled the offspring in the same experiment on a daily basis throughout the entire spawning season, and found that the size of the parent did actually have a positive influence on the number and quality of offspring, their spawning duration and the number of egg batches produced. However, if maternal effects were an important driver behind the results in my study, one might not expect to observe crossing reaction norms where the hybrid larvae are smallest at the lowest temperature and largest at the highest temperature. Instead, I consider temperature as the main driver behind the observed differences in growth.

Larval survival was explored using two approaches. First, survival was quantified using data from both day 2 and day 28, as the proportion of larvae alive at the end of the experiment (day 28). Model selection supported a temperature effect on survival, but no population effect or interaction effect, meaning that there is no statistical support for population difference in how temperature affect survival. Due to sample size limitations, only the inner population and the hybrid population was included in this first analysis. Both these populations survived better at the low (6°C) and intermediate (9.5°C) temperature compared to the high temperature (13°C). Second, an alternative analysis was applied for comparing relative survival between the outer and inner population, as well as the hybrids. Here, I used only data from day 28 where sample size from the outer population was somewhat better, and compared the proportion of larvae from each population at each temperature. I detected a significant interaction effect between population and temperature on relative survival, suggesting that the hybrid population performs better than the inner and outer populations at the highest temperature. Earlier studies have detected differences in larval survival as a response to temperature among populations of

cod whose native thermal regime differ (Hutchings *et al.*, 2007; Oomen and Hutchings, 2015b; Oomen and Hutchings, 2016). In these studies, larval cod survival was found to be increasing, decreasing or unaffected by increasing temperatures, with larvae naturally experiencing comparatively warmer (6-10°C) or colder (3-7°C) temperatures not consistently showing the same survival response. How larvae from different populations of cod will respond to changes in temperature, cannot be intuitively inferred from the native temperature regime of the populations. Previously, larval survival of cod from comparatively cold waters was found to be enhanced by increasing temperatures (Planque and Frédou, 1999; Ottersen *et al.*, 2006; Hutchings *et al.*, 2007). However, Oomen and Hutchings (2015b) found that survival response of cod larvae within the same management stock can differ among spawning groups. Larvae from waters of temperatures intermediate between the cold and the warm, have not been found to have a consistent survival pattern of cod larvae (Planque and Frédou, 1999). These findings is a reminder, that although I found survival to be decreasing with increasing temperatures for cod larvae from the Risør fjord system, this response might not be the case for all cod populations along the Skagerrak coast.

Potentially, my samples could contain a mix of local fjord cod larvae and oceanic North Sea cod larvae, since earlier studies have detected both of these genotypes in samples of half-year old cod juveniles from the Risør fjord system and neighbouring coastal areas (Knutsen *et al.*, 2011; Mellerud, 2016). Specifically, Knutsen *et al.* (2011) found that cod from the outer areas of the Risør fjord system, where the outer population was sampled, to a higher degree resemble the North Sea cod. More recent analyses of juvenile cod origin revealed that over 50% of the juveniles from the outer skerries around Risør are of North Sea origin, in contrast to only 5% in the inner part of the fjord system (Mellerud, 2016). If North Sea cod exhibits a different response to temperature than coastal Norwegian cod, then the degree of which North Sea cod is present in my samples could affect observed lengths and survival.

The lack of statistical support for differences in growth plasticity between the inner and outer fjord larvae does not rule out the possibility that such differences exist. The difference detected for growth between the hybrids and the outer population, despite the very low sample size for outer cod larvae, suggests that this difference is substantial. The difference in plasticity between the inner and outer population might not be equally strong, and my study could therefore lack the power to unveil it. On the other hand, my result could also imply that there simply is no biological important difference in growth plasticity between these neighbouring populations. If the cod larvae experience similar temperature regimes at the two locations in the wild, one would not expect temperature to be a strong driver of local adaptive divergence. The temperature loggers deposited in the Risør fjord from February to May in this study indicated that temperatures in the inner fjord are more variable. However, longer-term temperature data suggests that inter-annual variations are more pronounced than the intra-

annual differences between the inner and outer part of the Risør fjord system (pers.com., Jon Albretsen, oceanographer at the Institute of Marine Research Flødevigen).

Interestingly, the hybrid population seem to perform better overall (in terms of growth as well as survival) compared to the inner and outer populations at the highest temperature. They experience a significantly higher growth at the high temperature than the inner and outer cod, and although survival is low at the high temperature, the hybrids survive better relative to the inner and outer populations. Previous studies on the terrestrial plant *Avena barbata* found that a hybrid cross between two populations adapted to slightly different environments, can exhibit a wider range of tolerance and perform better in a novel environment than the two parent populations (Johansen-Morris and Latta, 2007). Better performance of hybrids is a phenomenon known as hybrid vigour (Halliburton, 2004). It is commonly observed in crosses of breeding lines in crop plants (Schnable and Springer, 2013; Fu *et al.*, 2014), and can also be observed in the wild, for instance when two subspecies cross (Fitzpatrick and Shaffer, 2007). Although I observed a better performance at the high temperature for the hybrids in both growth and survival (relative to the inner and outer population), this might not be the case for hybrids in the wild. As mentioned previously, observed values of a trait in experimental conditions does not necessarily relate to values and performances in the wild (Buckley *et al.*, 2004).

Knowledge about the extent to which marine populations are spatially structured and how different populations will respond to changing climate is important for fisheries management in order to avoid overexploitation of subdivisions within a stock (Lindegren *et al.*, 2013). By affecting larval dispersal and survival (Beaugrand *et al.*, 2003; O'Connor *et al.*, 2007), increased temperatures can negatively affect recruitment. Knowing that many fish stocks, including cod, in the Northeast Atlantic are currently overexploited (Sparholt *et al.*, 2007), information about the spatial scales of population thermal tolerance could prove important for management towards recovery and conservation of valuable genetic variation (Crozier and Hutchings, 2014). Earlier studies suggest that such genetic variation in cod growth plasticity is present at large (600-800 km) and intermediate (~200 km) spatial scales (Hutchings *et al.*, 2007; Oomen and Hutchings, 2015b; Oomen and Hutchings, 2016). Working on an even finer scale (<30 km), my study did not detect clear differences in adaptive thermal traits. This could be due to small sample sizes in my study, and therefore lack of power to detect fine scale differences among the two populations, or it could also be because I am touching the limits of the spatial scale at which populations differ in thermal responses.

In conclusion, I did not find any clear difference in plasticity for neither growth nor survival for larval cod from the inner and outer part of the Risør fjord. However, I did find a population effect on growth, and a significant and marginally significant difference from the hybrid growth reaction norm for the outer and inner cod respectively. This indicates that there

are genetic differences between the inner and outer population, and perhaps a difference in plasticity, only that my study lacked the power to detect it. The similar reaction norms of the inner and outer population could also indicate that my study approached a lower spatial limit for adaptive differentiation in coastal cod. Knowledge about the degree of spatial differentiation within a species is important when it comes to defining practical management units. I further found that larval growth and survival for the inner and outer population was lowest at the highest temperature (13°C). This implies that at some temperature below 13°C, the growth performance and the survival of the cod larvae from the Risør fjord system start to decrease. This could have implications for how to manage the species from a climate change perspective. For instance, Brander (2007) points out that because of potentially strong interactions between effects of fishing and climate change, reducing fishing mortality is perhaps the most important means of mitigating climate change effects on wild fish. Additional studies are needed, to say whether the larval temperature responses and the lack of thermal fine-scale structuring found in my study apply to coastal cod in general, or if they are distinct properties of the Risør fjord system.

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APPENDIX A

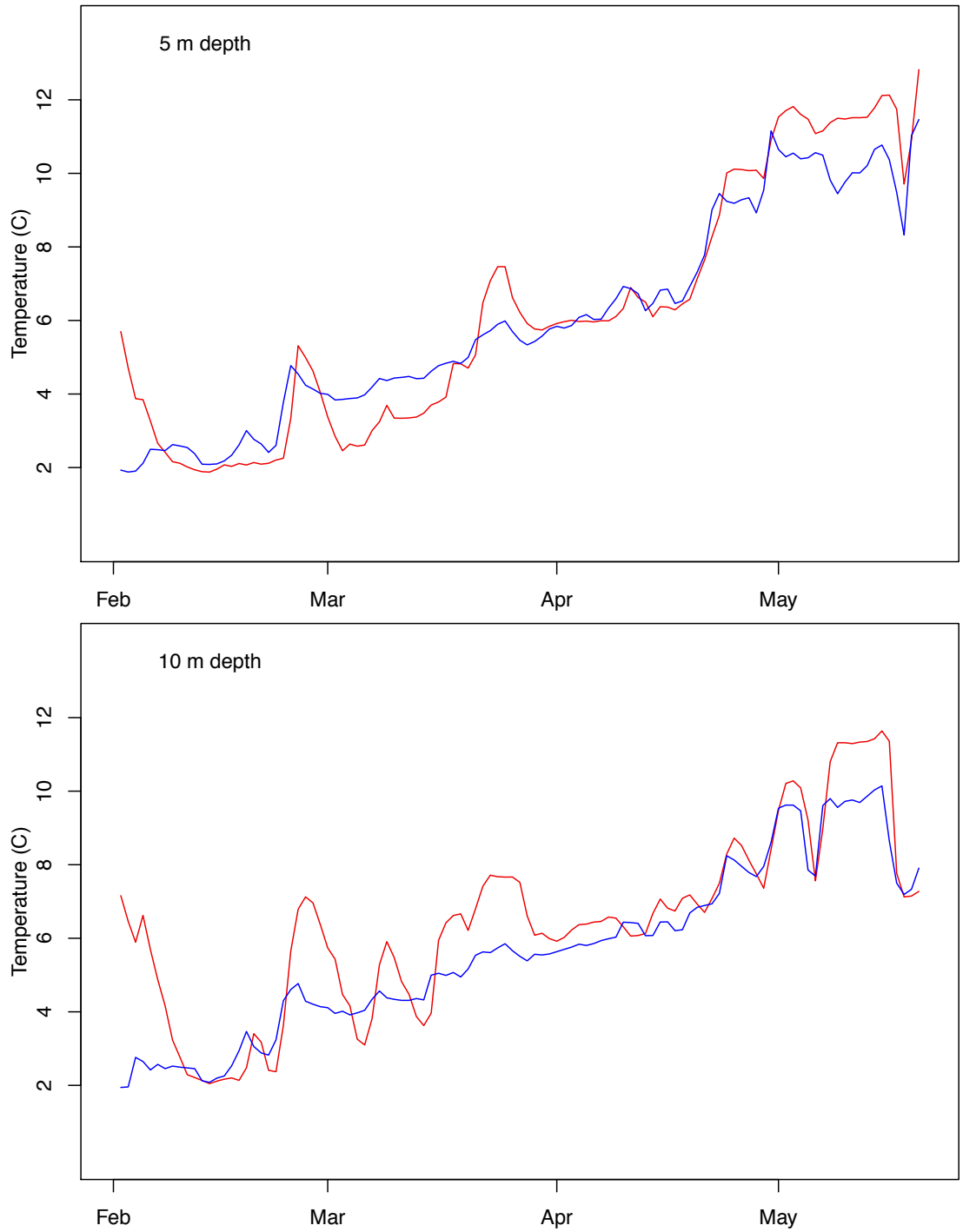

Figure A1. *Temperatures for the inner part of the Risørfjord (Sørfjorden) (red) and the outer part of the Risør fjord (Østerfjorden) (blue) from February 2014 throughout May 2014, at 5 m (top graph) and 10 m depth (bottom graph).*

APPENDIX B

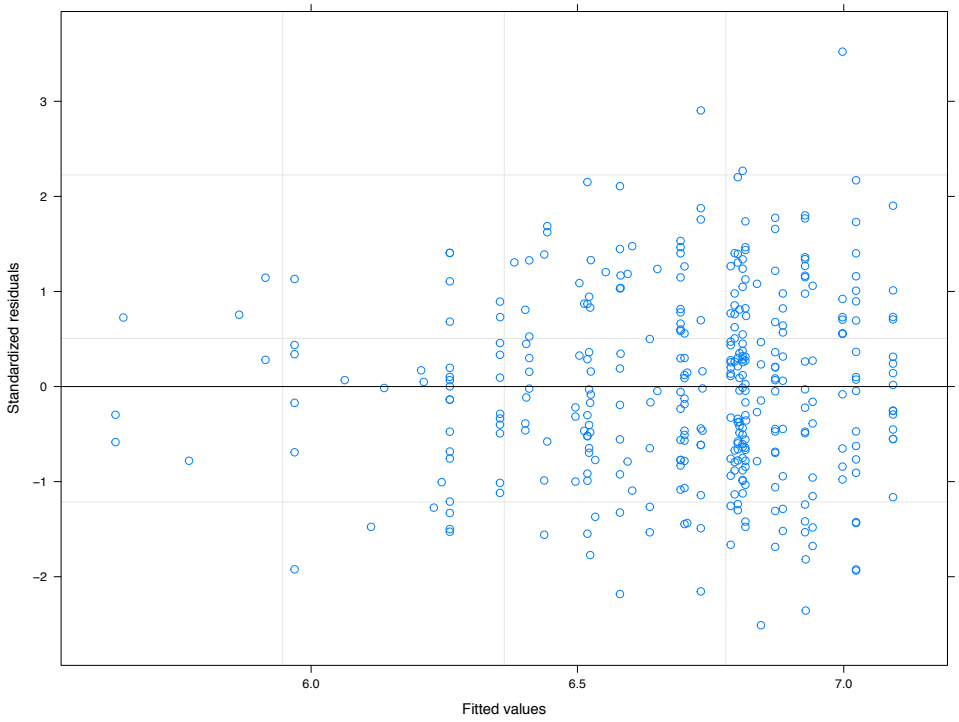

Figure B1. *Plot of model residuals from the best growth model.*

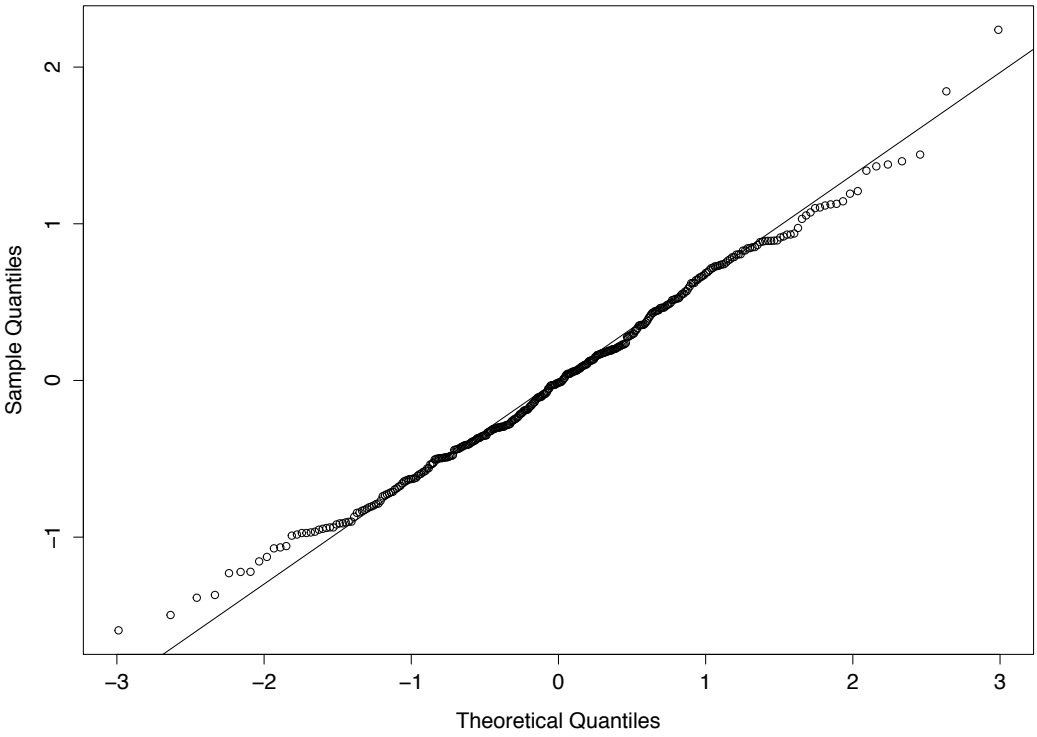

Figure B2. *Q-Q plot of model residuals for larval length at day 28 from the model including population, temperature and their interaction as fixed effects, and tank as a random effect. A quantile-quantile plot plots the ranked model residuals against a similar number of ranked quantiles from a normal distribution (Crawley 2007).*

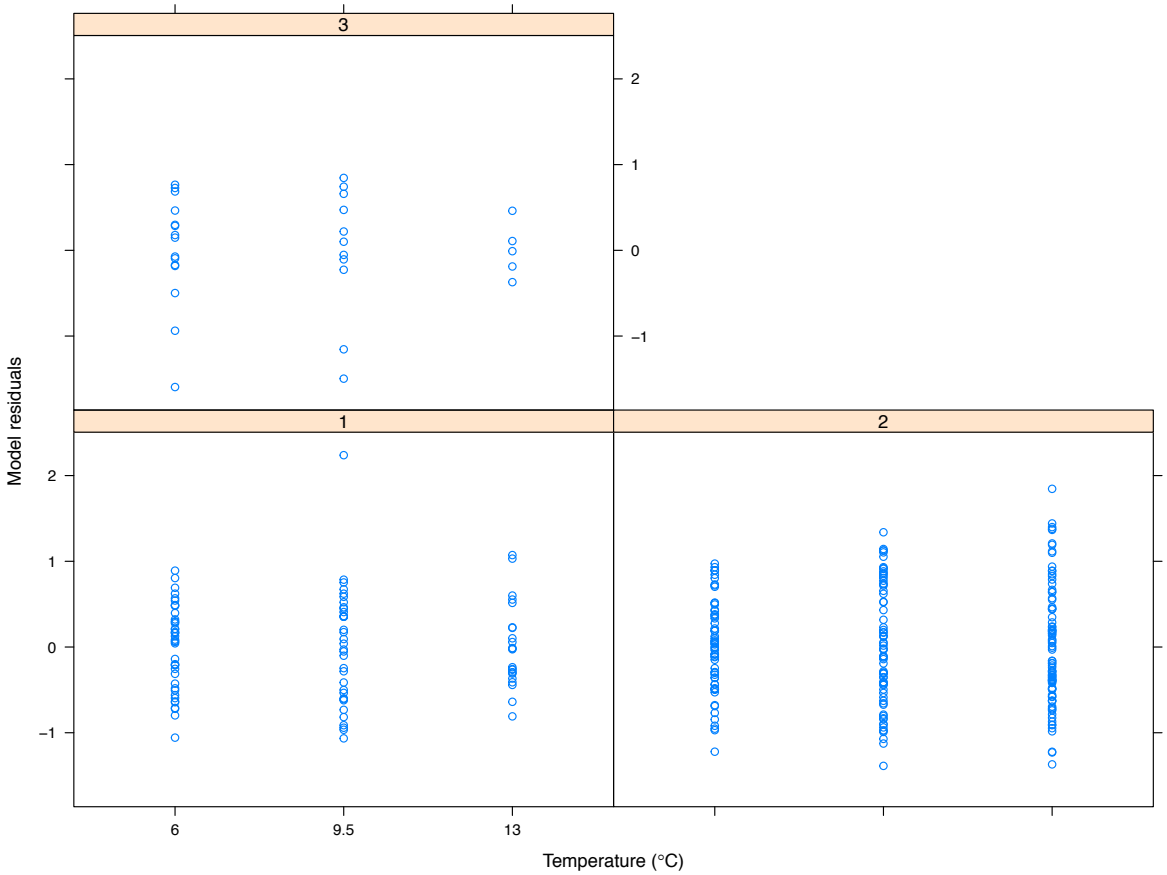

Figure B3. *Plot of model residuals by population and temperature for larval length at the end of the experiment (day 28) to assess homogeneity of variances´. 1: inner population. 2: hybrid population. 3: outer population.*

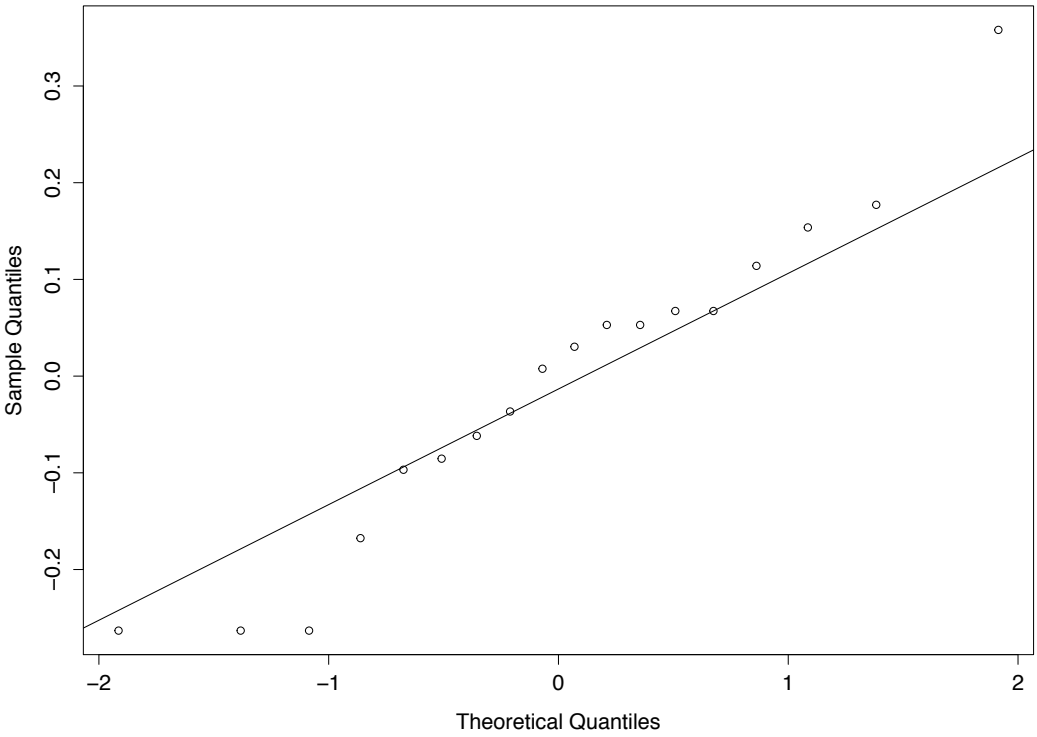

Figure B4. *QQ-plot of model residuals from the best model for larval survival (model including only temperature and a quasi-binomial distribution was used).*

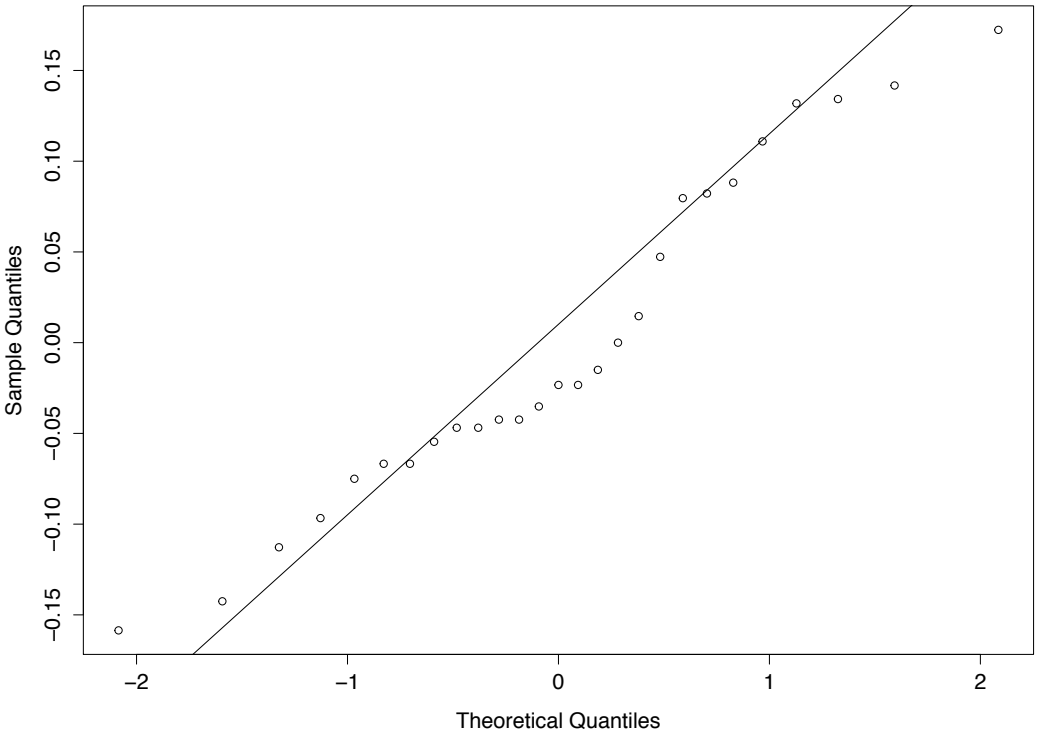

Figure B5. *QQ-plot of residuals from the best model for the alternative approach to survival (percentage alive from each population relative to the other populations, at each temperature in each tank). The model included an interaction effect of temperature and population, and a quasi-binomial distribution was used.*

APPENDIX C

Table C1. ANOVA table for the best growth model. Effect of curvature, population, temperature and the interaction between temperature and population on larval length at the end of the experiment. Numerator and denominator degrees of freedom are given in the second and third column respectively. F-values and P-values are also given.

Model term	NumDF	DenDF	F	P-value
curvature	2	340	1.504	0.2237
population	2	340	1.344	0.3294
temperature	2	6	9.777	0.0001
population x temperature	4	340	3.068	0.0167