Population variation in the life history traits and thermal responses of Atlantic cod, *Gadus morhua* L.
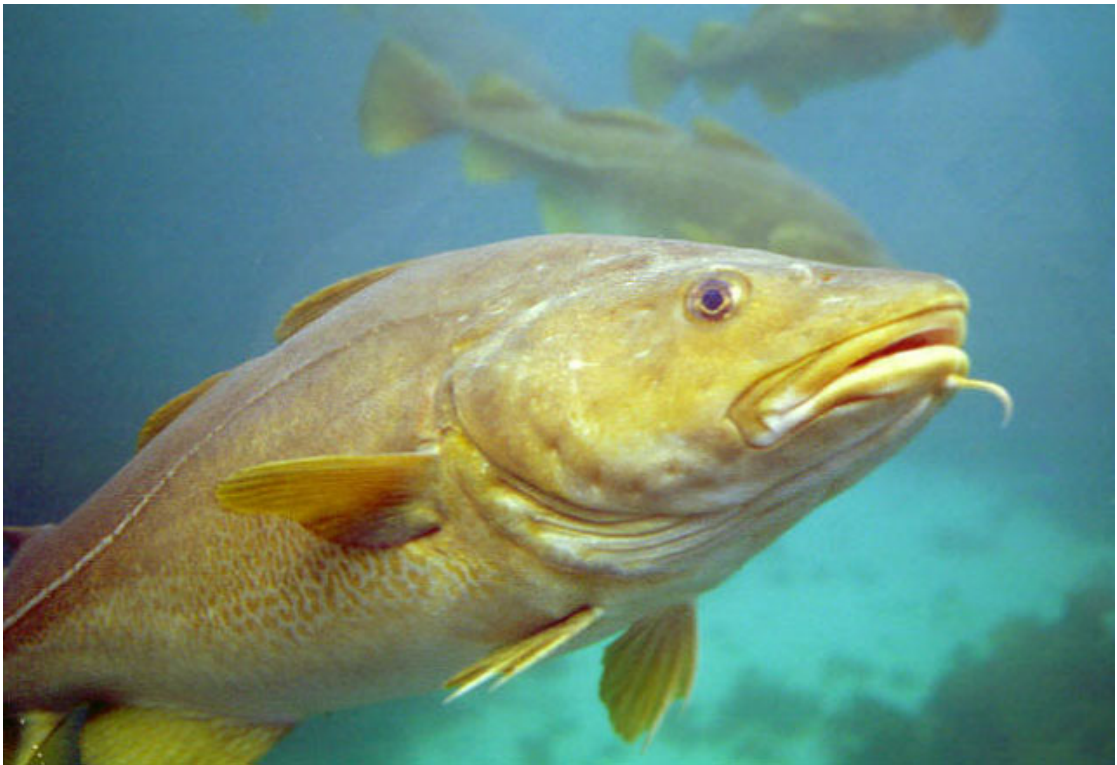
Marion Perutz

Thesis submitted for the degree of Doctor of Philosophy

Division of Environmental and Evolutionary Biology

University of Glasgow

DECLARATION

This thesis has been composed in its entirety by the candidate. Except where specifically acknowledged the work described in this thesis has been conducted independently and has not been submitted for any other degree.

Signature of the candidate _____

(Marion Perutz)

Date _____

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both fish = 27.6 - 0.142 polarisation score). Significance is given in Table 2.8.

somatic weight (SW) = residuals of liver weight (LW) vs. total weight (TW) - LW + population (significance given in the figures).

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using a linear mixed effects model on log $_{10}$ weight (g) using an antedependence order 2 model. Random and fixed effects are given in the table along with the Wald statistic and associated significance levels.

ACKNOWLEDGEMENTS

Tobin, James Orpwood, Liz Galley and Phil Boulcott. The members of the B Block coffee room have been fabulous in restoring my spirits with their wild sense of humour throughout my three years at the Marine Laboratory. Also thank you to the Philbricks for their never-ending hospitality in making me welcome in Glasgow during my visits to the University.

A special thank you to my family and to Brent for their support and encouragement throughout, and one final thank you to the most essential contributor: the fish!

SUMMARY

- Studies of the phenotypes of animals at different parts of their geographic range often reveal striking variability. It is of considerable fundamental and applied interest to discover the extent to which such variation depends on genetic as opposed to environmental differences. A first step towards disentangling these effects is to use an empirical approach known as the common environment method in which wild-caught juveniles from different regions are reared under common laboratory conditions. I used this approach to determine the population and thermal responses of Atlantic cod, a species with a wide distribution and geographic variation in life history traits.

- Life history traits were investigated in cod from three areas around the British Isles of differing thermal regimes, namely St Andrews Bay on the Scottish east coast, the Clyde Sea on the Scottish west coast, and from near Lowestoft in the southern North Sea. Concurrently haemoglobin genotype and behaviour were also studied.

- Spatially significant differences in life history traits and thermal responses were revealed in juvenile and adult growth rate, gonadal investment and behaviour, suggestive of population differentiation. Behavioural differences between cod of differing haemoglobin genotypes were also demonstrated.

- Results suggested that juvenile growth rates may be modified by competitive interactions. At a group level, growth rate of cod from the Clyde Sea was suppressed in the presence of cod from St Andrews Bay. Pairwise trials demonstrated that cod from the Clyde Sea consumed a higher prey share than those from St Andrews Bay but that those from St Andrews Bay were more aggressive and thus could potentially restrict feeding of cod from the Clyde Sea, resulting in a reduced growth rate.

- There were no population differences in the distribution of haemoglobin genotype, but haemoglobin genotype did have a strong influence on behaviour in pairwise contests. Cod of the HbI-2*2 genotype displayed a higher level of aggression than other genotypes and this effect was stronger than the population difference.

- Juvenile cod from the Clyde Sea exhibited a growth rate 24 % higher than those from St Andrews Bay. Cod from the Clyde Sea and from Lowestoft expressed higher growth rates as adults than those from St Andrews Bay. Body size and thus growth appeared to be the main driver of fecundity in the females and body size and liver were the main influences on gonadosomatic index (GSI) in the males. Females from the Clyde Sea invested more into fecundity than those from St Andrews Bay and males from St Andrews Bay had a higher testis investment than those from the Clyde Sea and Lowestoft.

- Temperature had a large influence on both the juvenile growth and egg development. Growth rate increased linearly and in parallel over the experimental temperatures, within their normal range. Egg development was strongly affected by temperature, resulting in a decrease in hatch time and an increase in embryonic cardiac rate, and a smaller larval size at hatch for a given temperature at higher temperatures. Temperature did not directly influence fecundity or GSI in males but warmer temperatures resulted in higher growth rates and thus a larger body size, which in turn resulted in a greater fecundity or GSI.

- These differences in life history traits, demonstrated under controlled environment conditions, raises the possibility that there may be a genetic basis to the variation and that cod may be locally adapted to their thermal environments in areas around the British Isles. However, effects of environmental differences prior to capture, including maternal effects, cannot be ruled out.

- This greater understanding of life history variation in cod will be important in the conservation of phenotypic diversity, vital for the long-term persistence of the species, while the findings of plasticity in response to temperature will enhance predictions of responses to sea temperature rise.

CHAPTER 1

Introduction

1.1   Diversity in life history strategies in fishes

Fishes exhibit a huge array of life history strategies. Age at maturity can vary from weeks in cyprinodonts (pup fishes) (Simpson, 1979) to decades in squalids (dogfish sharks) (Saunders and McFarlane, 1993). Fecundity varies from only two in some elasmobranchs to millions in broadcast-spawning marine teleosts (Hutchings, 2002). However, life history diversity is not only limited to between-species variation. In recent years studies have also focussed on the diversity within a species. A very well studied example is the Trinidadian guppy, *Poecilia reticulata*, which shows extensive natural variability in reproductive traits (Reznick and Endler, 1982; Reznick and Bryga, 1987; Magurran, 1998) and offspring size in response to predator presence (Reznick and Endler, 1982). For example, in a time span of only two years (approximately 15 generations, estimated by Endler, 1983) following a transplant of the guppies to a predator free site upstream of their original habitat, males gradually became more colourful (Endler, 1980), the proportion of body mass devoted to reproduction increased, and offspring became larger and less numerous (Reznick and Endler, 1982).

The ability to cope with environmental variability enables the long-term persistence of a species in the face of environmental change. Conservation practices are increasingly recognising the value of preserving intraspecific variability. Such variation within a species is moderated by the interplay between the strength of selection and the smoothing forces of gene flow (Mayr, 1963; Conover, 1998). For example, in the case of the Trinidadian guppies, groups subject to high or low predation pressure were separated by waterfalls, which act as barriers to migration (Magurran, 1998). In the most insular of populations, there is no exchange of individuals between populations and each population has independent dynamics. At the other end of the scale are panmictic populations, in which there is random breeding and mixing throughout the species range. In between these two extremes, a metapopulation (a term first coined by

Levins, 1970) may be defined as a network of partially closed populations, which persist due to a balance of local extinction and between-patch migration (Freckleton and Watkinson, 2002). In other words, a metapopulation is a set of interdependent local populations, the size of which can change independently of each other but the likelihood of one existing is dependent on processes of extinction and mutual recolonisation between other local populations (Harrison *et al.*, 1988).

1.2 Adaptive divergence in life history traits in fishes

Analysis of the distribution of heritable molecular variation among individuals can result in the identification of isolated local populations with limited or no exchange of genes (individuals) between populations. These molecular population studies tend to focus on selectively neutral markers, which accumulate random DNA base-pair changes caused by mutation or drift over many thousands of generations. In the absence of gene flow these markers enable differences among isolated populations to be identified (Conover *et al.*, 2006). However, adaptive divergence that could be occurring concurrently and perhaps at a faster rate (Conover, 1998), will not necessarily be correlated with neutral genetic change and should be studied as an additional tool for gaining insight into population structuring.

In a number of cases there is strong evidence for adaptive divergence in the life history, development and morphological traits among populations in which molecular variation has not been detected. This is the case for four sympatric morphs of the Arctic charr, *Salvelinus alpinus*, in Iceland (Skulason *et al.*, 1989; Snorrason *et al.*, 1994) and the pumpkinseed sunfish, *Lepomis gibbosus*, where divergence in body form has been found across 26 populations in Ontario and New York (Jastrebski and Robinson, 2004). Such variation is more likely to be uncovered by examining quantitative genetic traits (Conover and Schultz, 1995; Conover, 1998). Expression of quantitative traits (including life history traits, meristic and morphometric traits) is controlled by many genes and the interaction with the environment in which they are expressed (Falconer, 1981; Conover *et al.*, 2006). Such traits arise from both natural and sexual selection, being closely related to fitness, and thus can reflect adaptation to the local environment and socio-biological system respectively (Conover, 1998). Local adaptation is a process

acting within populations that increases the success of individuals for a given trait in their native habitat relative to individuals originating from elsewhere (Conover, 1998), and is dependent on interactions between genotype and the environment. A number of factors can act against local adaptation. For example, gene flow may homogenise variation across habitats (Mayr, 1963), random genetic drift may cause non-adaptive genetic heterogeneity, or a fluctuating environment may make the optimal phenotype a moving target and trade-offs among traits may constrain their divergence (Conover *et al.*, 2006).

Despite the lack of obvious physical barriers to gene flow in the marine environment, there is still considerable evidence for adaptive divergence in marine fishes, although molecular genetic studies report a lower genetic diversity in marine fishes compared to anadromous and freshwater species (Ward and Grewe, 1994). A well-studied example is the Atlantic silverside, *Menidia menidia* (Conover and Present, 1990; Billerbeck, 1997; Billerbeck, 2000; Conover *et al.*, 2006). This is a migratory species that is widely distributed along the west coast of North America. This environment features a steep latitudinal gradient with associated changes in temperature and photoperiod and few physical barriers. While molecular genetic studies offer little evidence of population differentiation (Conover, 1998), studies of morphological, meristic and life history traits have produced extensive evidence of local adaptation (Conover *et al.*, 2006). Recent studies have shown that such adaptive variation can exist despite gene flow if selection is strong enough to distinguish inferior genotypes. This is the case for sympatric rainbow smelt, *Osmerus mordax*, in which two ecotypes, a semelparous dwarf form and an iteroparous normal form, occur in two genetically distinct populations that use the same spawning habitat. It is suggested that divergent selection is strong enough to maintain the phenotypic differentiation despite the gene flow through the synchronous migration to a common spawning site (Saint-Laurant *et al.*, 2003). Moreover, the speed and scale at which adaptive divergence can occur is quite remarkable. Divergence in early life history traits (larval size at hatch, larval development and survival), was demonstrated in three grayling populations that had shared a common ancestor only 80-90 years ago and had therefore evolved in only 13-18 generations (Haugen and Vøllestad, 2000). The spatial scale over which adaptive divergence in the marine

environment can occur can be as little as 60 km in the juvenile growth of turbot *Scophthalmus maximus* (L.) off Norway (Imsland *et al.*, 2001).

1.3 Environmental effects on adaptive divergence

Adaptive divergence may occur in response to many environmental factors, such as food availability (Marcil *et al.*, 2006a and b), predation pressure (Magurran, 1998) and temperature (Purchase and Brown, 2000; Haugen and Vøllestad, 2000; Salvanes *et al.*, 2004). The Trinidadian guppies, discussed earlier, evolved relatively quickly due to the presence of predators that directly affected the survival of different phenotypes (Reznick and Bryga, 1987; Reznick *et al.*, 1997). In animals of indeterminate growth, such as fishes, the effect of the environment is to a large extent reflected through the inter-related variables of body size and rate of growth (Arendt, 1997). Body size can be influenced by temperature, food (Winemiller and Rose, 1992; Yoneda and Wright, 2005a and b) and predation pressure (Werner *et al.*, 1983), while growth may be limited by length of the growing season (Schultz and Conover, 1997). Larger females are reported to produce larger eggs in species such as turbot, herring and capelin. They are likely to be more fecund (Wootton, 1998; Roff, 2002) and to mature earlier (Trippel *et al.*, 1995).

In the marine environment temperature varies widely and is the driver of many physiological processes (Brander, 1995). Numerous studies have shown temperature in particular to be a vital factor in determining the characteristics of fish species and populations. Temperature has a direct effect on metabolic rate and thus determines energy expenditure (Blaxter, 1988; Claireaux, 1995; Helle, 2002). It can also affect time to hatching, efficiency of yolk utilisation, time to metamorphosis, behaviour, rates of feeding and digestion and metabolic demands (Blaxter, 1988), and may have indirect effects on larvae via the oxygen capacity of the water, viscosity and timing of the phytoplankton bloom (Blaxter, 1988; Beaugrand *et al.*, 2003).

1.4 Genotypic and environment effects on the phenotype

It is a combination of genotype and the environment and interactions between them that generates such continuous variation among phenotypes. As explained in Conover and Schultz (1995), this relationship can be expressed by the equation:

$$V_P = V_G + V_E + V_{G*E} + 2\text{Cov (G,E)},$$

where V_P is the phenotypic variance; V_G and V_E are the genotypic and environmental variances respectively; V_{G*E} is the non-additive interaction between the environmental and genotypic effects and Cov (G,E) is the covariance between the genotypic and the environmental sources of variation.

The set of phenotypes produced across a varying environmental gradient of a genotype is known as its reaction norm. The shape of the reaction norm allows for comparison between populations, as shown in Figure 1.1 (Conover and Schultz, 1995).
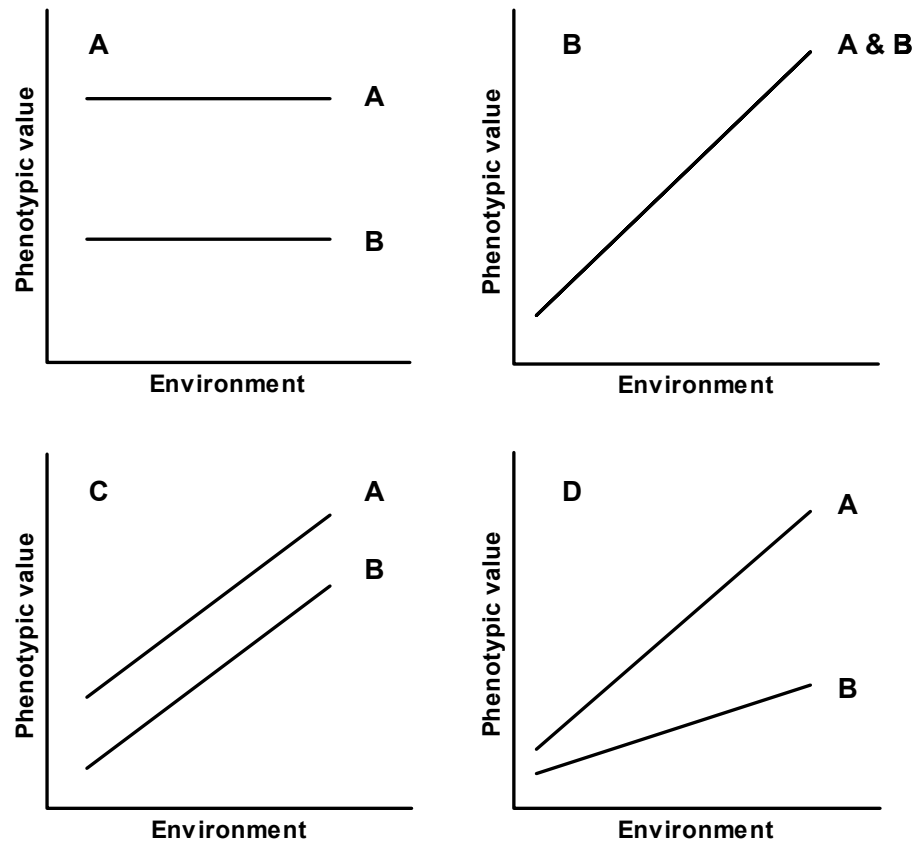
Figure 1.1. Hypothetical reaction norms for populations A and B, showing A) a genetic effect but no environmental effect, B) an environmental effect but no genetic effect, C) a genetic effect and an environmental effect but no genotype x environment interaction and D) a genotype x environment interaction.

If the contribution of V_E is zero, then V_P is attributed completely to the genotype (Figure 1.1A). If V_G is zero then V_P is entirely due to environmental plasticity (Figure 1.1B). The interaction term expresses the degree to which the different genotypes vary in their reaction to the environment. The magnitude of the interaction term can be used to express how the phenotypic value changes in response to the environment, and is known as the phenotypic plasticity. The covariance between the reaction norms of the genotype and the environment can be used to explain the unequal representation of genotypes in different environments (Figure 1.1C and D) (Conover and Schultz, 1995).

1.5   Patterns of phenotypic variation

Selection can generate differences in patterns of the reaction norms, leading to population differences in response to the environment, although the genetic variation

must be heritable (additive) for selection to act upon it. Different patterns in the covariance term have been identified in nature, some of which are highly cryptic (Figure 1.2). Species that live across an ecological gradient may appear similar, but in fact counteracting effects may be masking variation. If the genetic influences on a plastic trait are distributed across the ecological gradient, such that they oppose the effect of the environmental influence, this generates similarity and stabilising selection, known as countergradient variation (Figure 1.2A) (Levins, 1969; Conover and Schultz, 1995). Countergradient variation has only been identified in 26 species to date (Conover *et al.*, 2006). Most demonstrations of countergradient variation have been in growth rates (Conover and Present, 1990), egg production (Klahre, 1998), reproductive traits (Klahre, 1998; Kokita, 2003) or in body shape (Marcil *et al.*, 2006a and b), over a latitudinal (Purchase and Brown, 2000) or altitudinal gradient (Berven *et al.*, 1979) that may covary with temperature.

Alternatively when selection favours different phenotypes over an ecological gradient, variation may be cogradient (Figure 1.2B) (Marcil *et al.*, 2006). In this case genetic influences act in the same direction as environmental effects and thus accentuate the character in question (Levins, 1968; Conover and Schultz, 1995). In such a situation in the wild, the genetic effect serves to accentuate any environmental effects and maximise the phenotypic differences between the populations. Examples of cogradient variation are mostly restricted to morphological traits (Levins, 1968; Berven *et al.*, 1979; Parsons, 1997), such as the distinct benthic and pelagic morphs that occur in a number of freshwater fishes (Robinson and Wilson, 1994). However, Otterlei *et al.* (1999) demonstrate faster growth rate in larval and juvenile cod of the warmer Norwegian coast than those of the cooler Northeast Arctic, consistent with the idea of cogradient variation.

A third type of adaptive variation can occur, in which the genotype is most effective under the conditions of its origin (Figure 1.2C). An example is provided by the mummichog, *Fundulus heteroclitus*, in which more northerly, cold-adapted genotypes have a higher swimming endurance than their warm-adapted conspecifics but only at lower temperatures. The heritability of swimming performance is also confirmed by its

correlation with allozyme variation in lactate dehydrogenase (Powers and Schulte, 1998).
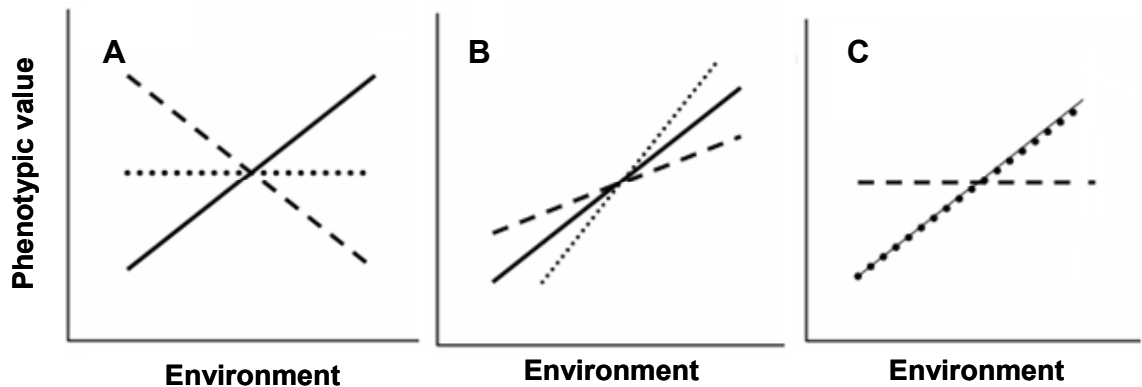
Figure 1.2. Environmental influence is depicted by solid line, genotypic influence by dashed line and pattern of phenotypic variation by dotted line. A illustrates countergradient variation, in which the phenotypic effect is equal across all environments due to the opposing influence of the environmental and genetic effects. B depicts cogradient variation in which the phenotypic effect is enhanced by both the environmental and the genetic effects, which act in the same direction. In C there is no genotypic influence and thus no covariance. The phenotypic pattern follows the same slope as the environmental influence. Adapted from Conover and Schultz (1995).

1.6   Empirical approaches to the study of population differentiation

While such studies of phenogeography can reveal intraspecific diversity across a species range (e.g. Brander, 1995; Yoneda and Wright, 2004), they are correlational. As a consequence, the relative contribution of phenotypic and genotypic effects cannot be quantified, due to uncontrollable environmental effects in the wild, such as food availability (as discussed by Kokita, 2003) that may also influence the phenotype. There are two main approaches to uncovering such effects; a reciprocal transplant or a common-garden experiment. A reciprocal transplant experiment can be performed, in which individuals from different environments are transferred together to each of the original habitats. Many of the experiments carried out on the Trinidadian guppies were performed using such an approach. Endler (1980) transferred guppies from a high-predation site to one upstream that had not been colonised by guppies or other predators. The introduced guppies became rapidly more colourful and more attractive to females, in line with the expectation of a low-risk habitat (Magurran, 1998). This

method has the advantage that the response to multiple parameters in the natural environment can be investigated, but population introductions are generally undesirable due to concerns over 'genetic pollution'. For example, two sub-species of salmon, the masao salmon, *Oncorhynchus masou masou* and the amago salmon *O. m. ishikawae* from Japan naturally occur parapatrically but introduction in some rivers has resulted in hybridisation between the two sub-species, the hybrid being smaller and less valuable commercially than the amago salmon (Yamazaki *et al.*, 2005).

Alternatively, adaptive variation can be identified through a common garden experiment, in which individuals from different points in the natural range are reared from the egg stage under standardised conditions. Using this approach, the response to specific variables can be tested without other confounding factors. Both reciprocal transplant and the common garden methods can however be affected by early environment effects (prior experience by the test individuals) and also maternal effects. A female allocates energy to the yolk in the egg (Wootton, 1998) and it is likely that the condition of the female affects egg size or quality (Heath and Blouw, 1998). To control such undesirable effects, experimental subjects should be reared under standardised conditions from the beginning of their lifecycle, not simply during the period of interest for the study. Secondly, family effects should be accounted for in the design and ideally the parents should also be reared under controlled conditions. The experiments should also be carried out across the full range of environmental conditions experienced by the target species in nature, as genotype x environment effects may only be seen under a certain range of conditions. Lastly individuals from a minimum of three locations are required from across the ecological gradient to ensure that the variation observed is adaptive and not due to chance (Conover *et al.*, 2006). A compelling example of the use of a common garden design was in the demonstration of adaptive divergence in the grayling, *Thymallus thymallus* (Haugen and Vøllestad, 2000). Larvae from three populations and 28 families were reared from hatch under common conditions, in three temperature treatments, representative of the temperatures in their natural environment. Reaction norms revealed population variation in a number of early life history traits, in which larvae from each population performed best at the temperature experienced in nature. Sire-effects demonstrated a strong genetic component to this variation.

1.7   Implications for conservation

Evidence of adaptive divergence has widespread implications for conservation, in particular to target species. Fish conservation usually focuses on identifying evolutionary significant units (Waples, 1991), in order to identify biologically significant management units for stock assessment and to protect genetic diversity. Studies of molecular diversity, phenogeography and migration are used to identify such units, but common garden studies are required to test whether apparent phenotypic differences represent genetic adaptations (Conover *et al.*, 2006). It is such adaptive phenotypic traits that influence the viability of populations and that has enabled many species to persist in an ever-changing environment (Hutchings *et al.*, 2007). Therefore, identification of adaptive divergence and an appreciation of how it will change in response to future environmental change is an essential component of fisheries management (Conover *et al.*, 2006). Such an approach is especially pertinent in over-exploited stocks, where in addition to responding to the environment, selection will also be induced by commercial fishing. Fishing can be a major source of mortality and is highly size selective. Both empirical studies (Ricker, 1981) and experimental studies (Conover and Munch, 2002) confirm a fisheries-induced adaptive change. One such example of an over-exploited species that could be experiencing phenotypic changes in accordance to the theory is the Atlantic cod, *Gadus morhua*. Several studies have suggested a shift in age of maturation, with maturation at smaller sizes and a younger age in exploited stocks (Olsen *et al.*, 2004; Yoneda and Wright, 2004). Therefore, artificially induced selection such as that imposed by heavy fishing pressure must also be taken into account in conservation practices. It has been suggested that a lack of appreciation of the variation in dynamics between an inshore and an offshore component of a stock has accelerated the collapse of cod stocks in the northwest Atlantic. Studies of adaptive variation in Atlantic cod may enable improved conservation measures to be taken.

In response to the decline in fisheries and subsequent high market value of cod, interest in intensive culture of cod has increased dramatically over the past 10 years (Brown *et al.*, 2003). Cultured cod has also been used for restocking purposes, although as yet attempts have had little success (Brown and Day, 2002; Salvanes and Braithwaite,

2006). Prime concerns in the aquaculture of cod, amongst others, are to minimise mortality (Brown *et al.*, 2003) and aggression-induced growth reduction (Hatlen *et al.*, 2006). Cod are aggressive and often exhibit cannibalism throughout the early life history stages (Folkvord, 1989; Folkvord and Ottera, 1993), especially in a restrictive environment such as that experienced in culture. Likewise, one of the many concerns with restocking is to enable competition with the native stock, such as in obtaining food (Brown and Day, 2002). Studies have shown better foraging in certain haemoglobin genotypes of cod (Salvanes and Hart, 2000) and higher rates of growth in those dominant fish (Hart and Salvanes, 2000), but no known studies have explored the potential for population interactions. Experiments (similar to those suggested by Salvanes and Braithwaite, 2006) are required in order to discern the effects of contact among populations which would not otherwise have mixed, such as might happen through aquaculture or restocking. In carrying out controlled environment experiments to investigate life history responses, it is important to understand social interactions between individuals sharing a tank, which may mediate individual responses.

1.8   Study species: Atlantic cod, *Gadus morhua* (L.)

The Atlantic cod, *Gadus morhua* (Linnaeus, 1758), is one of the most valuable commercial species of the northern Atlantic (Imsland *et al.*, 2003) and is also a key component of the north Atlantic ecosystem (Link and Garrison, 2002). It has been fished to commercial extinction in some regions of the northwest Atlantic and is very heavily exploited elsewhere and as a consequence is listed as vulnerable by the IUCN. Both the North Sea (ICES area IV) and West of Scotland stock (ICES area VIa) are very depleted and the spawning stock biomass is reported as being at a historic low (ICES, 2004).

1.9   Life history of Atlantic cod

The life history of cod is highly variable as will be described in detail throughout this thesis. A brief life history of cod from the North Sea is given to provide a background to the thesis. Cod can grow to a length of over 120 cm at an age of nine or ten years and are one of the largest species of fish in the North Sea (Hislop, 1984). Cod are widely

distributed but in many areas they congregate to spawn (Brander, 1975; Robichaud and Rose, 2001). Spawning grounds have been identified throughout the North Sea in a number of aggregations (Wright *et al.*, 2006a; Fox *et al.*, 2007). The main spawning time is February and March but some will spawn later in the year (Wright *et al.*, 2003; Hislop, 1986). Maturity can now be attained as early as age 2 (36 cm) and most are mature by four years of age (Yoneda and Wright, 2004). The proportion of mature individuals increases gradually with age and, within any age group, mature individuals are usually larger than immature ones. Absolute and relative fecundity is exceptionally high, in the order of tens of millions (Hislop, 1984; Kjesbu *et al.*, 1998). Fertilisation is external and its efficiency is increased by the spawning embrace, whereby the vents of the couple are in close proximity prior to emission of eggs and sperm (Hislop, 1984). The eggs are pelagic and upon hatching the larvae descend for several weeks or months in the upper water column. Mortality is extremely high during the first year of life. At this stage cannibalism can play an important role in regulating numbers (Hislop, 1984). A period of extensive feeding, lasting for approximately four months, takes place prior to gonadal maturation. Cod have a protracted spawning period, spawning many batches of eggs (Kjesbu and Kryvi, 1989). The eggs of an individual female are ripened and shed in discrete batches at intervals of 24 to 48 hours to several weeks (Hislop, 1984; Kjesbu *et al.*, 1996). For cod to complete the maturation process within the subsequent reproductive cycle, some of the oocytes need to reach pre-vitellogenesis by late autumn (Holdway and Beamish, 1985). The precise timing and extent of life history events can only be understood by examining the factors that contribute to variation in those traits.

1.10 Environmental variability in Atlantic cod

The phenogeography of Atlantic cod is highly variable. Cod are distributed over a wide range of environmental conditions. Their mean annual ambient temperatures can range between 11 ºC in the Celtic Sea (towards their more southerly extent) to 1 ºC in the Labrador Sea in their extreme northerly range (Brander, 1994a) but the full thermal range can vary between -1 and 19 ºC (Sundby, 2000; Neat and Righton, 2007). They also exhibit a high degree of environmentally-induced plasticity in a number of life history traits. Mean weight-at-age can vary by an order of magnitude. For example, mean weight-at-age for 4-group cod can vary between 0.6 kg in the Labrador Sea to 7.3

kg in the Celtic Sea and age at maturation varies from 2 to 3 years in the Baltic and southern North Sea (Rijnsdorp *et al.*, 1991) to 7 years of age in Icelandic waters (Brander, 1994a). Relatively high heritabilities have been reported for traits such as growth in cod (Gjerde *et al.*, 2004) and early life history traits in other fishes (Haugen and Vøllestad, 2000). Cod are a migratory species, yet tag-recapture studies have shown a high degree of site fidelity to particular spawning grounds in the adults (Robichaud and Rose, 2004; Wright *et al.*, 2006a) and limited annual movements in some areas (Neat *et al.*, 2006). Moreover, molecular genetic methods have identified large genetic differences across the species range (Dahle *et al.*, 2006). Differentiation at the pantophysin (*Pan* I) locus, a nuclear RFLP locus, has revealed genetic differences in cod from Norway (Fevolden and Pogson, 1997; Dahle *et al*, 2006), while microsatellite loci have revealed a more complex structure in the eastern (Hutchinson *et al*, 2001; Jónsdóttir *et al.*, 2002) and western Atlantic (Pogson *et al.*, 1995; Taggart *et al.*, 1998; Ruzzante *et al.*, 1998). This mounting evidence of population structuring together with the plasticity of phenotypic traits and evidence for heritability, demonstrates that cod is an ideal candidate for studies on local adaptation.

1.11 Revealing phenotypic variation in Atlantic cod

Studies have shown the capacity for local adaptation in cod in response to a number of environmental conditions in several life history traits from the eastern (Otterlei *et al.*, 1999; Salvanes *et al.*, 2004) and western Atlantic (Puvanendran and Brown, 1998; Purchase and Brown, 2000; Marcil *et al.*, 2006a and b; Hutchings *et al.*, 2007). Under common environment conditions, cod larvae from the Grand Banks have a higher genetic capacity for growth than those from a more southerly population (from the Gulf of Maine), despite the reverse trend being observed in the wild. This is evidence of countergradient variation (Purchase and Brown, 2000). Similarly, Salvanes *et al.* (2004) document higher juvenile growth rates and greater success in competition for food in a more northerly population of cod from the Norwegian coast than a more southerly population, using a controlled environment approach. In both of these studies, a response to temperature was documented, although the difference may also have resulted from the variation in photoperiod and growing season along a north-south gradient. Puvanendran and Brown (1998) demonstrated that cod larvae are adapted to

the ambient light levels at the sites where they would naturally hatch. Larvae from a more northerly site on the Scotian Shelf grew and foraged faster under higher light levels, while those from Newfoundland performed better under lower light intensities. Body shape in cod from Nova Scotia and Newfoundland also shows countergradient variation in response to food and temperature variation. Cod measured from the wild showed no difference in body form relative to each other but those reared under controlled environment conditions did (Marcil *et al.*, 2006a and b). Divergent reaction norms in larval growth and survival demonstrate that warm-water adapted cod populations from the east coast of Canada are more sensitive to food availability, whereas cold-water adapted populations are more sensitive to temperature changes. These traits differed across a finer spatial scale than that detected by microsatellite DNA variation (Hutchings *et al.*, 2007).

1.12 Molecular variability in Atlantic cod

Additionally, local adaptation has been shown with respect to genotype, such as haemoglobin genotype (HbI), (Nævdal *et al.*, 1992; Imsland *et al.*, 2004), first described by Sick (1961), and *Pan* I (Gjerde *et al.*, 2004; Case *et al.*, 2005, 2006). The distribution in frequency of the haemoglobin genotype (HbI-1*1, HbI-1*2 and HbI-2*2), associated with differences in oxygen affinity (Karpov and Novikov, 1981) and the transport of oxygen from the gills to the respiring tissue, is strongly influenced by temperature (Brix *et al.*, 2004). Some of the highest frequencies of the HbI-1*1 genotype (0.72) are found in the southern North Sea (Appendix 1.2, Figure 1.1), where water temperatures are high, and frequencies gradually decline (to 0.40) with declining temperature along the Norwegian coast (Frydenberg *et al.*, 1965; Dahle and Jørstad, 1993; Petersen and Steffensen, 2003). Likewise, the frequency of the HbI[1] allele is approximately 0.1 in the cooler waters of the Barents Sea and 0.2-0.5 on the northern and west Norwegian coast (Frydenberg *et al.*, 1965; Petersen and Steffensen, 2003). Haemoglobin genotype has been demonstrated experimentally to influence many traits, such as growth (Nævdal *et al.*, 1992; Imsland *et al.*, 2004), feeding efficiency (Jordan *et al.*, 2006), temperature preferenda (Petersen and Steffensen, 2003) and feeding behaviour (Salvanes and Hart, 2000). The *Pan* I* genotype has also been linked to growth rate (Case *et al.*, 2006) and

it has been suggested that, like the haemoglobin locus, it too is under positive selection (Jónsdóttir *et al.*, 2002; Case *et al.*, 2006).

1.13 Environmental factors influencing variability in phenotype of Atlantic cod

A number of factors have been shown to influence cod development; these include food (Yoneda and Wright, 2005a and b; Marcil *et al.*, 2006a and b, Hutchings *et al.*, 2007), light intensity (Puvanendran and Brown, 1998), photoperiod (Norberg *et al.*, 2004; Skjæraasen *et al.*, 2004) and temperature (Otterlei *et al.*, 1999; Purchase and Brown, 2000; Salvanes *et al.*, 2004; Marcil *et al.*, 2006b, Hutchings *et al.*, 2007). Which of these environmental factors has the most important influence is dependent on the trait and population in question (Hutchings *et al.*, 2007). However, temperature certainly has an overarching influence in many aspects of development in marine fishes including cod, being a direct determinant of vital physiological processes (Jobling, 1985; Brander, 1994b, 1995). It also has direct effects on metabolic rate, thus determining energy expenditure (Blaxter, 1988; Claireaux, 1995; Helle, 2002), hatching time, efficiency of yolk utilisation, time to metamorphosis, activity (Neuman *et al.*, 1996), rates of feeding (Brett, 1979; Koskela *et al.*, 1997) and digestion and metabolic demands. Temperature may have indirect effects on larvae via the oxygen capacity of the water and viscosity.

Sea temperatures are thought to be changing in response to climate change. How cod will respond to such changes in temperature requires a detailed understanding of the extent of thermal plasticity in vital traits from different populations, in combination with an understanding of potential oceanographic changes. Factors such as salinity (Brander, 2000), winds and currents (O'Brien *et al.*, 2000) can all have a profound effect on life history. If temperature causes a 'mismatch' in timing of the planktonic food supply and the hatching larvae, this could result in insufficient food and poor survival (Cushing, 1975; Blaxter, 1988). Thermal change could also result in a shift in cod distribution towards a more optimal climate. It has been proposed that such a shift has taken place in cod in the North Sea over the past 25 years (Perry *et al.*, 2005). However, this has recently been questioned as evidence from cod tagged with electronic devices suggests they do not avoid warmer waters in the southern North Sea (Neat and Righton, 2007). Another possibility is that a thermal shift may result in a change in development rates

and thus alter the timing of life history events. A parabolic (domed) pattern exists in many traits, such as egg development (Jordaan and Kling, 2003) and growth (Jobling, 1988, 1997; Imsland *et al.*, 1996, 2005; Jonassen *et al.*, 1999), in which the trait rises with temperature to a thermal optimum and subsequently drops at higher temperatures. Therefore if environmental temperatures exceed the degree of thermal plasticity for a specific trait, development rates may decline and result in local declines in populations.

1.14 Population structure in Atlantic cod from around the UK

Around the British Isles, there is evidence from a variety of techniques for population structuring in cod. At a broad geographic scale, all European stocks were found to be genetically distinct from Canadian stocks (Hutchinson *et al.*, 2001). At a local scale, four genetically isolated populations in the North Sea were identified including Bergen Bank, the Moray Firth, Flamborough Head and the Southern Bight. The stock in the central English Channel was found to be significantly distinct from the stocks in the Celtic Sea and Outer Hebrides and northern North Sea (Hutchinson *et al.*, 2001). A highly structured population also appears to exist in the northern North Sea. Differences in otolith shape, an indicator of environment, between spawning groups from the Viking bank, Moray Firth, Clyde Sea and Irish Sea, suggests that there is a high degree of spatial segregation (Galley *et al.*, 2006). Tag-recapture studies in the 1960s and 1970s and the present day have found evidence of residency of spawning stocks in the Clyde, Moray Firth, the Minch (Wright *et al.*, 2006a) and Shetland (Neat *et al.*, 2006). Otolith chemistry has also demonstrated differentiation between cod from nursery areas in the Clyde Sea, Moray Firth, Buchan and west Shetland (Gibb *et al.*, 2007). Present day tag-recapture studies in the region suggest a high degree of natal fidelity, particularly in cod from the Clyde Sea, consistent with a metapopulation structure (Wright *et al.*, 2006b). Evidence for adaptive divergence has not yet been reported in cod from British waters, but Yoneda and Wright (2004) do report differences in reproductive traits between cod from the Moray Firth and the Firth of Clyde from field studies.

1.15 Thermal variability in the marine environment around the UK

The environment around the British Isles differs considerably, particularly in relation to temperature, with a milder and a less variable climate on the west coast than in the North Sea (Figure 1.3; Appendix 1, Figures A1.1 and A1.2; Hughes, 2004). The west coast of Scotland especially, and to some extent the northern North Sea, is strongly influenced by the relatively warm water of the Atlantic inflow from the Slope Current (Turrell, 2006). The northern North Sea is deeper than the southern North Sea and has a lower and more stable thermal regime. The shallower waters of the southern North Sea (rarely in excess of 50 m) result in a higher degree of mixing and together with a strong influence of continental air temperature, the thermal environment is more variable than that of the northern North Sea (Turrell *et al.*, 1992; Neat and Righton, 2007). From the extensive evidence of population structuring, suggestions for phenotypic differences and the natural variability in thermal environments, it was hypothesized that there may be local thermal adaptation in life history traits in cod from around the British Isles and one of the aims of this thesis is to test this hypothesis experimentally.
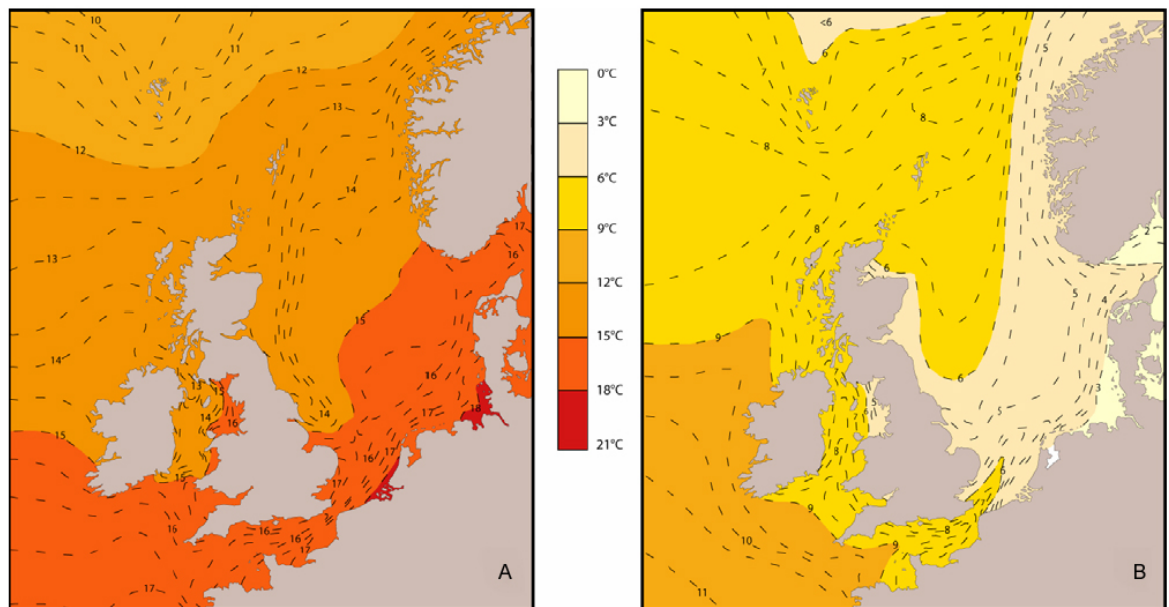
Figure 1.3. Mean sea surface temperatures (°C) in summer (A) and winter (B) in seas around Britain and Ireland (after Lee and Ramster, 1981; FSBI, 2007).

1.16 Experimental design

A controlled environment approach was taken to investigate the potential for adaptive divergence. As in a common garden experiment, the experimental subjects were maintained under standardised controlled conditions. Ideally a full common garden experiment would have been carried out, in which the F_1 generation of wild caught stock would have been used in order to fully control for all maternal or early environmental effects. However, it is debatable how important such effects are as there have been very few studies that have demonstrated maternal effects in life history stages beyond the larval stage. Constraints of time and the difficulties of rearing eggs through to larvae made such an approach impractical. A controlled environment method was used rather than the full common garden approach in which fish were captured from the wild at the earliest possible stage and subject to common environment conditions. While this approach cannot entirely control for maternal or early environmental effects, the sample of fish being used in the experiment may be more representative of wild fish. In contrast to using cultured fish, in which survival is unnaturally high, by using wild-caught fish which have been subject to natural selection pressures, this problem is avoided. A common environment method has been used in similar studies on cod, such as in discerning differences in growth rates of juvenile cod from two sites on the west coast of Norway (Salvanes *et al.*, 2004) and also in investigating differences in spawning times in cod from four sites also along the Norwegian coast (Ottera *et al.*, 2006). Specifically this thesis will address how population of origin and/or temperature will influence juvenile behaviour, juvenile growth rate, adult growth rate, condition and the onset of maturation and development from egg to larvae. The influence of haemoglobin genotype in these experiments was also accounted for.

1.17 Sample locations

Three sample populations were selected (Figure 1.4) from different thermal regimes; St Andrews Bay on the Scottish east coast (56º22'N 2º41'W), the Clyde Sea (55º22'N 4º58'W) on the Scottish west coast, and Lowestoft (52º27'N 1º45'E) in the southern North Sea. Details of the study sites, the thermal regimes and the trawl positions from where the cod were sampled are given in Appendix 1.
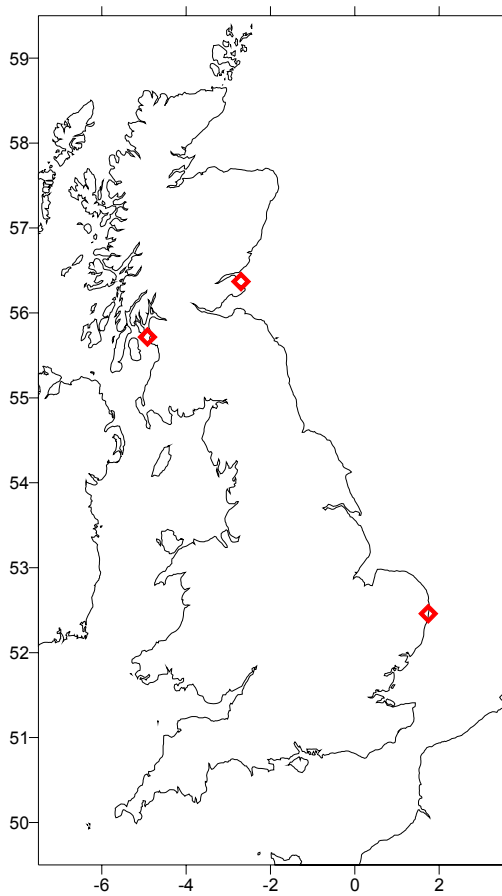
Figure 1.4. Locations of study groups from around the British Isles; St Andrews Bay in the northern North Sea/ Scottish east coast (2004 and 2005 year classes), the Clyde Sea on the Scottish west coast (2004 and 2005 year classes) and Lowestoft in the southern North Sea (2005 year class). The cod were caught as wild 0-groups.

1.18 Aims

This thesis will deal with a number of life history stages in turn. The aims are as follows:

• To investigate juvenile behaviour in cod and its potential influences on growth and how population and haemoglobin genotype affect behavioural interactions.

• To examine population and thermal responses on juvenile growth rate, taking into account haemoglobin genotype effects.

- To explore population and thermal effects on adult growth rate and maturation of virgin spawning cod, with consideration of haemoglobin genotype.

- To investigate thermal responses of egg development and larval hatch size. A study on the effect of temperature on the prevalence of spinal malformations in the larvae (Fitzsimmons and Perutz, 2006) is also given in Appendix 2.

- To describe thermal regimes of the study populations, their respective geography, and their habitats.

CHAPTER 2

Competitive interactions between populations of wild Scottish Atlantic cod

2.1 INTRODUCTION

Variation in competitive ability among individuals has direct consequences for resource acquisition and ultimately fitness. Winning or losing can be related to intrinsic features of the opponents such as their relative size (Turner and Huntingford, 1986; Koops and Grant, 1993), energy reserves, relatedness to each other (Waldman, 1988) and genotype (Salvanes and Hart, 2000), or to behavioural factors such as prior residency (Johnsson and Forser, 2002; Metcalfe *et al.*, 2003) and presence of predators (Persson and Greenberg, 1990). These factors influence an individual's resource-holding potential, i.e. the ability of a combatant to fight or to defend (Maynard Smith, 1982). Winning a conflict may also depend on how much an opponent values the disputed resource and the costs it is prepared to incur in order to secure it (Huntingford and Turner, 1987). Appetite would alter the value of food, for example. The study of competitive asymmetries and conflict is dealt with by Game Theory (Maynard Smith, 1974), an aspect of which is how animals use differences in certain traits between opponents to settle a fight, known as competitive asymmetry. When opponents are matched for one trait they may use another to settle a dispute, or fighting may escalate until resolved by overt aggression rather than assessment (Huntingford and Turner, 1987). Juvenile Atlantic cod, *Gadus morhua* L., vary both within and between populations, in terms of growth rate (Purchase and Brown, 2001; Chapter 3 and 4) size (Höglund *et al.*, 2005), condition (Lambert and Dutil, 2003; Chapter 4) and genotype (Sick, 1961; Case *et al.*, 2006). Furthermore, juvenile cod are believed to be highly competitive, particularly at the juvenile stages when size variation commonly results in physical aggression (Folkvord, 1989; Folkvord and Ottera 1993; Hatlen *et al.*, 2006). However, the ways in which the behaviour of juvenile cod affects the outcome of competitive interactions are poorly understood.

Natural populations are also composed of individuals who are of varying degrees of familiarity to each other. The ability of a fish to recognise familiar conspecifics has been found to play an important role in determining intraspecific interactions, such as schooling behaviour in guppies (Magurran and Seghers, 1991), feeding (Seppä *et al.*, 2001), mating preferences (Kelley *et al.*, 1999) and aggression (Höjesjö *et al.*, 1998). How recognition of familiar fish occurs is less clear but such recognition can develop in only 12 days (Griffiths and Magurran, 1997). The "Dear Enemy" idea was based around a model whereby when individuals are retained together it pays to cooperate (Jaeger, 1981; Ward and Hart, 2003). Thereby aggression is greatest between competitors that infrequently interact. In juvenile sea trout, *Salmo trutta*, it has been reported that familiarity reduces aggression (Höjesjö *et al.*, 1998). Often the long-term consequences of competitive differences are reflected in growth performance and monitoring growth can be an indirect way of assessing competition. Weight gain or growth has been used as a direct indictor of dominance in fishes, being related to increased social status (Abbott *et al.*, 1985), decreased predation (Williams and Brown, 1991) and better ability to defend resources (Abbott *et al.*, 1985). Studies on fishes including stickleback, *Gasterosteus aculeatus* (Utne-Palm and Hart, 2000), and juvenile brown trout (Höjesjö *et al.*, 1998) have shown that growth rate is greater and less variable in the company of familiar as opposed to unknown individuals, although such findings may be environment dependent. Under non-favourable conditions, such as restrictions in food supply, greater variability may develop (Waldman, 1988).

Three factors that may affect the outcome of competitive interactions in cod are body size, haemoglobin genotype and feeding success. Variation in access to food can lead to size advantages and larger individuals can become cannibalistic, resulting in an amplification of size heterogeneity (Folkvord and Ottera, 1993; Hart and Salvanes, 2000). Physical conflicts, whereby the opponents interact, are known as interference competition (Miller, 1967; Huntingford and Turner, 1987). Competition over food in cod is apparently in the manner of scramble competition, however. In this case, an animal may become increasingly efficient at obtaining the resource in a non-aggressive manner. It has been shown in Norwegian cod that when given restricted access to food, those from the Northeast Arctic population were more successful in gaining food than those from the Norwegian coastal population (Salvanes *et al.*, 2004). Haemoglobin

genotype has also been shown to influence competition in pair-wise encounters in juvenile cod (Hart and Salvanes, 2000), the HbI-2*2 genotype out-competing the HbI-1*1 and the heterozygote. Furthermore, the performance of individuals of the differing genotypes has been found to be temperature dependent. The HbI-2*2 genotype functions more efficiently, in terms of transport of oxygen at temperatures above 14 ºC, while the HbI-1*1 genotype functions better at temperatures below 10 ºC (Karpov and Novikov, 1981). Moreover this temperature dependent response of haemoglobin genotype has been linked to metabolic rate (Weber, 1990) and growth (Nævdal e*t al.*, 1992; Imsland *et al.*, 2004; Jordan *et al.*, 2006), both of which have also been related to indicators of competitive performance (Nakano, 1994; Yamamoto *et al.*, 1998; Cutts *et al.*, 1998; Höjesjö, 2002).

In addition to genotypic variation, putative populations of cod are now commonly reported with distinct phenotypic characteristics, suggestive of local adaptation (Purchase and Brown, 2001; Salvanes *et al.*, 2004 and Perutz *et al.*, submitted; Chapter 3). Such differences could potentially result in competitive advantages in specific environments. Conversely, behavioural differences between populations may also result in restriction of gene flow and may actually help maintain spatial population structure (Jenkins, 1969; Huntingford and Turner, 1987; Mendelson and Shaw, 2002). Climate change may result in changes in geographic distribution of species, which could result in interactions between populations that have not previously co-existed. Additionally, cod aquaculture is now of considerable economic importance and mixing individuals from different populations may enhance or suppress cod production (as is discussed in Griffiths, 2003). Similarly, following the decline in cod in many areas, there are attempts to restock populations, but restocking with a non-local population may provoke competition with the remaining native individuals (Brown and Day, 2002). While there have been numerous demonstrations of intraspecific interactions in fishes, relatively few have studied competition between individuals of differing populations. The aim of this study was therefore to investigate such interactions in Atlantic cod from different geographical regions.

I investigated two geographically separated populations of cod, one from the Clyde Sea on the west coast of Scotland and one from St Andrews Bay on the east coast. The Clyde Sea and St Andrews Bay differ in a variety of factors that could provide

opportunities for local adaptation, such as thermal regime (Slesser and Turrell, 2005; Perutz *et al.,* submitted), habitat of juvenile cod (Appendix 1) and interactions with other species (Temming *et al.*, 2007). Any such local adaptations might influence competitive abilities.

The first study measured growth rates in juvenile cod held in groups composed of individuals originating from either the same populations (pure) or from different populations (mixed), to investigate the prediction that population interactions have consequences on their growth rate. The second study involved direct behavioural observations of pair-wise competitive interactions, comparing pairs of fish originating from either the same or from different populations, to examine the behavioural mechanisms that could potentially underlie the consequences identified in the first experiment. The influence of haemoglobin genotype on growth and the behaviour was also assessed. The aim of these experiments was firstly to ascertain whether there were population and haemoglobin genotype interactions on growth and behaviour, and secondly to provide a link between growth at a group-level and aggression and feeding at an individual level. According to the Dear Enemy hypothesis (Jaeger, 1981), it would be predicted that growth rate in the pure population groups (provided they have been held together previously) should be greater than when mixed. Furthermore in the pair-wise experiments a lower level of aggression would be expected in the pure population pairs than the mixed and a more equal prey share between opponents.

EXPERIMENT 1. Growth in juvenile Atlantic cod held in pure and mixed population groups

The aims of this study were as follows: -

- Are there differences in growth rate according to whether a group of fish are in a mixed or a pure population condition?

- Is growth rate influenced by haemoglobin genotype?

2.1   MATERIALS AND METHODS

2.1.1 Fish collection and husbandry

Wild juvenile 0-group cod were collected from two locations from the east coast of Scotland in the North Sea and the west coast of Scotland in the northeast Atlantic. The east coast cod were caught from the bay at St Andrews by the Fisheries Research Services (FRS) vessel 'Clupea' using a fine mesh trawl. Cod were collected in trawls from the same area (centre point at 56°21.64'N 2°44.04'W), in October of 2004 and 2005, both towed at a depth of between 7 and 15 metres for 30 minutes. The west coast cod were caught from the Clyde Sea on a commercial vessel crewed by the Millport Marine Biological Station using a fine mesh cod end. They were caught in November 2004 and 2005 (centre point at 55°43.15'N 4°54.9'W) at a mean depth of 21 metres again for a 30 minute period. The pair-wise study used the fish caught in 2005. The cod were transferred to the FRS Marine Laboratory in Aberdeen and each stock housed in a separate cylindrical tank (3000 L) under a flow-through system with between 250 and 300 cod per tank. Fish from both populations were kept under identical conditions. Temperature was maintained at ambient and fluorescent green lighting was provided with a photoperiod adjusted to ambient (57°16.7'N). Each group of fish was given the same feeding regime, in which they were fed to satiation with commercial dry pellets twice daily in the early morning and late afternoon (TROUW; protein 50 %, oil 18 %, ash 11.5 %, fibre 0.6 %, phosphorus 1.8 %).

2.1.2 Experimental design

The experiment was carried out twice, once in December 2004 and once in February 2006. A sub-sample of fish from both stocks was transferred into a tank of equal dimensions two weeks prior to the experiment for acclimation and recovery from tagging. Before transferral, fish were anaesthetised using a standard protocol with tricaine methanesulphonate (MS-222, 1 g 10 L^{-1}) and tagged, weighed (g) and measured (cm). The 2004 replicate were individually marked with visible fluorescent alpha-numeric tags (Northwest Marine Technology) inserted immediately below the skin on the upper lateral surface near to the second dorsal fin on an area of pale coloured skin

and an ultraviolet torch was used to identify the tags. The 2006 replicate were tagged internally using Trovan Passive Integrated Transponder (PIT) tags, which were implanted into the peritoneal cavity. Procedures were carried out under a UK Home Office Animals (Scientific Procedures) license.

The experimental design consisted of three identical flow-through tanks (550 L), one of which contained 15 cod from each stock (mixed) and the other two contained 30 cod from one stock (pure). Cod were fed small-sized pelleted food and were fed to excess to give all fish the opportunity to feed. Temperature was kept at ambient and varied between 9.15 and 10.5 °C. Cod were measured fortnightly and were weighed and measured under anaesthetic and returned to the tank. During the experiment six fish died overall in both years, spread over both treatments and both populations. The slight reduction in density is unlikely to have had an effect on the growth of the remaining fish in the tank, as the difference in densities were far lower than that shown to affect growth (Lambert and Dutil, 2001).

2.1.3 Haemoglobin genotype

Haemoglobin genotype was determined only in the 2006 experiment. Fresh blood samples were collected from individuals upon sacrifice with an overdose of anaesthetic (MS-222, 1g 10 L^{-1}). Blood was taken from the caudal vein using a heparinised syringe. Haemoglobin genotype was determined by agar gel electrophoresis details of which are in Fyhn *et al.* (1994). In brief, 250 μl of blood was centrifuged at 3000 g, the plasma discarded and replaced by the same amount of distilled water. Samples were loaded on to an agar gel and dissolved in Smithies buffer diluted with 1:1 distilled water. Samples were electrophoresed for two hours and stained with Co-massie Brilliant Blue. The juveniles were grouped into three haemoglobin genotypes based on the electrophoretic pattern interpreted as two homozygotes (HbI-1*1 and HbI-2*2) and one heterozygote (HbI-1*2) (Figure 2.1).
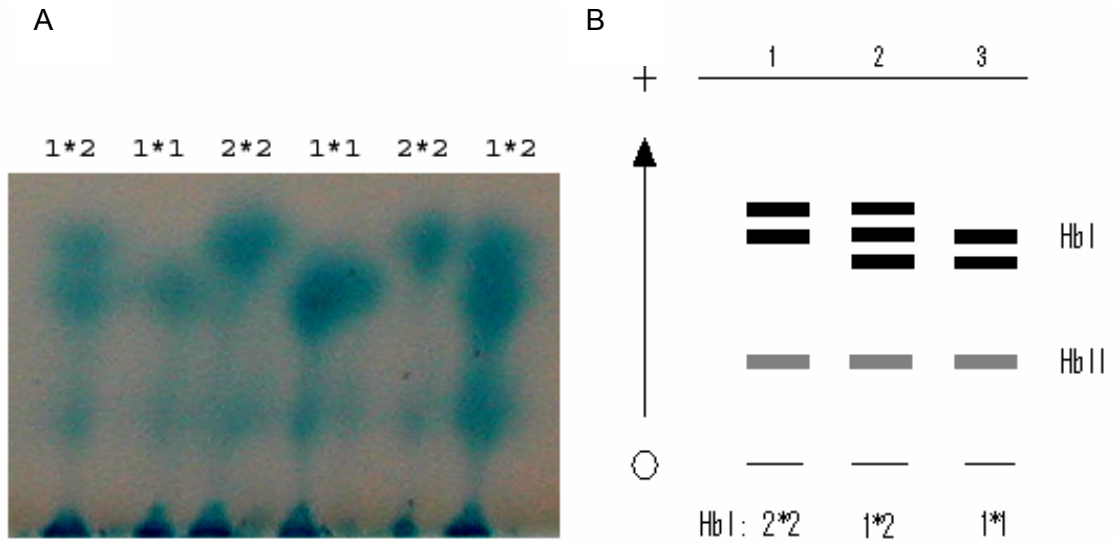
Figure 2.1. Cod haemoglobin patterns on an agar gel (A) and zymogram illustrating HbII and HbI loci (B) with three banding patterns at the HbI locus; 1*1, 1*2 and 2*2.

Genotype frequencies and numbers for each population for the 2006 replicate are given in Table 2.1. No significant difference in frequencies were found between populations (G-like test, $P = 0.706$, SE = 0.0027) or treatments ($P = 0.560$, SE = 0.0049).

Table 2.1. Haemoglobin numbers and frequencies by population.

Haemoglobin genotype	Population (frequencies and numbers)	
	Clyde Sea	St Andrews Bay
HbI-1*1	0.121, n = 4	0.181, n = 6
HbI-1*2	0.606, n = 20	0.545, n = 18
HbI-2*2	0.273, n = 9	0.273, n = 9

2.1.4 Data analysis

Individual daily weight-specific growth rates (G_s, % day^{-1}) were calculated after each two week period according to Jobling (1988) as,

$$G_s = (LN (W_2) - LN (W_1) / D) * 100,$$

where W_2 = final weight (g), W_1 = initial weight (g) and D = number of days.

Individual data points were excluded from fish that appeared to be in ill health (characterised by having growth of less than two standard deviations below the mean; one from the Clyde Sea in the mixed treatment in 2004 and the other from St Andrews Bay in the pure treatment in 2006). Data were checked for normality and homogeneity of variance assumptions and parametric or non-parametric techniques performed as appropriate. A General Linear Model with Tukey Simultaneous tests was used to test for differences between treatments with treatment and year as fixed effects. A Mann-Whitney test was performed on G_s in the mixed treatment and a Kruskal-Wallis Test to test for differences in initial length. Correlation between length and G_s was carried out using Pearson's Correlation. All analyses were carried out using the statistical software, Minitab and GenStat, 9th edition.

2.2   RESULTS

To ascertain the effect of mixing populations on specific growth rate (G_s), the G_s was measured on 160 fish from two populations replicated over two years in 2004 and 2006. There were differences in lengths between treatments at the start of the experiment (Table 2.2, Kruskal-Wallis Test, H = 29.0, d.f. = 2, $P < 0.001$), but differences were between populations (median for St Andrews Bay = 17.8 cm, median for Clyde Sea = 15.9 cm), rather than between treatments, and initial length did not have a significant effect on G_S (Pearson Correlation = -0.002, n = 145, $P = 0.979$).

Table 2. Daily weight-specific growth rate, G_s (% day^{-1}), initial length and numbers of cod (post-acclimation period) in either a pure or a mixed population treatment for experiments in both years (2004 and 2006).

Replicate	Treatment	Population	Initial numbers	Initial length (cm, mean $\pm$ 95% C.I.)	G_s (% day^{-1}) (mean $\pm$ 95% C.I.)
2004	Pure	St Andrews Bay	28	16.9 $\pm$ 0.5	0.38 $\pm$ 0.14
		Clyde Sea	26	15.8 $\pm$ 0.4	0.71 $\pm$ 0.19
	Mixed	St Andrews Bay	14	16.3 $\pm$ 0.7	0.45 $\pm$ 0.21
		Clyde Sea	14	15.7 $\pm$ 0.7	0.34 $\pm$ 0.17
2006	Pure	St Andrews Bay	27	19.1 $\pm$ 0.5	0.39 $\pm$ 0.10
		Clyde Sea	26	16.3 $\pm$ 0.5	0.83 $\pm$ 0.17
	Mixed	St Andrews Bay	14	19.6 $\pm$ 0.7	0.50 $\pm$ 0.15
		Clyde Sea	14	18.6 $\pm$ 0.9	0.40 $\pm$ 0.24

2.2.1 Treatment effects

G_s varied significantly with treatment for both the 2004 and the 2006 study (ANOVA, F_{2-65} = 4.96, P = 0.01 and F_{2-77} = 12.08, P < 0.001 respectively). No difference was found between years (F_{1-142} = 1.07, P = 0.303) and a significant effect of treatment was also found when both years were combined (F_{2-142} = 16.49, P < 0.001) (Table 2.3, Figure 2.2). Combining data from both years, in the pure treatment the Clyde Sea cod achieved a G_s of 0.78 % day^{-1} $\pm$ 0.13 (mean and 95 % C.I.), 52 % higher than the St Andrews Bay cod (0.40 $\pm$ 0.08) (Mann Whitney test, n = 93, W = 2573, P < 0.001, Table 2.3, Figure 2.2). In the mixed treatment the G_s for the Clyde Sea cod was 53.6 % lower than when in the pure treatment (0.78 % day^{-1} $\pm$ 0.25 and 0.42 $\pm$ 0.12 respectively). There was no significant difference in G_s between cod from the different sites in the mixed tank (Mann-Whitney test, W = 558, N = 50, P = 0.596. Nor were there any significant differences between the growth rate of St Andrews Bay cod in the pure and the mixed tank (t = -0.715, P = 0.755).

Table 2.3. Results of testing for differences in G_s (% day^{-1}) between mixed and pure treatments for both years combined, using ANOVA and Tukey simultaneous tests.

Source of variation	Difference of Means	SE of difference	·Value	*P*
Pure Clyde – mixed	-0.33	0.07	-4.66	<0.001
Pure Clyde – pure St Andrews	-0.38	0.07	-5.36	<0.001
Pure St Andrews – mixed	-0.05	0.07	-0.71	0.760
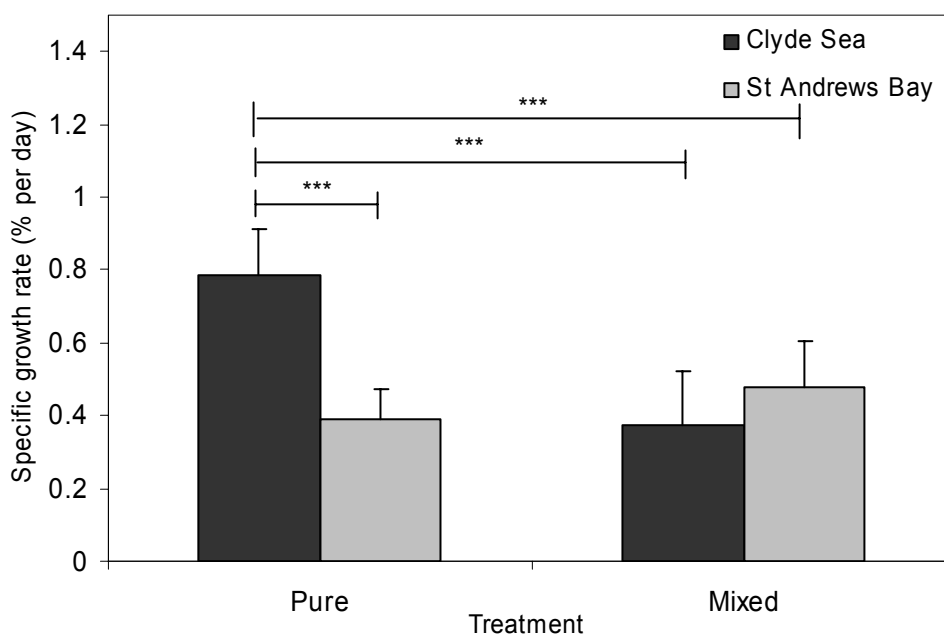
Figure 2.2. G_s (% day^{-1}) (mean $\pm$ 95 % C.I.) for pure and mixed treatments for Clyde Sea and St Andrews Bay populations. Results are combined for two studies carried out once in December 2004 and secondly in February 2006 (*** signifies *P* < 0.001, no line signifies non-significance between populations or treatments).

2.2.2 Haemoglobin effects

G_s of fish of different haemoglobin genotypes in the pure treatments are illustrated in Figure 2.3. No significant effect was found of haemoglobin genotype on the G_s once population of origin was taken into account (ANOVA, $F_{1-65} = 0.65$, *P* = 0.528) and no interaction was found.
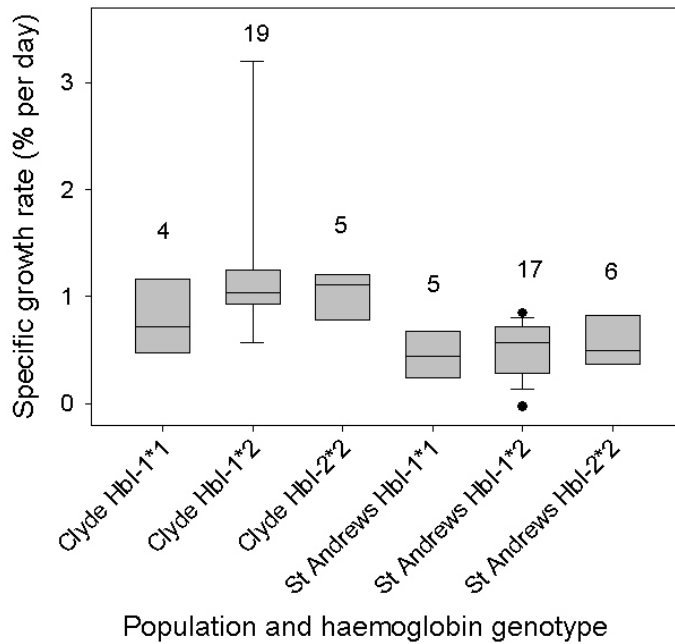
Figure 2.3. Box plots (mean, 95th and 5th percentiles) of G_s (% day^{-1}) for haemoglobin genotypes of populations from the pure treatment only.

3. EXPERIMENT 2. Behavioural interactions in pairs of juvenile cod

The aim here was to understand the mechanisms that might generate the differences in growth rates described in experiment 1 by direct observations of pair-wise interactions. Two indicators of competition were used; aggression and competition for food in mixed and pure population size-matched pairs. The experiment was balanced for population and not genotype, as it was only possible to assign haemoglobin genotype to the cod retrospectively.

The specific questions to be addressed were:

- What is the nature of the social interactions between juvenile cod and how much do they vary in nature?

- Are any differences in aggression due to population or genotype?

- Are there differences in food intake and can this be predicted by the aggressive interactions?

- How do population and genotype influence food intake?

3.1 MATERIALS AND METHODS

3.1.1 Experimental design

Fish from the 2005 stock were selected in size-matched pairs (maximum difference of 2.2 cm and mean and SE of difference of 0.9 ± 0.1 cm, for fish of a mean size of 25.5 ± 0.4 cm). 27 trials were performed. 15 of these were from the same population and 12 from different populations. Fish were marked with visible implant elastomer tags (Northwest Marine Technology) on their cheeks under anaesthetic (MS-222). These could be read and identified while in the tank under the UV light. Each fish was identifiable by a unique combination or positioning of colour markings. Experiments were carried out in April and May 2006 on 1-group cod in three glass tanks of equal dimensions (76 cm x 45 cm x 40 cm, 137 L). The UV light was set on ambient photoperiod for 57°16.7' N and provided a low level of lighting to imitate conditions at depth. The fish were illuminated from above and the observer stood at a distance of approximately 50 cm away in a darkened room. No reaction to the experimenter was observed during the trials. Temperature was maintained at between 7.9 and 8.5 ℃ (mean and SEM, 8.3 ± 0.0). The tank was split into two using an opaque, PVC removable separator in the centre of each tank. Two pieces of plastic drain pipe, one on either side of the separator, provided a shelter for each fish.

Two fish were transferred into the tanks, one on each side of the separator. The fish were either both from the same population (pure) or from different populations (mixed). An acclimation period of 48 hours followed, which was considered ample time for recovery. Experiments on recovery after handling juvenile cod using opercula beat report that only 1.5 hours is needed to recover from handling stress (Artigas *et al.*, 2005). Social interactions were observed first, as the majority of the interactions

occurred immediately upon exposure of the fish to each other. The foraging experiment was performed two days later, at which time the fish would take food readily. The haemoglobin genotype could not be determined until after the fish were sacrificed, so genotype combination of pairs could not be controlled for.

3.1.2 Social interactions

The plastic separator was carefully removed from the tank to allow both fish access to the whole tank. Fish were observed for a 20 minute period and interactions noted. During this period aggressive encounters were observed. In most cases aggressive encounters ceased after 20 to 40 minutes. The observer stood at a distance of approximately 50 cm from the tank. No reaction to the observer was noted, nor did the fish hesitate to feed in the presence of the observer. Four types of interaction were observed; obstruction (whereby the initiator swam towards the receiver and the receiver moved to avoid the initiator), touch (similar to obstruction, but the receiver did not move to avoid the initiator and was touched by it), slow chase (a chase at a slow speed), fast chase (a chase involving a dart around or up and down the tank). These were combined for analysis to give the total number of aggressive interactions, which gave an indicator of intensity. The initiator and the recipient were recorded in each case.

3.1.3 Feeding trials

The cod were fed ten pellets from a distance of 50 cm. Pellets were selected rather than a more natural food because the cod had already been acclimated to pellets and so they would be instantly recognized as food. The order in which food was given to each of the three tanks was randomized, as was the position of the food thrown in to the tank with respect to the position of the fish. This minimized the effect of prior residence, i.e. whereby an individual that has been in a specific area of the tank, has a competitive advantage over another individual subsequently entering that area (Huntingford and Turner, 1987; Krebs and Davies, 1987; Metcalfe *et al.*, 2003). Food was introduced one pellet at a time and only when a pellet had been eaten was another one introduced. The first fish to consume the pellet was recorded, even if it subsequently rejected it. For each fish the prey share (proportion of total pellets eaten) and the order in which prey were taken were recorded. Three feeding trials were performed on the first 10 pairs with a 48

hour period in between trials. A significant concordance between individual scores in the three trials was found (Kendall's coefficient of Concordance, χ^2 = 38.5, d.f. = 19, P = 0.005), and so only one feeding trial was conducted on the remaining 17 samples. After each trial the fish were given another 5 g of food to ensure that they were not food deprived while in the experimental tanks.

3.1.4 Haemoglobin genotyping

At the end of the pair-wise trial, the fish were transferred to another tank and sacrificed to enable sex determination and screening for haemoglobin genotype. The genotype frequencies within the samples of this study (determined as described above) are given in Table 2.4, and no population differences in genotype frequencies were found for the samples in this experiment (Genepop, G-like test, P = 0.305, SE = 0.0036). The numbers of trials for each genotype combination are given in Table 2.5.

Table 2.4. Frequencies and numbers of haemoglobin genotypes by population.

Haemoglobin genotype	Population (frequencies and numbers)	
	Clyde	St Andrews
HbI-1*1	0.0796, n = 2	0.25, n = 7
HbI-1*2	0.654, n = 17	0.50, n = 14
HbI-2*2	0.269, n = 7	0.25, n = 7

Table 2.5. Numbers of trials by haemoglobin genotype combination.

Haemoglobin genotype combinations		Number of trials
Pure	HbI-1*1 : HbI-1*1	1
	HbI-1*2 : HbI-1*2	9
	HbI-2*2 : HbI-2*2	2
Mixed	HbI-1*1 : HbI-1*2	3
	HbI-1*1 : HbI-2*2	4
	HbI-1*2 : HbI-2*2	7

3.1.5 Data analysis

No correlation was found between individual frequencies of fast chase, slow chase and obstruction, which indicated that these were measuring different aspects of the

interaction, so all were included in subsequent analyses. There was however, a correlation between obstructions and touches (Pearson Correlation: 0.385, $P = 0.004$), so these were combined and labelled as 'obstructions'. The total number of interactions for each fish were summed together and used to calculate the following scores:

Proportionate initiation = $\dfrac{\text{attacks initiated}}{\text{attacks initiated + attacks received}}$

Attacks initiated = $\sum$ (all attack types initiated)

Differential initiation = $\sum$ (attacks initiated) - $\sum$ (attacks received)

Total attacks = $\sum$ (all attacks initiated from both individuals)

Polarisation = 'differential initiation' in winners – 'differential initiation' in losers

Analyses were conducted to determine the influence of fish size on the behaviour, using: individual weights and lengths of each fish, mean weights and lengths of both fish in the pair, weight and length differentials between individuals in the pair and condition factor (Fulton's K), according to the following equations: -

Weight differential = $\dfrac{\text{weight of larger fish – weight of smaller fish}}{\text{weight of smaller fish}}$

Fulton's condition factor (K) = $100\ W\ L^{-3}$,

where K is condition factor, W is weight and L is length.

Data were checked for normality and homogeneity of variances and analysed by parametric or non-parametric techniques as appropriate, using the programme Genstat.

3.1.6 Gender

Gender could not be controlled for because it could only be determined upon sacrifice. However, no differences in behaviour were found according to sex within the pair (Table 2.6) and between the combinations of sexes in the pair, so sex was excluded from later analyses.

Table 2.6. Aggression scores and prey share by sex of individuals within a pair for 11 trials.

Score / measure	Aggression score or prey share (mean and 95% C.I. for t-test, medians for Wilcoxon tests, proportion for chi-squared)		Test	d.f.	Significance
	Female	Male			
Attacks initiated	2	6	Wilcoxon matched-pairs test	10	$W = 15.5$, $P = 0.270$
Differential initiation	-4.0 ± 5.8	4.0 ± 5.8	Paired t-test	10	$t = -1.74$, $P = 0.113$
Prey share for big eaters (%)	70	40	Wilcoxon matched-pairs test	10	$W = 17$, $P = 0.316$
Proportion of winners in aggressive encounters	0.3	0.7	Pearson's Chi-squared	1	$\chi^2 = 1.6$, $P = 0.206$

3.2. RESULTS

Aggressive encounters

3.2.1. Variability in aggressive encounters

Histograms of the frequencies of social interactions are given in Figure 2.4 A-D. All variables were highly skewed, with some fish showing no interactions but some showing many. There was also high variability in levels of aggressive interactions (Figure 2.4 E).
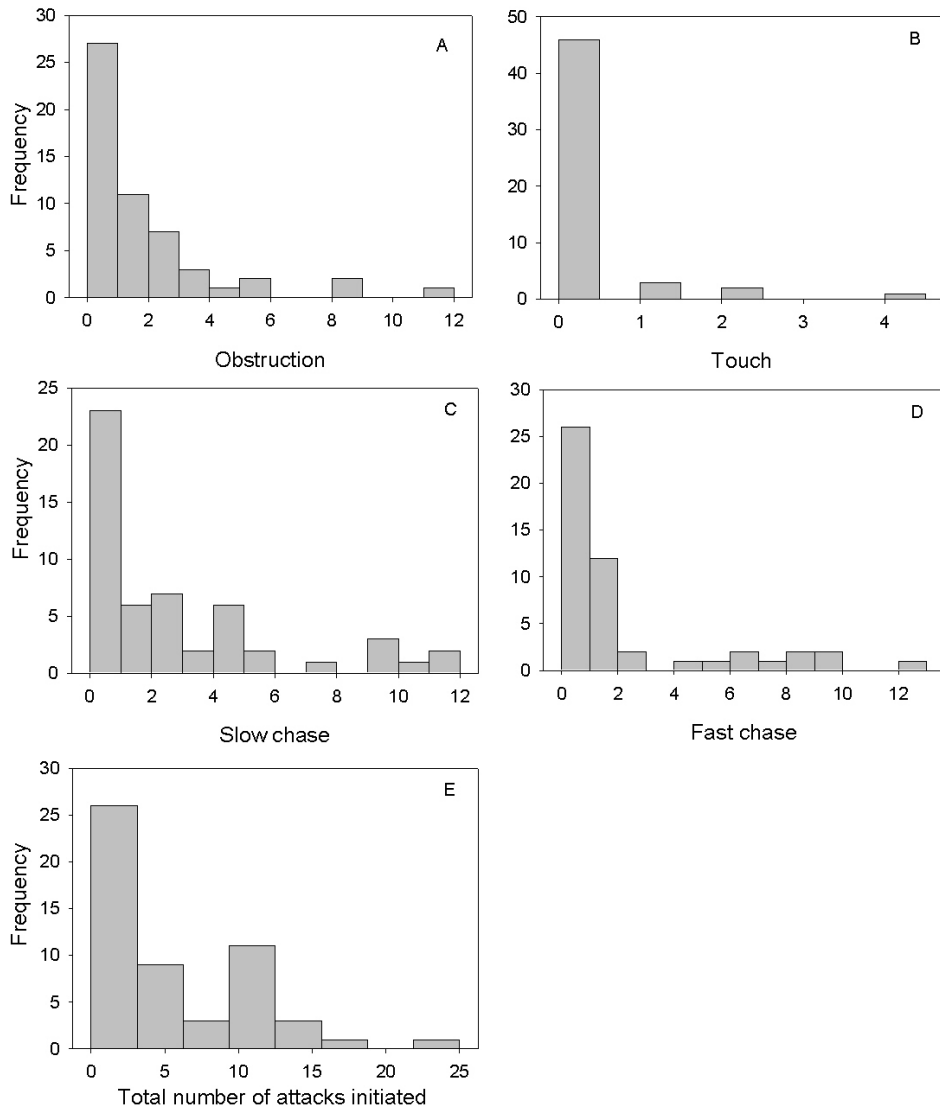
Figure 2.4. Histograms of frequency of types of behavioural observations: obstructions (A), touches (B), slow chases (C) and fast chases (darts) (D), and total number of attacks initiated by both fish in each pair (E).

Within pairs, the aggressive interactions were for the most part highly polarized, with one individual in the pair initiating most of the attacks. The proportionate initiation score shows polarisation between the higher scoring and lower scoring trials in the aggressive encounters (Figure 2.5). On this basis the individuals could be classed as winner or loser according to their aggressive interactions (Table 2.7). Two types of draw were identified; where neither fish attacked or where both fish attacked equally. In cases of the latter, the winner and loser were defined according to the type of interaction, whereby a chase was considered more aggressive than an obstruction.
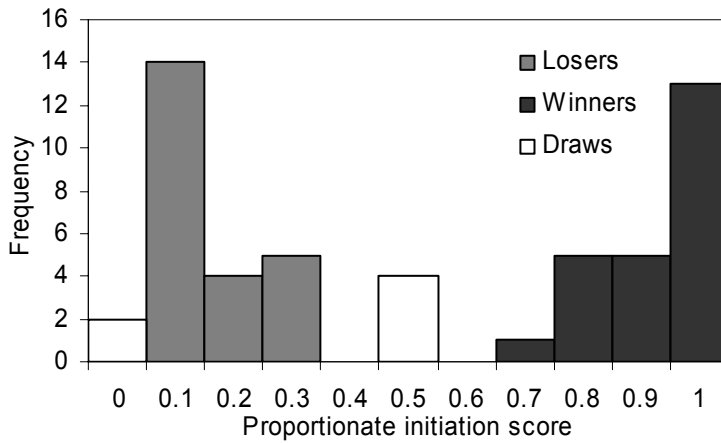
Figure 2.5. Histogram of proportionate initiation scores for all fish by winners, losers and draws.

Table 2.7. Aggressive encounter scores for cod defined as winners and losers on the basis of their proportionate initiation score), for n = 26 pairs (median, mean and 95 % C.I.).

Scores	Aggressive encounter scores (median for Wilcoxon test or mean & 95 % C.I. for t-test)		Tests	Significance
	Winners	Losers		
Attacks initiated score	10	1	Wilcoxon Matched-Pairs test	W = 0, *P* < 0.001
Differential initiation score	7.7 ± 2.1	-7.4 ± 2.1	Paired t-test	t = -7.14, *P* < 0.001
Proportionate initiation score	0.91	0.09	Wilcoxon Matched-Pairs test	W = 0, *P* < 0.001

3.2.2 Role of size difference in determining the nature of the interactions

Relationships between mean size of the pair of fish and the size differential within the pair were investigated using the total number of attacks and the degree of polarisation in attacks between the individuals. Differences in weights and lengths between winners and losers of the aggressive encounters were also checked.

3.2.3 Effect of size within a pair in determining aggressive encounters

No significant relationship between weight differential of fish within a pair and total attacks of both fish was found (F_{1-26} = 2.89, P = 0.103). Nor were there relationships between length differential and total attacks (F_{1-26} = 0.06, P = 0.811) or between condition factor (K) and total attacks (F_{1-26} = 0.27, P = 0.608). There was also no difference in weight (median weights were 143.6 g and 131.0 g for winners and losers respectively, Wilcoxon matched pairs test, n = 24, t = 126, P = 0.901) or length between winners and losers within a pair (median lengths were 24.3 cm for both winners and losers respectively, Wilcoxon matched pairs test, n = 24, t = 118, P = 0.288). Therefore there is no evidence to suggest that, within the small range of size differences used here, size of individuals within a pair had an effect on the outcome of the trial.

3.2.4 Effect of size of individuals between and within pairs on the intensity of the attack

A significant negative regression was found between the mean length of both fish in the pair with the total number of attacks (Figure 2.6A, Table 2.8) and the polarisation score (Figure 2.6B, Table 2.8) (F_{1-25} = 5.88, P = 0.023, r^2 = 0.197 and F_{1-25} = 8.29, P = 0.008, r^2 = 0.257 respectively). Pairs of a smaller size tended to have more fierce fights than pairs of a larger size and there was a greater difference between the winning and losing individual in smaller sized pairs. A marginally significant negative relationship was also found between condition (Fulton's K) and total number of attacks (F_{1-25} = 4.00, P = 0.057, r^2 = 0.143); pairs of a higher condition (Fulton's K) had less fierce fights.
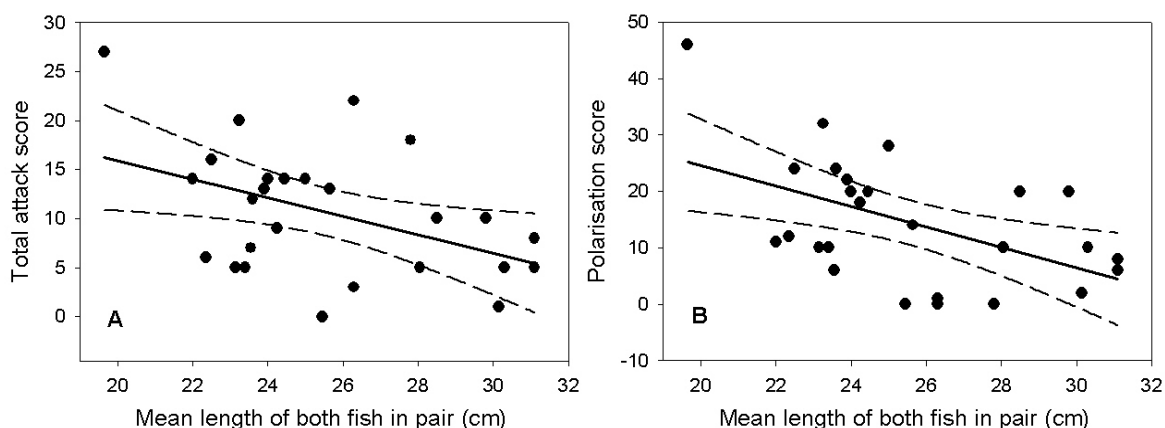
Figure 2.6. Relationship between (A) mean length of both fish in the pair and total attack score (regression equation: total attacks of both fish = 35.9 - 0.982 mean length of both fish) and (B) mean length of both fish in the pair and polarisation score (regression equation: mean length of both fish = 27.6 - 0.142 polarisation score). Significance is given in Table 2.8.

Table 2.8. Relationships between mean length of both fish in the pair and mean condition factor (Fulton's K) against total attack score and polarisation score, and between length differential within pairs against total attack and polarisation score. Slopes are given in Figure 2.6 where significant.

Regressions between:	Slope	d.f.	P (ANOVA)
Mean length and total attack score	-0.209	1-25	$F = 5.88$, $P = 0.023$, $r^2 = 0.197$
Mean length and polarisation score	0.142	1-25	$F = 8.29$, $P = 0.008$, $r^2 = 0.257$
Condition factor and total attack score	0.00016	1-25	$F = 0.01$, $P = 0.928$, $r^2 = 0.0$
Condition factor and polarisation score	-0.00188	1-25	$F = 4.00$, $P = 0.057$, $r^2 = 0.143$
Length differential and total attack score	-0.000449	1-25	$F = 0.34$, $P = 0.565$, $r^2 = 0.014$
Length differential and polarisation score	0.000150	1-25	$F = 0.11$, $P = 0.747$, $r^2 = 0.004$

No significant relationship was found in the regression between the length differential and the total attack or polarisation score (Table 2.8). Therefore the degree of variance in the aggression appears to be dependent on the absolute size rather than the size difference between individuals within the pair.

Feeding trials

3.2.5 Prey share

The prey share (proportion of delivered pellets eaten by each fish) differs significantly from the 50 % expected if fish were eating equally (t-test, t = 11.3, d.f. = 25, $P < 0.001$). Thus individuals within the pair did take a significantly different proportion of the food given (Figure 2.7). This polarisation in prey share taken between individuals was used to identify the big eaters (> 50 % taken) from the little eaters (< 50 % taken). No aggression was observed during the feeding trials.
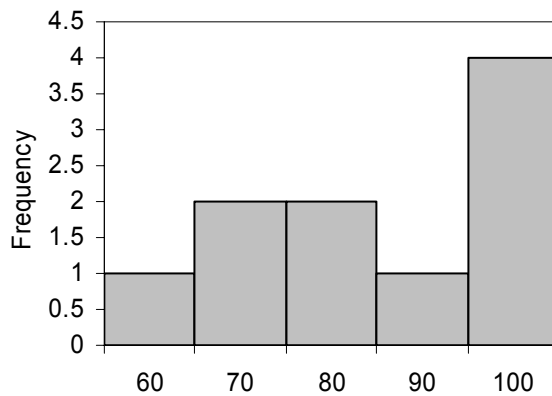
Figure 2.7. Histogram of percent of pellets taken (10 pellets per pair given) by the big eater only.

3.2.6 Feeding dynamics of each trial

In each feeding trial 10 pellets were fed to the pair sequentially and the individual that fed on each was recorded. The total number of pellets consumed by the big and the little eater summed over all the trials is illustrated in Figure 2.8. The big eater tended to take the majority of pellets introduced throughout the trial. The little eater tended to feed more towards the latter end of the trial than at the beginning.

Figure 2.8. Total number of pellets consumed by the big eaters in all the trials (n = 26), showing data separately for each pellet in the sequence one to ten, dotted line (-----) indicates an equal number of pellets consumed.

3.2.7 Predictors of prey share

There was no difference in weight, length or condition factor (Fulton's K) between the big and little eater in each pair (Table 2.9). Additionally, since there was no effect of weight of the big eater on the proportion of food taken (ANOVA, $F_{1-25} = 0.25$, $P = 0.625$), there is no evidence to suggest that smaller fish were hungrier than larger ones.

Table 2.9. Size (weight, length and condition factor, Fulton's K) in big and little eaters.

Test	Median of big eaters	Median of little eaters	Wilcoxon matched pairs test (W) and n	P
Weight (g)	131	147	W = 125, n = 26	0.208
Length (cm)	24.9	24.9	W = 116, n = 24	0.343
Condition factor	0.88	0.94	W = 106, n = 26	0.080

No significant difference was found in the prey share between winners and losers of the aggression trials (median percent eaten was 60.0 for both the winner and loser, Mann Whitney test, $W = 639$, $P = 0.992$) and thus aggression does not predict prey share.

Population differences

3.2.8 Aggressive encounters

No differences in aggressive encounter scores according to population treatment were found (Table 2.10). Nor were there differences in aggression scores between populations in trials of mixed population types (Table 2.11). No significant interactions were found.

Table 2.10. Aggressive encounter scores (mean and 95 % C.I.) by population treatment, with significance (ANCOVA with length as covariate). Draws have been excluded.

Score	Scores by population treatment (n, mean score and 95 % C.I., adjusted for covariate)			d.f.	*P*-value of covariate (length)	*P*-value of treatment
	Clyde : Clyde	Clyde : St Andrews	St Andrews : St Andrews			
Attacks initiated for winners	n = 7, 9.8 $\pm$ 3.4	n = 11, 9.8 $\pm$ 2.7	n = 8, 8.8 $\pm$ 3.1	2,25	0.003	0.867
Differential initiation for winners	n = 7, 7.9 $\pm$ 3.5	n = 11, 7.8 $\pm$ 2.8	n = 8, 7.4 $\pm$ 3.2	2,25	0.004	0.975
Polarisation score for each pair	n = 7, 14.3 $\pm$ 7.6	N = 12, 14.4 $\pm$ 5.8	n = 8, 14.8 $\pm$ 7.0	2,26	0.012	0.994
Total attack score	n = 7,11.7 $\pm$ 4.7	n = 12, 10.8 $\pm$ 3.6	n = 8, 10.2 $\pm$ 4.3	2,26	0.020	0.900

Table 2.11. Aggressive encounter scores for individuals in the mixed population trials (means and 95 % C.I. with significance) for 11 trials (draws excluded).

Score (winners only)	Mean score and 95 % C.I. (in paired t-test), median (in Wilcoxon test) and n (in Chi-squared test) by population		Test	d.f.	Significance
	Clyde	St Andrews			
Attacks initiated	2	9	Wilcoxon matched-pairs	10	W = 16, *P* = 0.287
Differential initiation	-4.6 $\pm$ 6.2	4.7 $\pm$ 6.2	Paired t-test	10	t = -1.46, *P* = 0.176
Number of wins	4	7	Pearson's Chi-squared	1	χ^2 = 0.820, *P* = 0.366

3.2.9 Feeding

In the mixed population trials, a chi-squared test was conducted on the association between population and the number of trials in which the fish obtained a larger or a smaller prey share.

Table 2.12. Proportions of big and little eaters in the prey share trials (mixed populations) with significance using a Pearson's Chi squared test.

Foraging test	Population	Proportion of big eaters	n	Significance
Prey share	Clyde	0.818	9	χ^2 = 4.55, d.f. = 1, P = 0.0329
	St Andrews	0.182	2	

The Clyde Sea cod ate a significantly higher proportion of the food in more of the trials than the St Andrews Bay cod (Pearson's Chi squared, χ^2 = 4.55, d.f. = 1, P = 0.033). No effect of the combination in the trial (Clyde vs. Clyde, St Andrews vs. St Andrews or St Andrews vs. Clyde) on the prey share of the big eater was found (Kruskal-Wallis Test, H = 0.0, d.f. = 2, P = 1.00).

The performance of individuals from each population, according to the proportion of aggressive encounters won and the proportion of big eaters is illustrated in Figure 2.9. No link between aggression and prey share was found.
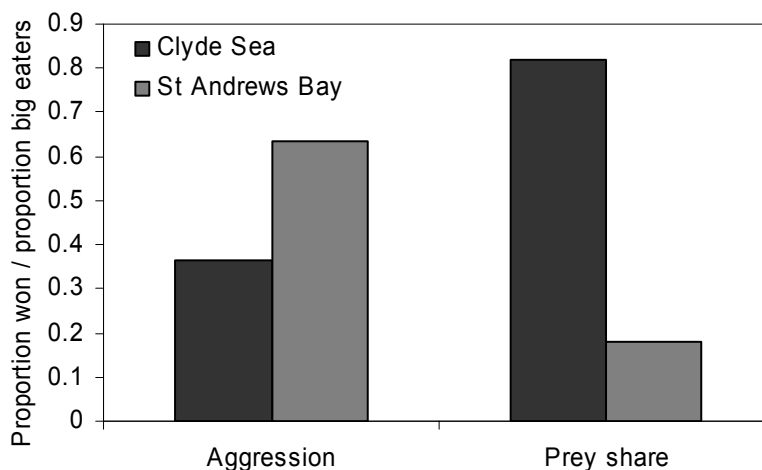
Figure 2.9. Performance of individuals (in mixed population trials) from each population according to proportion of aggressive encounters won and proportion of big eaters in prey share trials (n =11 trials).

Haemoglobin genotypes

3.2.10 Comparison across haemoglobin genotype combination of pairs

Differences in aggressive encounter scores between pairs of haemoglobin genotype combinations are illustrated in Figure 2.10 and given in Table 2.13. A marginally significant difference was found using the differential initiation score (ANCOVA, F_{1-24} = 2.67, P = 0.064), although sample numbers were low and significance values should merely illustrate a trend. These differences were found between HbI-2*2 : HbI-2*2 and HbI-1*2 : HbI-2*2 (Tukey simultaneous tests, T = -2.81, P = 0.074), HbI-2*2 : HbI-2*2 and HbI-1*1 : HbI-2*2 (T = -2.74, P = 0.0847) and HbI-2*2 : HbI-2*2 and HbI-1*1 : HbI-1*2 (T = -2.72, P = 0.088).
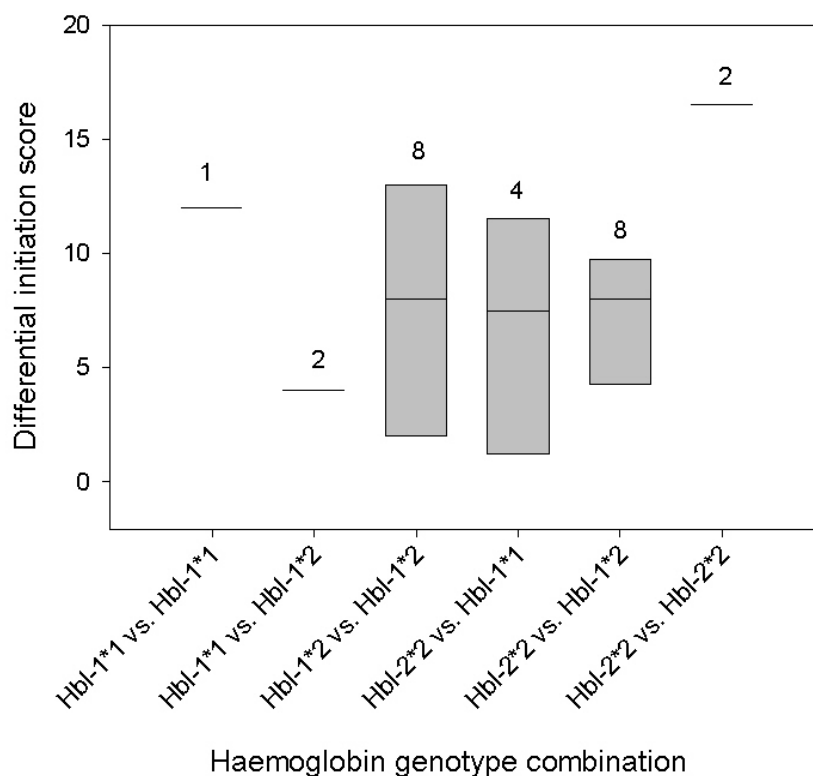
Figure 2.10. Boxplots of differential initiation scores (mean, 95th and 5th percentiles) for the winners according to haemoglobin combinations of the pair.

Table 2.13. Aggressive encounter scores for haemoglobin genotype combinations (mean, n and results of ANCOVAs with length as covariate). HbI-1*1 against HbI-1*1 has been excluded from the analysis due to low sample size. A Box-Cox transformation was used on the attacks initiated score.

Score	Scores (mean values and 95 % C.I. of mean adjusted for covariate and n)					d.f.	Signific - ance of treatment	Signifc - ance of covariate
	HbI-2*2 : HbI-2*2	HbI-2*2 : HbI-1*2	HbI-2*2 : HbI-1*1	HbI-1*2 : HbI-1*2	HbI-1*2 : HbI-1*1			
Attacks initiated (winners)	2.6, n = 2	2.0 ± 0.2, n = 8	2.1 ± 0.3, n = 4	1.9 ± 0.2, n = 8	2.1± 0.4, n = 2	1-24	F = 5.06, P = 0.716	F = 0.53, P = 0.037
Differential initiation (winners)	15.3, n = 2	6.4 ± 1.4, n = 8	5.8± 2.0, n = 4	8.6 ± 1.4, n = 8	5.0 ± 2.4, n = 3	1-24	F = 2.67, P = 0.064	F = 10.2, P = 0.005
Total attacks of both fish in the pair	17.1, n = 2	9.7 ± 2.2, n = 8	11.5 ± 3.1, n = 4	8.8 ± 2.1, n = 9	13.4 ± 3.7, n = 3	1-25	F = 0.86, P = 0.527	F = 4.04, P = 0.059
Polarisation	30.7, n = 2	11.6 ± 3.3, n = 7	12.2 ± 4.6, n = 4	14.9 ± 3.0, n = 9	9.7 ± 5.5, n = 3	1,24	F = 1.58, P = 0.213	F = 4.89, P = 0.039

3.2.11 Genotype effects of aggressive encounters in the mixed genotype groups

Differences in aggressive encounter scores according to haemoglobin genotype in the mixed genotype groups were investigated (Table 2.14, Figure 2.11). A significant difference in the numbers of aggression trials won or lost was shown (Table 2.14, Figure 2.11, χ^2 = 7.97, d.f. = 2, P = 0.019). Within the winners (those of the HbI-2*2 and HbI-1*2 genotypes), there was a marginally significant effect of haemoglobin genotype on the number of attacks initiated (median score for HbI-2*2 = 10, HbI-1*2 = 5, Mann Whitney U test, U = 4.5, P = 0.077).

Table 2.14. Aggressive encounter scores (median) for individual genotypes in the mixed genotype groups with significance (Mann-Whitney U test) or numbers of trials won with significance (Pearson's Chi-squared test). N.B. Those of the HbI-1*1 genotype did not win any of the aggressive encounter trials.

Score	Medians of scores or numbers won			Test	Significance of main factor
	HbI-2*2	HbI-1*2	HbI-1*1		
Attacks initiated (winners only)	10.0	5.0	-	Mann-Whitney U test	$U = 4.5$, $P = 0.077$
Differential initiation (winners only)	10	5.0	-	Mann-Whitney U test	$U = 6.5$, $P = 0.132$
Proportionate initiation	0.86	0.80	-	Mann-Whitney U test	$U = 15$, $P = 0.846$
Numbers won	11	3	0	Pearson's Chi-squared	$\chi^2 = 7.97$, d.f. = 2, $P = 0.019$
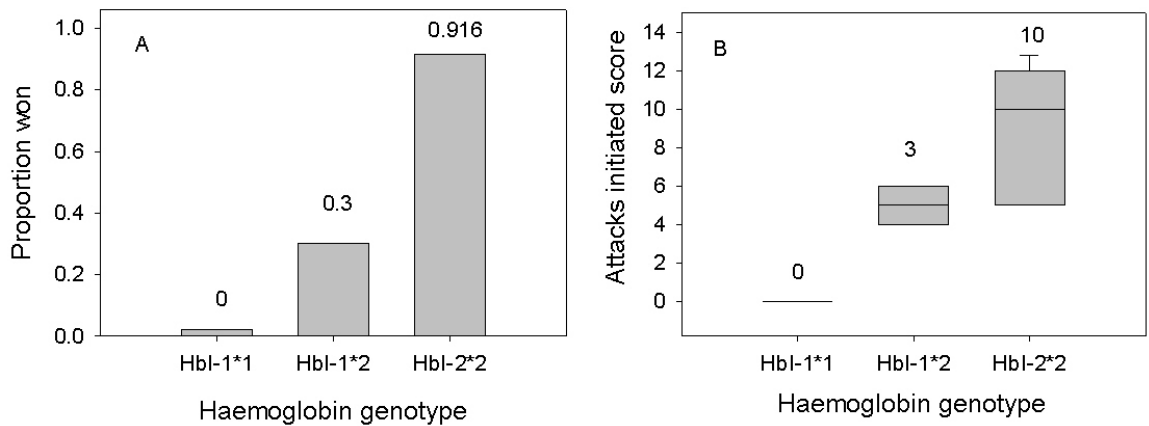
Figure 2.11. Proportion of aggression trials won by haemoglobin genotypes in mixed genotype trials with proportions above bars (A) and boxplots of attacks initiated score for haemoglobin genotypes in mixed genotype trials for the winners, showing means and 95th and 5th percentiles (B) and numbers above bars.

3.2.12 Differences between mixed and pure haemoglobin genotype pairs

Since differences between aggressive encounter scores were weak when accounting for individual combinations of genotype within a pair (Table 2.14, Figure 2.11), an analysis was performed with all the mixed genotype trials and all the pure trials grouped together (Table 2.15, Figure 2.12). A higher level of aggression was found in pure genotype pairs than in mixed when using the differential initiation score (ANCOVA, $F_{1-25} = 5.17$, $P = 0.033$). Also interactions between fish of the same genotype trials were marginally

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more polarized in their aggression than when mixed (F_{1-26} = 3.37, P = 0.079). However, this result could have been influenced by the HbI-1*2 : HbI-1*2 combination of which there were 9 trials, compared to only one case of the HbI-1*1 : HbI-1*1 and HbI-2*2 : HbI-2*2 combinations. This effect was still present upon removal of the pure HbI-1*1 combination (F_{1-23} = 4.75, P = 0.041) but not after removing the HbI-2*2 : HbI-2*2 combination (F_{1-22} = 1.63, P = 0.153). Therefore the result is due to a combination of the HbI-1*2 and HbI-2*2 pure combinations.

Table 2.15. Differences in aggressive encounter scores between mixed and pure genotype scores (n, mean and SEM or median depending on test) with significance of treatment and covariate (length) in ANCOVA.

Score	Score by haemoglobin genotype combination, (n, mean and SEM adjusted for covariate, or median for Mann Whitney U test)		Test	d.f.	Significance
	Mixed	Pure			
Attacks initiated (winners)	n = 14, 9	n = 11, 10	Mann-Whitney U test	n/a	W = 167, P = 0.412
Differential initiation (winners)	n = 15, 6.4 ± 1.1	n = 11, 10.1 ± 1.2	ANCOVA (length as covariate)	1-25	Covariate: F = 15.40, P < 0.001, treatment: F = 5.17, P = 0.033
Polarisation between both fish in pair	n = 15, 11.6 ± 2.4	n = 12, 18.2 ± 2.7	ANCOVA (mean length of both fish as covariate	1-26	Covariate: F = 9.94, P = 0.004, treatment: F = 3.37, P = 0.079
Total attacks score for both fish	n = 15, 11.0 ± 1.6	n = 12, 10.6 ± 1.8	ANCOVA (mean length of both fish as covariate	1-26	Covariate: F = 6.58, P = 0.017, treatment: F = 0.03, P = 0.865
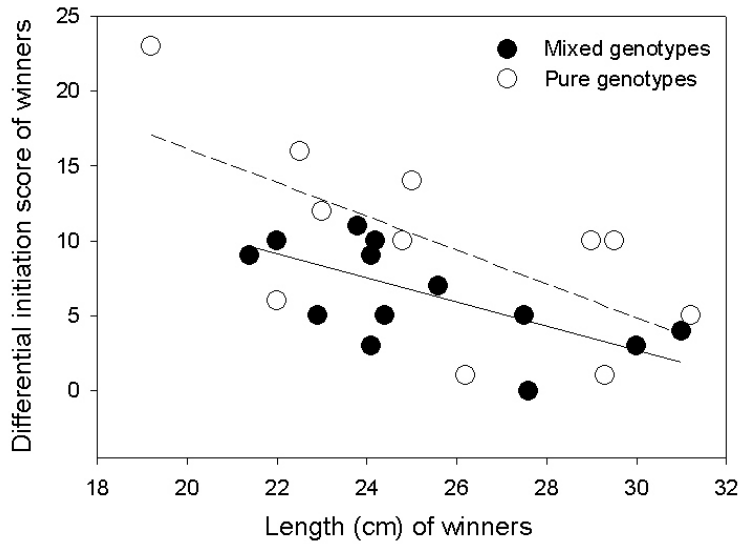
Figure 2.12. Differential initiation score against length by pure or mixed haemoglobin genotype combinations, for winners only. Significance (ANCOVA) given in section 3.2.12.

Feeding trials and haemoglobin genotype

Prey share was examined for differences between pairs of different haemoglobin genotypes combinations and individuals of different haemoglobin genotypes.

3.2.13 Prey share

Unlike in the observations of aggression, no significant difference between haemoglobin genotypes was found in mixed haemoglobin genotype trials with respect to actual prey share, nor the proportion of times a genotype did take a higher prey share (Figure 2.13, Table 2.16).
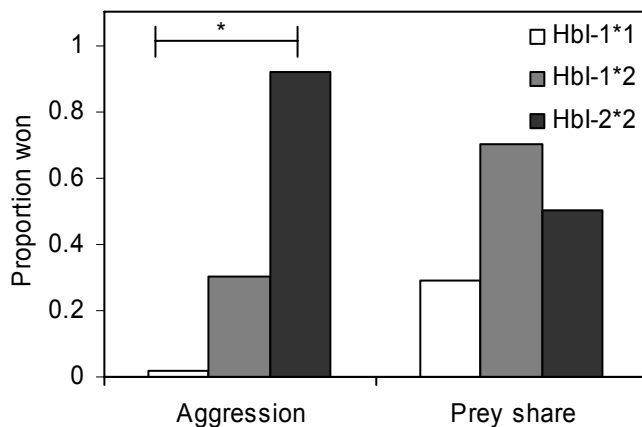
Figure 2.13. Proportion of trials won by different haemoglobin genotypes in the mixed genotype trials according to aggression and prey share (* = $P < 0.05$).

Table 2.16. Prey share of big eaters (proportion of pellets eaten in a trial) and proportion of occasions in which an individual is the big eater by haemoglobin genotype (mixed haemoglobin genotype trials only). Chi-squared test was performed on numbers of times a genotype was the big eater.

Score	n and mean score or proportion			Test	d.f.	Significance
	HbI-1*1	HbI-1*2	HbI-2*2			
Prey share of big eaters	n = 3, 0.6	n = 16, 0.9	n = 7, 0.9	Kruskal-Wallis	2	H = 3.76, 0.153
Proportion of times big eater	0.29	0.70	0.50	Pearson's Chi-squared	2	χ^2 = 1.49, P = 0.475

3.2.14 Differences in prey share according to haemoglobin genotype

A significant difference in prey share was found between mixed and pure genotype trials (F_{1-10} = 5.88, P = 0.038). The winning individuals in the mixed groups took a larger prey share than those from the pure groups.

3.2.15 Genotype and population interactions

The interaction between genotype and population was investigated at the level of the individuals of each pair (Tables 2.17, 2.18 and 2.19). When both individuals had the same genotype but were of different populations, the St Andrews Bay population won on 5 out of 5 occasions (Table 2.17, exact binomial test, z = 2.236, P = 0.0253). When both individuals were matched for population but were of different genotypes, the individual with more of the HbI^2 allele won on 7 out of 8 occasions (Table 2.18, z = 2.121, P = 0.0339). When both genotype and population were mixed in the pair, the genotype effect was stronger than the population effect (i.e. the stronger genotype would win over the stronger population), in 4 out of 4 cases (Table 2.19, z = 2.0, P = 0.0455).

Table 2.17. Outcome of aggression trials when haemoglobin genotypes are matched but populations are mixed.

Haemoglobin genotypes of cod in pair	Number of winners of a fight		Number of pairs	Exact binomial test
	St Andrews	Clyde		
HbI-2*2	1	0	1	
HbI-1*2	3	0	3	
HbI-1*1	1	0	1	
Totals	**St Andrews** > **Clyde**		**5**	**z = 2.236, P =**
	5	**0**		**0.0253**

Table 2.18. Outcome of aggression trials when populations are matched but haemoglobin genotypes are mixed.

Population of cod in pair	Number of winners of a fight			Number of pairs	Exact binomial test
	HbI-2*2	HbI-1*2	HbI-1*1		
St Andrews	1	-	0	1	
St Andrews	2	0	-	2	
St Andrews	-	1	0	1	
Clyde	1	1	-	2	
Clyde	2	-	0	2	
Totals	**Greater HbI^2 > lesser HbI^2**			**8**	**z = 2.121, _P_ =**
	7	**1**			**0.0339**

Table 2.19. Outcome of aggression trials when both populations and haemoglobin genotypes are mixed.

St Andrews HbI-2*2	St Andrews HbI-1*2	St Andrews HbI-1*1	Clyde HbI-2*2	Clyde HbI-1*2	Clyde HbI-1*1	Number of pairs	Exact binomial test
		Number of winners of a fight					
1	-	-	-	0	-	1	
-	0	-	1	-	-	1	
-	-	0	-	1	-	1	
1	-	-	-	-	0	1	
Totals	**Genotype > population**					**4**	**z = 2.0, _P_ =**
	4	**0**					**0.0455**

4 DISCUSSION

A summary of the main results from both the group-level and the pair-wise experiment is given in Table 2.20.

Table 2.20. Summary of main results from both experiments on the effect of mixing populations on growth and on the effect of mixing populations and haemoglobin genotypes on behaviour.

Experiment	Test	Population effect	Haemoglobin genotype effect
Group	Effect of mixing populations or haemoglobin genotype on growth	yes	no
Pair-wise	Effect of mixing populations or haemoglobin genotypes on aggression	no	yes
	Effect of mixing populations or haemoglobin genotypes on feeding	no	yes
	Effect of population or haemoglobin genotype on aggression	yes	yes
	Effect of population or haemoglobin genotype on feeding	yes	no

This is one of the first studies to reveal an intra-specific difference in behaviour in cod from different populations and explores the links between growth, dominance and feeding behaviour in fishes. The study extends the findings of Salvanes and Hart (2000) on the effect of haemoglobin genotype on the behaviour of juvenile cod. It also offers further insights into the theory of familiarity. I firstly discuss results from each experiment in turn, before bringing the two studies together to discuss the links between each.

4.1 Growth in juvenile Atlantic cod held in pure and mixed population groups

The suppression in growth rate by an average of over a half of the Clyde Sea cod when in the presence of those from St Andrews Bay is in agreement with the "Dear Enemy" phenomenon (Jaeger, 1981), in which levels of aggression are higher amongst non-familiar individuals. Similar findings have been reported in sea trout, in which fish in familiar groups had a more stable dominance hierarchy and reduced aggression. This is thought to result in a higher food intake and growth rate, and thus result in greater fitness (Höjesjö *et al.*, 1998).

Contrary to this theory, however, the St Andrews Bay cod did not experience the same decrease in growth rate when in the mixed population situation. This suggests that the competitive ability is an asymmetric trait in which the St Andrews Bay cod will be more competitive than those from the Clyde Sea and result in a restricted growth rate. Hatlen *et al.* (2006) demonstrated a link between such competitive interactions and growth, in which slower growing cod were often those that suffered most from physical attacks. Therefore feeding by individuals from the Clyde Sea population may have been restricted by those from St Andrews Bay. The lack of a difference in growth rate by the St Andrews Bay cod when in the mixed treatment could have been because they were already at their maximum level of growth when in the pure population treatment. Therefore growth rate could not be raised even if they had access to more food in the mixed treatment. The 52 % higher growth rate achieved by the Clyde Sea cod relative to those from St Andrews Bay is in accordance with other studies (Perutz *et al.*, submitted; Chapter 3) in which there is evidence to suggest a population difference in growth rate between individuals of the same age and origins.

The fact that this experiment was repeated using two year classes and similar results were obtained in both years suggests that the effect seen is not simply a year class effect, although there are alternative explanations, such as size and haemoglobin genotype. There were no body size trait (length, weight and condition, Fulton's K) differences between treatments. There were minor but significant differences in initial length between populations in the pure treatment, with cod from the Clyde Sea being slightly larger than those from St Andrews Bay, but such a small difference in size range between populations (mean of 1.9 cm) is unlikely to have had a significant effect on growth rate, as has also been found in Perutz *et al.* (submitted; Chapter 3). Therefore it is unlikely that this population difference in initial length could explain the differences in growth rate between the St Andrews Bay and the Clyde Sea cod in the pure treatment. Contrary to many studies (Mork *et al.*, 1984; Nævdal *et al.*, 1992; Imsland *et al.*, 2004), there was no effect of haemoglobin genotype on growth rate within treatments. Haemoglobin effects have usually been demonstrated at the higher and lower thermal preferences of cod (greater than 14 °C or less than 5 °C) (Imsland *et al.*, 2004), whereas this study was designed to take place in the mid-range of temperatures experienced by both groups. Additionally, no differences in genotype or

allele frequencies were found between populations (Appendix 2) or treatments, so any effect of haemoglobin genotype would have been equal between populations.

4.2 Behavioural interactions in pairs of juvenile cod

Unlike in the group-level experiment, the pair-wise experiments revealed striking asymmetries in haemoglobin genotype as well as population, which influenced the outcome of competition. Aggression was strongly influenced by haemoglobin genotype and population, while population affected feeding. Salvanes and Hart (2000) reported that the HbI-2*2 genotype was dominant in feeding. In the present study however, the HbI-2*2 genotype was again dominant, but only in terms of aggression. The feeding was dependent on population rather than haemoglobin genotype. Contrary to the familiarity theory and the group-level study, no difference was found in aggression or feeding according to whether they were in mixed or pure population combinations.

Aggressive interactions took the form of obstructions, touches, slow chases and fast, dart-like chases and it was usually one individual that initiated the attacks in a pair. While other studies report on damage inflicted by conspecifics in which a range of different sized fish are present (Hatlen *et al.*, 2006) and cannibalism (Folkvord, 1991; Folkvord and Ottera, 1993), there have been few reports of visual observations of interactions on size-matched individuals in cod. Observations have been made on other species, such as Arctic charr, *Salvelinus alpinus*, in which similar agonistic attacks are reported, although these involved more physical contact (Adams *et al.*, 1995). The feeding behaviour observed was similar to other studies on juvenile cod (Hart and Salvanes, 2000). Usually only one individual swam after a pellet and no aggressive interactions were observed during feeding. While there was high variability in levels of feeding between individuals, it was usually one individual in the pair that ate the majority of the pellets.

Size differences between individuals in a fight, can often overwhelm other effects (Turner and Huntingford, 1986; Koops and Grant, 1993). Even though the fish were size-matched, differences as little as 5 % in juvenile steelhead trout, *Onchorhynchus mykiss,* have been found to influence experimental outcome in dominance trials (Abbott *et al.,* 1985). In the present study no effect of relative size was found on aggression or

feeding, even though difference in length between pairs was sometimes greater than this. However, other studies have also found that size does not always determine dominance. For example, Huntingford *et al.* (1990) found that prior experience was a more important indictor than size, even in fish with a size difference of greater than 5 %. Although no effect of size was found within pairs, between pairs there was an effect; interactions in smaller sized pairs being more aggressive and more polarised than in larger sized pairs. This is in agreement with Hatlen *et al.* (2006), who investigated effects of size on aggression in cod over a similar size range. Groups of smaller cod (55g) were more aggressive, as reflected in fin damage, than groups of larger sized cod (250 and 450g). Since there was no relationship between fish size and prey share, we can preclude the possibility that the findings were due to size-related differences in appetite, even though pairs of fish did vary in size by up to 13 cm.

Contrary to the prediction of there being a higher level of aggression in the mixed population trials compared to the pure, no such difference was found. Findings of familiarity may, however, be context dependent. For example, Ward and Hart (2003) suggest that the advantages of associating with familiars are greater in shoaling than in aggressive or solitary species. This could also explain why a classic familiarity result was found in the group level experiment but not at a pair-wise level. Alternatively the lack of differences between trials with mixed and pure population combinations may have resulted from an underlying effect of haemoglobin genotype masking the familiarity effects, despite the low and variable sample sizes in the different categories of haemoglobin genotypes. When separating pair combinations according to mixed or pure haemoglobin genotype rather than population, it was found that encounters between pairs of fish involving the same genotypes (pure HbI-1*2 and HbI-2*2 combinations) were significantly more aggressive than mixed. The data suggests that, despite low sample numbers, this result was largely driven by the fights involving HbI-2*2 genotypes paired against each other, as these fights were marginally fiercer than other combinations. These findings hint towards a behavioural asymmetry, in which fights were more intense when the trait most closely aligned with winning (in this case haemoglobin genotype) was equal.

The findings of aggression being related to population and haemoglobin genotype have not been reported previously. Cod of the HbI-2*2 genotype are reported to have a

higher efficiency as an oxygen carrier at lower temperatures, in the temperature range of this study (between 7.9 and 8.5 °C). Many studies find a relationship between aggression and metabolic rate (e.g. Nakano, 1994; Yamamoto *et al*., 1998; Cutts *et al*., 1998) and haemoglobin genotype is linked to metabolic rate through ability to carry oxygen (Weber, 1990). Therefore if those cod of the HbI-2*2 genotype had a higher metabolic rate under these experimental conditions, this may have resulted in their greater capacity for aggression. However, Salvanes and Hart (2000) suggest that the link between haemoglobin genotype and growth is through feeding behaviour, following the idea that fish of the HbI-2*2 genotype support a more active metabolism. Contrary to results of the present study, they found that those of the HbI-2*2 genotype were better feeders. In this study, no effect of haemoglobin genotype was found on feeding. However, there was a population effect, in which the Clyde Sea cod took a higher prey share in 82 % of the trials.

Other studies suggest that more aggressive fish would also take more food (Grand and Grant, 1994). More food might support a more active metabolism and enable greater aggression. Fish with a higher metabolic rate have also been found to possess faster swimming speeds (Brett, 1965), which could increase the likelihood of reaching food first. The finding that aggression does not predict prey share, has also been reported in salmonids (Höjesjö *et al*., 2005) in simultaneously feeding juvenile brown trout, *Salmo trutta,* and salmon, *Salmo salar*. The brown trout were dominant but the salmon were the more efficient at feeding of the two species with regard to prey capture efficiency and were described as 'sneaky feeders'. Similarly, the Clyde Sea cod may have also been more efficient at prey capture, perhaps in terms of their speed or capture success. Also dominance may not necessarily reflect timidity to feed and less timid fish may take a higher prey share.

Another possibility is that aggression may have predicted feeding had the location and timing of food been predictable, as it was in Salvanes and Hart (2000). In this study the fish had learned the experimental procedure over a 6-week acclimation period (Salvanes and Hart, 2000). In agreement with this suggestion, Grand and Grant (1994) found that only under a predictable food supply was feeding correlated with dominance status. Prior experience in fighting in fish has also been shown to alter a contestant's fighting ability (e.g. Parker, 1974; Hsu *et al*., 2006). Therefore, had the cod in the present study

learned that feeding would follow shortly after the aggression trial was over, aggression could have predicted feeding.

4.3   Synthesis

Using the results from the pair-wise study, it is possible to speculate about the mechanisms that may have resulted in the differences in population-level growth rate in the pure and the mixed treatments. In the pair-wise trials, cod from the Clyde Sea, gained a larger prey share than those from St Andrews Bay. Supposing this was also the case at a group level, this may explain the higher growth rate of cod from the Clyde Sea. This higher growth rate of the Clyde Sea cod in the pure trials and also in Chapter 3, could have been due to an inherited tendency for a greater appetite in cod from the Clyde Sea compared to those from St Andrew Bay. Unlike in the pair-wise experiments, this study found no effect of haemoglobin genotype. As there were no differences in genotype frequencies between populations, any effect of genotype, however minor, should have been equal between the two populations. In the pair-wise trials, the St Andrews Bay cod of the HbI-2*2 genotype were more aggressive than the same genotype from the Clyde Sea. Supposing a similar occurrence at a group-level, the St Andrews Bay cod may have been dominant over those from the Clyde Sea. This could have restricted the feeding of the Clyde Sea cod and resulted in a suppressed growth rate. Similarly, the finding of Salvanes *et al.* (2004) demonstrated that under common-environment conditions with both stocks mixed together, a faster growing more northerly Norwegian stock had higher rates of juvenile growth, at least partly due to their greater success in competition against the more southerly stock.

It has been reported that feeding behaviour of subordinates has been suppressed in the presence of more dominant individuals (Jobling, 1985; Metcalfe, 1989). In feeding trials in cod, Hatlen *et al.* (2006) propose that those that received most aggression were prevented from feeding and that the slower growing fish received most aggression towards them, in terms of fin damage. While the St Andrews Bay cod may have restricted feeding of the Clyde Sea cod in the mixed tank, they did not themselves grow at a faster rate than when in the pure treatment. The pair-wise experiments indicated that the St Andrews Bay cod did not feed as much despite their higher level of aggression. Even if they had fed as much at the group level, they may not have been as efficient at

converting energy into growth. The cost of greater aggression may be a higher metabolic rate (Pucket and Dill, 1985) and an associated higher energy demand, and so the St Andrews Bay cod may have had to eat more than the Clyde Sea cod to grow an equivalent amount.

The finding of a familiarity effect in the group level experiment but not at the pair-wise level could be explained by the difference in tank environment as has been reported in other studies (Höjesjö *et al.*, 1998). For example, in the bluegill sunfish, *Lepomis macrochirus*, tendency to associate with familiar conspecifics was greater after already having foraged with that individual (Dugatkin and Wilson, 1992). Consistent with these ideas, in the group level experiment, the cod had been living in these groups for over a month, thus giving ample time for a dominance hierarchy to be established. In the pair-wise experiment, the cod had only just been exposed to each other and thus may not have been able to distinguish between familiar and unfamiliar conspecifics.

Alternatively haemoglobin or population dependent interactions may have superseded those of familiarity. This study has found evidence for population differences in the suppression of growth rate in the Clyde Sea cod, the greater aggression in those from St Andrews Bay and the higher feeding ability of those from the Clyde Sea. These behavioural differences suggest that there are competitive asymmetries between individuals from the different populations, which could have led to differences in resource-holding power. Other studies have identified possible asymmetries, such as gonad size in male cichlids, *Tilapia zillii*, was found to determine outcome of fights (Neat *et al.*, 1998). These differences could have evolved through partial isolation over a period of time together with differences in phenotypic traits (Chapter 3 and 4), habitat (Appendix 1) or density.

Contrary to other studies on the relationship between dominance and feeding (Höjesjö *et al.*, 2002 in brown trout), dominance in aggression did not predict growth rate, although both can be indicators of fitness (Huntingford and Turner, 1987). Dominance has not always been found to indicate higher growth rate, however. In a study of growth rates of wild stream-dwelling Atlantic salmon, no correlation was found between dominance status and specific growth rate (Martin-Smith and Armstrong, 2002). Additionally, Huntingford and Garcia de Leaniz (1997) suggest that the link between

dominance and growth is environment dependent. Salmon that were more dominant in tanks did not have such high growth rates in streams. Similarly, the sample populations of the present study may have been adapted to their respective environments in differing ways according to the selection pressures experienced. Common environment studies have suggested that the higher growth rate of the Clyde Sea cod could be an adaptation in response to a higher thermal regime (Perutz *et al.*, submitted; Chapter 3). In salmonids aggression has also been demonstrated to be an inherited trait (Taylor, 1990; Dunbrack, *et al.*, 1996). Higher levels of aggression could result from factors, such as higher population densities or competition for limited resources (Huntingford and Turner, 1987). From this study it is not possible to determine whether the difference in aggression is adaptive or phenotypically plastic, or merely a year class effect, as the study was only carried out over one year. Common environment or reciprocal transplant studies are required to tease apart the genetic and environmental effects and to determine possible causes of phenotypic plasticity in aggression.

Previous studies also suggest there may be plasticity in the behaviour of cod from these areas. The areas differ in their thermal regime (Slesser and Turrell, 2005) and other environmental factors. The Clyde Sea is milder and less variable than St Andrews Bay. If the HbI-2*2 genotype advantage is temperature dependent (Imsland *et al.*, 2004), dominance in aggression may only exist at these temperatures (8 to 9 °C) or lower. At higher temperatures, where the HbI-1*1 genotype is favoured (> 14 °C, Imsland *et al.*, 2004), we would expect those of the HbI-1*1 genotype to have a competitive advantage. Between 10 and 14 °C the genotypes may be equal. Therefore the competitive advantage of an individual may be seasonal. In summer when temperatures can reach 14 °C in the areas studied, the Hb1*1 genotypes may be favourable, whereas in the winter and spring when this study was carried out, the sea temperature would have been between 5 and 10 °C and so the HbI-2*2 genotypes may have been favoured. Further experiments over a range of temperatures are required to test these predictions. Supposing sea temperatures continue to rise, we may see a rise of the HbI-1*1 genotypes as the water temperature becomes more unfavourable for the HbI-2*2 genotypes for more of the year. Other site-specific differences in the natural environment could also affect behaviour in addition to temperature and thus the advantage of being more aggressive or being more successful at feeding may be environment dependent. In brown trout, less aggressive individuals were found to be

more successful in a more heterogeneous habitat, than in the wild (Höjesjö *et al.*, 2002). Previous exposure to predator presence may affect subsequent boldness in feeding. Temming *et al.*, (2007) demonstrated high predation on juvenile cod by whiting, *Merlangius merlangus,* in open areas.

Whether these experimental results can be extrapolated to the wild is yet to be determined. Natural environments are far more complex than the laboratory and other factors such as predator avoidance and territoriality (Johnsson *et al.*, 2000) may allow for a more diverse range of behavioural strategies (Milinski and Parker 1991; Metcalfe *et al.*, 1995; Kadri *et al.*, 1996; Höjesjö, *et al.*, 2002). Studying pair-wise interactions rather than in a group may also be unrepresentative of the wild situation. Juvenile cod display loose shoaling behaviour (Temming *et al.*, 2007; pers. observation) and aggression and readiness to feed may take on a different form in a group situation, due to factors such as group co-operation or differences in boldness.

Results of the present study also suggest a need for caution when selecting fish for restocking programmes or for aquaculture purposes. If differences in competitive behaviour occur between populations, it may be advisable to separate stocks in order to maximise growth rate, as has been suggested by Griffiths (2003). It may also be worthwhile to separate cod by haemoglobin genotype to reduce intra-group competition. Similarly for restocking purposes, restocking with progeny from the native population of the area could minimise intraspecific competition.

5.0   CONCLUSION

The relatively higher growth rate of cod from the Clyde Sea also identified in Perutz *et al.* (submitted; Chapter 3) may have been at least partly related to a population difference in appetite or motivation to feed, cod from the Clyde Sea cod eating more at a pair-wise level than those from St Andrews Bay. The suppression in growth rate experienced by the Clyde Sea cod in the presence of those from St Andrews Bay appears to have been related to population dependent competition directed towards non-familiar individuals or those of a differing population genetic background. The cod from St Andrews Bay may be inherently more competitive than those from the Clyde Sea.

Among themselves this may have equilibrated but when placed together with the cod from the Clyde Sea they are in a state of disequilibrium and win a greater proportion of the fights than those from the Clyde Sea. Findings at the individual level could not predict those at the group level, and no familiarity effects were found in the pair-wise experiments. Haemoglobin genotype was the main driver behind competitive interference at an individual level. The individual with the more HbI^2 alleles was the more aggressive and when genotype was matched, the St Andrews Bay cod were usually dominant, although whether these effects were temperature dependent is yet to be determined. Effect of haemoglobin genotype was visible at the individual level as a main influence of competitive interference, but appears to have been masked at the group level, where population of origin rather than haemoglobin genotype affected growth rate. Future work is needed to confirm suggestions found in the present studies, to relate dominance, feeding and growth concurrently at both the individual and the group levels and to investigate temperature dependence on these effects.

CHAPTER 3

Differences in thermal growth responses of juvenile Atlantic cod from the east and west coast of Scotland

1 INTRODUCTION

Temperature directly determines many vital physiological processes in fishes (Jobling, 1985; Brander, 1994b, 1995) and is likely to be a critical factor in determining fish growth. Since an individual's growth rate directly affects body size in fish and body size influences factors such as survival, maturation, and fecundity (Hutchings, 2002), we can expect temperature to have significant consequences for individual fitness and population processes. Understanding the relationship between temperature and growth is complicated however, because growth rates may be influenced by a number of other factors including food consumption (Brett, 1979; Jobling, 1985; Koskela *et al.*, 1997; Yoneda and Wright, 2005a and b), photoperiod (Kadri *et al.*, 1997; van der Meeren and Jørstad, 2001), the duration of feeding (Biswas and Takeuchi, 2003) and time of year (Levesque *et al.*, 2005). In addition to environmental influences, growth rate in cod has a relatively high heritability (Gjerde *et al.*, 2004). Within and across population heritability was estimated to be 0.29 for body weight (Gjerde *et al.*, 2004). Genetic influences such as haemoglobin genotype (Nævdal *et al.*, 1992; Imsland *et al.*, 2004), and pantophysin, a nuclear RFLP locus (Gjerde *et al.*, 2004; Case *et al.*, 2006), may also affect the scope for growth. Haemoglobin genotype has been demonstrated experimentally to affect growth (Nævdal *et al.*, 1992; Imsland *et al.*, 2004). Thus regional differences in growth rate, as reported for cod by Brander (1994a), arise from a complex suite of intrinsic and extrinsic factors. A common environment method was used to partition the variance in rates of growth and to understand the relative contribution of external and internal factors (Conover and Schultz, 1995; Conover, 1998; Imsland and Jónsdóttir, 2002).

Patterns of adaptive divergence in Atlantic cod, using such controlled environment methods have been identified, although they are not always consistent across the species

range. Countergradient variation in growth of fishes has been recorded across latitudinal and altitudinal gradients whereby a faster growth rate at higher latitude or altitude compensates for the shorter growing season (e.g. Conover and Present, 1990; Conover and Schultz, 1995; Salvanes *et al.*, 2004; Marcil *et al.*, 2006a and b). An example of countergradient variation is documented in growth and condition factor in juvenile cod from along the coast of Norway (Salvanes *et al.*, 2004). However, the pattern of growth between Northeast Arctic and Norwegian coastal cod is consistent with the hypothesis of cogradient variation, in which the Norwegian coastal cod grew at a faster rate than those from the Northeast Arctic (Otterlei *et al.*, 1999).

Evidence for population structuring in cod from around the British Isles (Chapter 1), together with the variation in climate, with milder and less variable sea temperatures on the west than the east coast (Appendix 1; Hughes, 2004), led to the hypothesis that there may be local thermal adaptation of growth rate in cod. This is examined here using a series of controlled environment experiments carried out on wild juvenile cod. The juvenile stage is a good age to study growth rate, as energy is consistently put into growth, whereas in the adult stages energy is also allocated to reproduction and growth rates will vary according to stage of maturation. The primary aim of this study was to test whether differences in growth rate exist between cod from the two areas when wild-caught fish were held under constant conditions. The secondary aim was to gain insight into the response of growth to different temperatures in each population over the normal range of temperatures experienced. Two studies were performed. The first examined the specific growth rate of cod from the Clyde Sea and St Andrews Bay over their normal temperature range experienced, using a step-wise temperature regime between 7 and 13 °C. Results of this study informed a more detailed study focussing in on temperatures within 3 °C of the optimal, between 8 and 12 °C.

2   MATERIALS AND METHODS

Juvenile 0-group cod from St Andrews Bay on the Scottish east coast and the Clyde Sea on the Scottish west coast were collected by trawl as in Chapter 2. The size of fish collected was similar in the two areas, ranging between 13.5 and 19.2 cm total length. They were maintained at the FRS Marine Laboratory (see Chapter 2).

In both experiments fish were weighed and measured fortnightly, under anaesthesia (MS-222, 1g 10 L^{-1}). Individual growth was calculated as the daily weight-specific growth rate (G_s, % day^{-1}) as in Chapter 2. The interval between measurements was chosen to ensure that wet weight could be measured with sufficient accuracy and precision. The results presented as a part of the second study, in experiment 2 B, were the first two weeks of an eight-week period of measuring G_s at a near constant temperature. Short-term growth over the two-week period significantly predicted longer-term growth over the entire eight-week period of the experiment (regression, F_{1-29} = 10.6, P = 0.003, r^2 = 0.274). Measurement error based on the mean variance of measuring 10 fish five times each was 1.1 g (i.e. < 1 % wet weight), for fish of a mean weight of 187.4 g (comparable to experimental animals). The mean weight gain over the two week period for fish in experiment 2 B was 9.9 g $\pm$ 0.9 (SEM) (percentage increase of 15.5 %) for fish of a mean weight of 120.0 g, which amounts to a 0.7 g increase (1.1 %) per day. Therefore all data was standardised to a two-week time period. The first two weeks of growth were used rather than another later period, while the fish were still of a similar size distribution from being size-matched.

3 Experiment 1 – Population variation in juvenile growth rate over the normal thermal range

3.1 MATERIALS AND METHODS

In January 2005 a sub-sample of the cod collected in 2004 from each site was measured, weighed and tagged for individual identification before transferral to the temperature controlled experimental tanks. They were transferred two weeks prior to the experiment to give time to acclimate to the new tanks. Cod were anaesthetised and tagged with fluorescent alpha-numeric tags (Northwest Marine Technology), inserted immediately below the skin on the upper lateral surface near to the second dorsal fin on an area of pale coloured skin.

Experiments were run in four identical flow-through 1 m diameter tanks (volume of 550 litres). 20 cod were allocated to each tank; two experimental and two controls, one of each containing either St Andrews Bay cod or Clyde Sea cod. The control tanks were

kept at a mean of 9.5 $\pm$ 0.1 °C (SEM) throughout the experiment. The experimental tanks progressed through a range of temperatures starting with 7 °C on 17th February 2005, and increasing by 2 °C every two weeks until 13 °C and the final measurement was on 14th April (Figure 3.1). Photoperiod was kept on ambient for 57°16.7'N using fluorescent green lighting. Cod were fed to excess on medium sized pelleted food twice daily in the early morning and late afternoon, with the same amount in each tank. The cod were weighed ($\pm$ 0.1 g) and measured ($\pm$ 0.1 cm) fortnightly and sacrificed when the experiment was terminated. Miniloggers (Vemco) were used to record temperatures in each of the experimental tanks and in one of the control tanks (Figure 3.1). Water temperature in the control tanks was controlled by the same chilling unit and so the temperature in each was assumed to be the same.
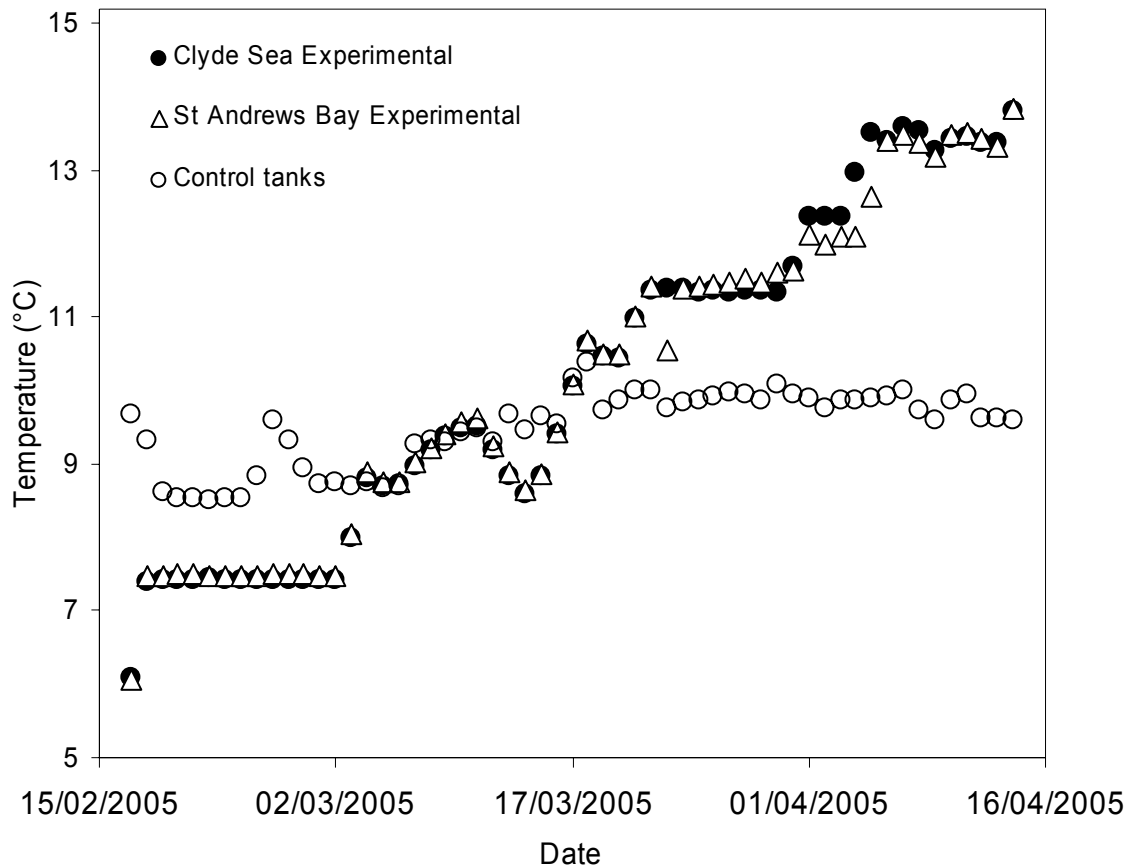
Figure 3.1. Temperature regimes for experimental tanks (Clyde Sea: ■ and St Andrews Bay: Δ) and both control tanks (x), recorded using miniloggers (Vemco).

Cod from each area were separated by tank, since prior work had shown competitive differences between cod from the two areas resulting in differing growth rates (Chapter 2). The cod were at a density lower than that shown to have an effect on growth

(Lambert and Dutil, 2001), so the slight difference in densities between tanks is unlikely to have had a significant effect on growth. Cod were size-matched to equalise size distributions between treatments. There were slight differences, however, in initial length between groups, due to some mortalities in the acclimation period after they had already been size-matched. There was a maximum mean difference of 2.1 cm, and cod in tank 3 were significantly shorter than those in tanks 1 and 2 ($F_{3\text{-}41} = 3.93$, $P = 0.015$). However, no effect of initial length was found on the growth rate ($F_{3\text{--}48} = 1.36$, $P = 0.250$), and thus these small differences are unlikely to have had a significant effect on growth. No differences in condition factor (Fulton's K) were found between groups ($F_{3\text{--}41} = 2.12$, $P = 0.113$). Details are given in Table 3.1.

Table 3.1. Summary data for control and experimental cod for each population including; dates, temperature, area, initial numbers in the tanks, initial lengths (cm), initial condition (K) and initial density (gL^{-1}) of fish in the tank.

Treatment	Area	Initial numbers	Mean initial length (cm) $\pm$ SEM	Mean initial condition (K) $\pm$ SEM	Initial density (gL^{-1}l)
Control – constant temperature	Clyde	12	19.8 $\pm$ 0.4	1.0 $\pm$ 0.0	1.7
	St Andrews	12	19.6 $\pm$ 0.4	1.0 $\pm$ 0.0	1.7
Experimental - stepwise temperature regime	Clyde	9	17.6 $\pm$ 0.4	1.0 $\pm$ 0.0	0.9
	St Andrews	9	18.4 $\pm$ 0.8	0.9 $\pm$ 0.0	1.0

Individuals were excluded in which fish had a G_s of less than two standard deviations below the mean and appeared to be in ill health. One fish was excluded from the Clyde Sea and one from St Andrews Bay in both of the control and experimental tanks.

Data were analysed using linear mixed effects models to account for repeated measures on the same fish at two time points. The individual fish and time (variate) was fitted as a random effect with treatment (control or experimental), area (place of origin), time (factor) and their interaction as fixed effects. The model was fitted by first inputting all parameters and then simplified by removing non-significant parameters at the 5 % level.

The auto-regressive structure of order 2 was selected as it produced the lowest Akaike Information Criterion (AIC) score in all models.

AIC = ¡2 log (maximum likelihood) + 2 (number parameters) (Burnham and Anderson, 1998) as estimated by the Wald test.

The large sample approximation to the Chi-squared distribution was accepted due to the large number of fish on which repeated measures were taken. The residuals were checked for normality and Levene's test was used to test for heteroscedascity. Analyses were conducted using the software package, Genstat 9.

3.2   RESULTS

Linear mixed effects modeling on the G_s (Table 3.2, Figures 3.2 and 3.3) of cod from both areas through time, revealed a highly significant difference between the experimental treatment and the controls (d.f. = 1,40, P < 0.001). In the controls, cod from both areas grew at a similar rate and growth rate was near constant through time. There was an effect of time (d.f. = 3,40, P = 0.003), but no consistent pattern through time as might be expected if there were a seasonal effect in the experimental period. There was also no effect of area (d.f. = 1,40, P = 0.423).

In the experimental data, however, a significant effect of temperature was found (d.f. = 3,20, P < 0.003) and growth rate was parabolic (Figure 3.3). G_s peaked at a similar maximum for the Clyde Sea and St Andrews Bay cod (1.0 % day^{-1} $\pm$ 0.2 and 1.0 $\pm$ 0.2 respectively), but at a higher temperature in those from St Andrews Bay (9 °C) than those from the Clyde Sea (11 °C). This is supported by an area x time interaction (d.f = 3,20, P = 0.032), although there was no effect of area on its own (d.f. = 1,20, P = 0.211). The peak G_s was more clearly defined in the Clyde Sea cod than those from St Andrews Bay. In the Clyde Sea cod, above the thermal optima, growth rate declined considerably and at 13 °C it was less than it had been at 7 °C. In the St Andrews Bay cod, however, growth rate was similar between 9 and 13 °C.
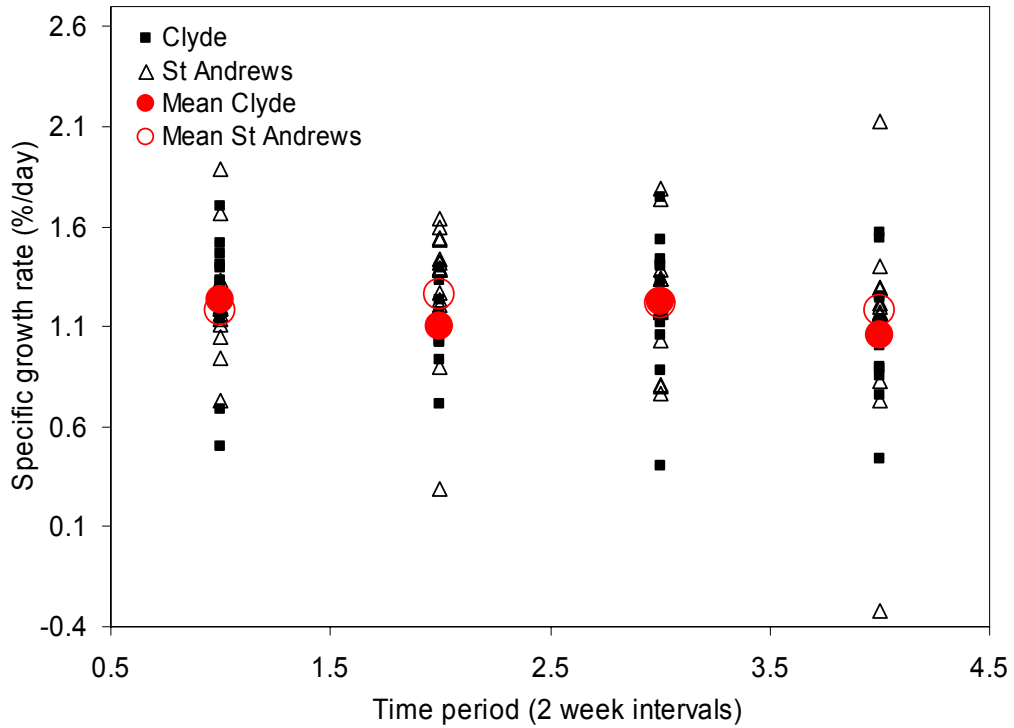
Figure 3.2. Daily weight-specific growth rate, G_s (% day^{-1}) of individual cod in the control treatment from each population held at 9.5 °C. Means at each time period are given for each population separately and significance given in Table 3.2.
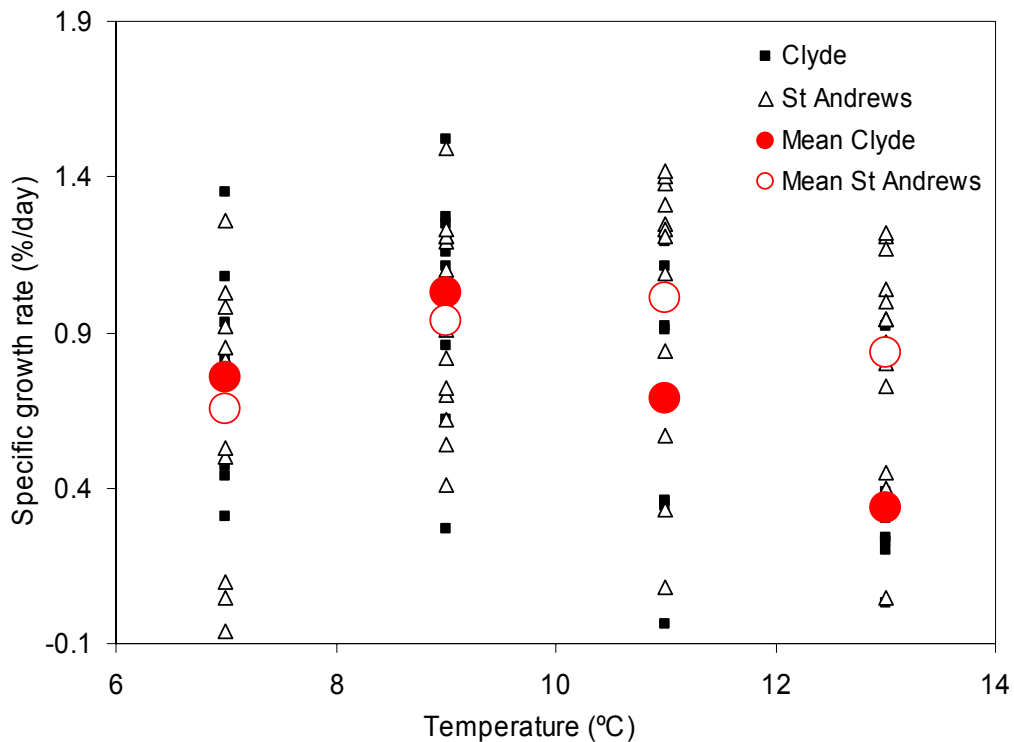
Figure 3.3. G_s (% day^{-1}) of individual experimental cod from each population progressing through a range of temperatures at two weekly intervals. Means at each time period are given for each population separately and significance given in Table 3.2.

Table 3.2. Effect of treatment, area and temperature on G_s using a linear mixed effects model (Auto-regressive order 2), for all controls and experimental data and then separately for each.

Data	Source of variation	d.f.	*P*
All	Treatment	1,40	< 0.001
	Area	1,40	0.531
	Time	3,40	0.00341
	Area.time (factor)	3,40	0.0257
Controls	Area	1,20	0.578
	Time	3,20	0.449
Experimental	Area	1,20	0.211
	Time or temperature	3,20	0.00331
	Area.time (factor)	3,20	0.0321

4 Experiment 2: Population variation in juvenile growth rate between 8 and 12 °C

4.1 MATERIALS AND METHODS

This experiment was carried out using a similar method to the first experiment with some exceptions to the treatment design and temperature regimes. In experiment A visible fluorescent alpha-numeric tags were used as in the first study, but in experiment B Passive Integrated Transponder (PIT) tags were used instead, implanted into the peritoneal cavity.

Between 20 and 30 cod were allocated to each group, depending on the specific experiment (Table 3.3). No significant differences in length or condition (Fulton's K, K = 100 (W/L^3)) were found in four of the cases. There were minor but significant differences, however, in initial length in experiment A run 1 (*t*-test, d.f. = 54, *P* = 0.001) and condition (*t*-test, d.f. = 47, *P* < 0.001), and in experiment B run 1 at the lower temperature (*t*-test, d.f. = 35, *P* = 0.007). This represented a maximum mean difference of 7.0 % in length and 12.2 % in condition. Nevertheless analysis of covariance showed no significant effect of either initial length or condition factor on growth rate in either of these experiments.

Table 3.3. Summary data for experiment A runs 1 and 2 including; dates, temperature, area, initial numbers in the tanks, initial lengths (cm), initial condition (K) and initial density (gL^{-1}) of fish in the tank.

Run	Date	Temperature (ºC) ± SD	Area	Initial numbers	Mean initial length (cm) ± SEM	Mean initial condition (K) ± SEM	Initial density (gL^{-1})
1	Dec 2004	10.1 ± 0.1	Clyde	30	15.5 ± 0.2	0.9 ± 0.0	1.9
		10.1 ± 0.1	St Andrews	30	15.7 ± 0.3	1.0 ± 0.0	2.5
2	Feb-Mar 2005	8.9 ± 0.4	Clyde	28	17.4 ± 0.3	1.0 ± 0.0	2.1
		8.9 ± 0.6	St Andrews	24	17.9 ± 0.3	1.0 ± 0.0	2.5

Table 3.4. Summary data for experiment B runs 1 and 2 including; dates, temperature, area, initial numbers, initial lengths (cm), initial condition (Fulton's K) and initial density (gL^{-1}) of fish in the tank.

Run	Date	Weeks	Temperature (ºC) ± SD	Area	Initial numbers	Mean initial length (cm) ± SEM	Mean initial condition (K) ± SEM	Initial density (gL^{-1})
1	Mar-Apr 2006	1 and 2	8.2 ± 0.5	Clyde	20	18.7 ± 0.4	0.9 ± 0.0	1.8
			8.3 ± 0.1	St Andrews	20	20.1 ± 0.3	0.9 ± 0.0	2.1
		3 and 4	12.0 ± 0.7	Clyde	20	18.3 ± 0.4	1.0 ± 0.0	2.5
			11.9 ± 0.5	St Andrews	20	19.7 ± 0.3	1.0 ± 0.0	3.1
2	May 2006	1 and 2	8.5 ± 0.1	Clyde	18	22.0 ± 0.5	1.0 ± 0.0	3.1
			8.5 ± 0.1	St Andrews	18	22.3 ± 0.3	1.0 ± 0.0	3.3
		3 and 4	11.7 ± 0.4	Clyde	18	21.1 ± 0.4	1.0 ± 0.0	3.5
			11.5 ± 0.4	St Andrews	17	21.6 ± 0.3	1.0 ± 0.0	3.7

Duplicate experiments were carried out, the first to examine population effects and the second to investigate temperature responses for a given population. Fortnightly

measurements of total length ($\pm$ 1 mm) and wet weight ($\pm$ 0.1 g) were taken. Details of the experiments are given in Table 3.3 and 3.4. In brief, two runs of experiment A were performed, both of which were carried out over a period of two weeks at an approximate mean temperature of 10 ºC in December 2004 and 9 ºC in February 2005. Two runs of experiment B were carried out, one conducted in March and the other in May 2006. Over the four-week period of each run, the first two weeks were conducted at approximately 8 ºC and the following two weeks at 12 ºC. The temperature was increased gradually over 2 days from the lower to the higher temperature. In all experiments cod were fed to excess on the same amount twice daily, in the early morning and late afternoon, with medium-sized pellet food.

The haemoglobin genotypes, HbI-1*1, HbI-1*2, HbI-2*2, were determined for fish only in experiment B. Fresh blood samples were collected from individuals upon sacrifice with an overdose of anaesthetic (MS-222). Blood was taken from the caudal vein using a heparinised syringe. Haemoglobin genotype was determined by agar gel electrophoresis details of which are in (Fyhn *et al.*, 1994, Chapter 2). Genotype frequencies and numbers for each population are given in Table 3.5.

Table 3.5. Haemoglobin genotype frequencies and numbers for cod from the Clyde Sea and St Andrews Bay in experiment two.

Area	Genotype frequencies and numbers		
	HbI-1*1	HbI-1*2	HbI-2*2
Clyde Sea	0.108, n = 8	0.621, n = 46	0.270, n = 20
St Andrews Bay	0.250, n = 16	0.375, n = 24	0.375, n = 24

As in the first study (section 3.1) certain data points were excluded from fish that appeared to be in ill health. These were equally spread across both areas (a total of six from the Clyde Sea and six from St Andrews Bay, from both experiments). The analysis of G_s in experiments A and B was performed using *t*-tests. A Box-Cox transformation was used in experiment A run 2 to provide a non-skewed distribution of the residuals (λ = 2.0). Experiment B was analysed using linear mixed effects models. The individual fish, haemoglobin genotype and time (variate) were fitted as a random effect with area and time (factor) and their interaction as fixed effects. Interactions are only given where significant.

4.2   RESULTS

Initial length and weight were not included in the main analysis, as no significant correlations were found between G_s and initial length or weight (Figure 3.4). In experiment A in both runs the G_s of the Clyde Sea cod was significantly higher than that of the St Andrews Bay cod (Table 3.6, Figure 3.5). For run 1, the mean ($\pm$ 95 % C.I.) G_s for the Clyde Sea and St Andrews Bay cod was 0.8 % day^{-1} $\pm$ 0.2 and 0.4 % day^{-1} $\pm$ 0.2 respectively (*t*-test, d.f. = 44, *P* = 0.002). For run 2, the mean G_s was 1.2 % $\pm$ 0.2 and 0.8 % day^{-1} $\pm$ 0.2 respectively, *t*-test, d.f. = 28, *P* = 0.003). The Clyde Sea cod maintained this difference over the St Andrews Bay cod for the full eight-week period of the experiment (1.2 % day^{-1} $\pm$ 0.1 and 1.0 % day^{-1} $\pm$ 0.16 respectively, *t*-test, d.f. = 29, *P* = 0.05).

Table 3.6. Results of effect of area and temperature on G_s for individual experiments are given using a *t*-test in experiment A (area effect only) and linear mixed effects models in experiment B. In experiment A run 2 G_s is Box-Cox transformed ($\lambda = 2.0$).

Experiment	Run	Source of variation	d.f.	*P*
A	1	Area	44	0.002
	2	Area	28	0.003
B	1	Area	1,35	0.600
		Temperature	1,35	< 0.001
	2	Area	1,32	0.011
		Temperature	1,32	0.046
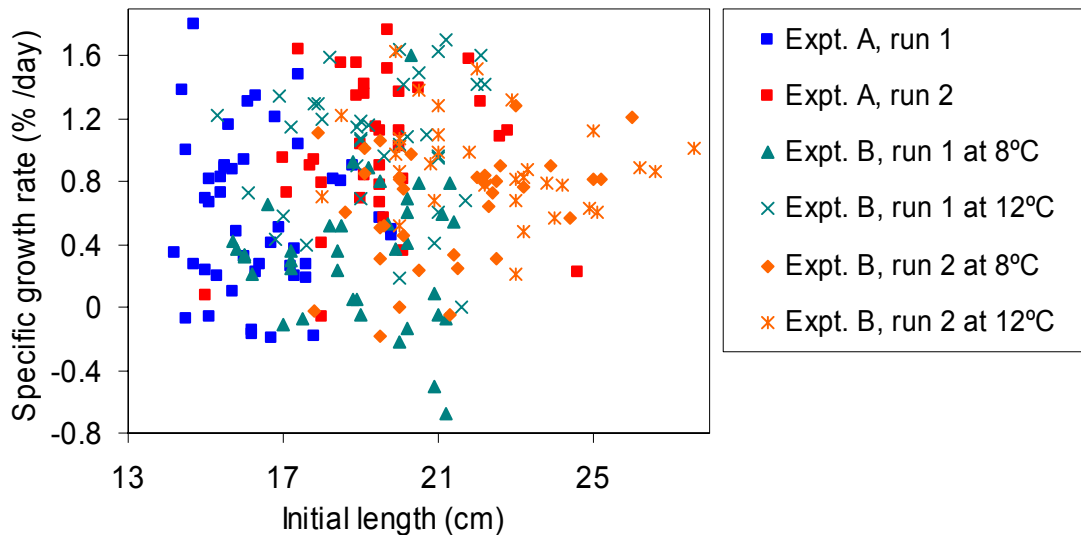
Figure 3.4. Relationship between G_s (% day^{-1}) and initial length (cm) for each run of each experiment (none of the relationships are significant).
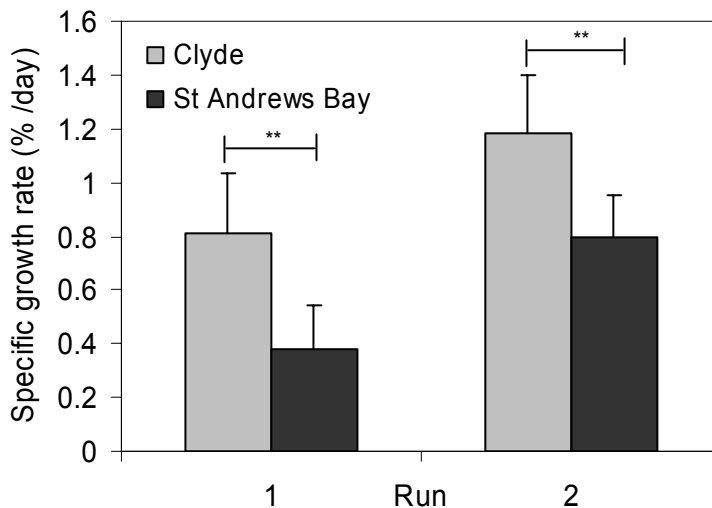
Figure 3.5. Mean G_s (% day^{-1}) for cod from the Clyde Sea and St Andrews Bay with 95 % C.I. for experiment A runs 1 and 2 (** signifies $P < 0.01$).

In experiment B runs 1 and 2, there was a significant effect of temperature over the difference of 4 ºC (linear mixed effects model, d.f. = 1,35, $P < 0.001$ and d.f. = 1,32, $P = 0.046$ respectively, Table 3.6, Figure 3.6A and B), and a significant effect of area in experiment B run 2 (linear mixed effects model, d.f. = 1,32, $P = 0.011$, Figure 3.6B). In experiment B run 2, the mean growth rate of the Clyde Sea and St Andrews Bay cod at 8.5 ºC was 0.7 % day^{-1} $\pm$ 0.3 and 0.7 % day^{-1} $\pm$ 0.1 respectively and at 11.7 ºC it was 1.0 % day^{-1} $\pm$ 0.1 and 0.8 % day^{-1} $\pm$ 0.1 respectively (Figure 3.6B). In experiment B run 1, where the effect of area was not significant, there was still a trend in the same direction as in the other cases (Table 3.6, Figure 3.6A). No temperature x area interaction was

found in either experiment or runs. Overall, across the range of temperatures tested, there was a trend consistent with a linear relationship between temperature and G_s (Figure 3.7). The difference in G_s between cod from the two areas increased marginally with rising temperature from 0.2 % day^{-1} at 8 ºC to 0.3 % day^{-1} at 12 ºC. The non-significant effect of area in experiment B run 1 indicates that population responses can differ between runs. This unaccounted component in experimental differences does not relate to initial length or temperature and there is no clear pattern between growth rate and time of year (Table 3.4).
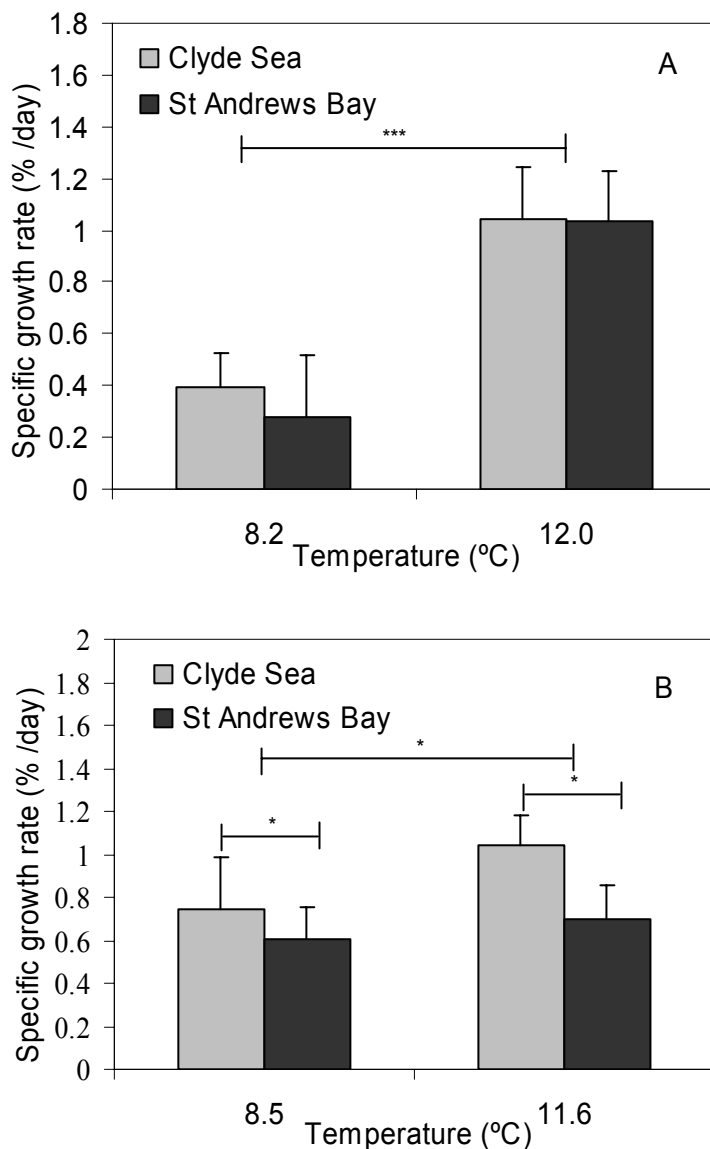
Figure 3.6. Mean G_s (% day^{-1}) for cod from the Clyde Sea and St Andrews Bay with 95 % C.I. for A) experiment B run 1 at 8.2 and 12.0 ºC and B) experiment B run 2 at 8.5 and 11.6 ºC (*** signifies $P < 0.001$, * signifies $P < 0.05$).
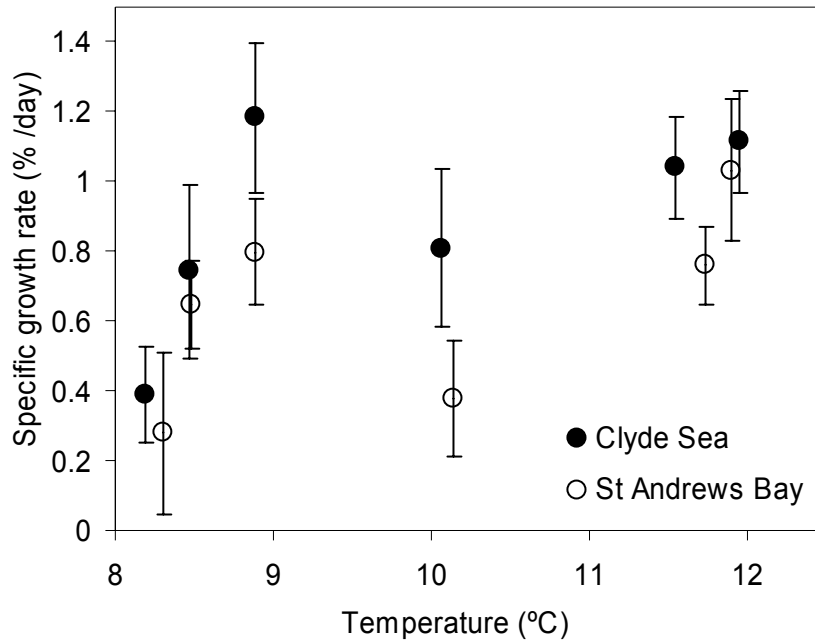
Figure 3.7. Mean G_s (% day^{-1}) versus temperature for all experimental trials with 95 % C.I. for Clyde Sea and St Andrews Bay cod.

The genotype effect was slightly significant in experiment B run 1 (d.f. = 2,33, P = 0.03) and was non-significant in experiment B run 2 (d.f. = 2,30, P = 0.22) and no genotype x area interaction was found in either run. Since no consistent pattern of G_s between genotypes was found in either run (Figure 3.8), it was not included in the analysis of growth rate.
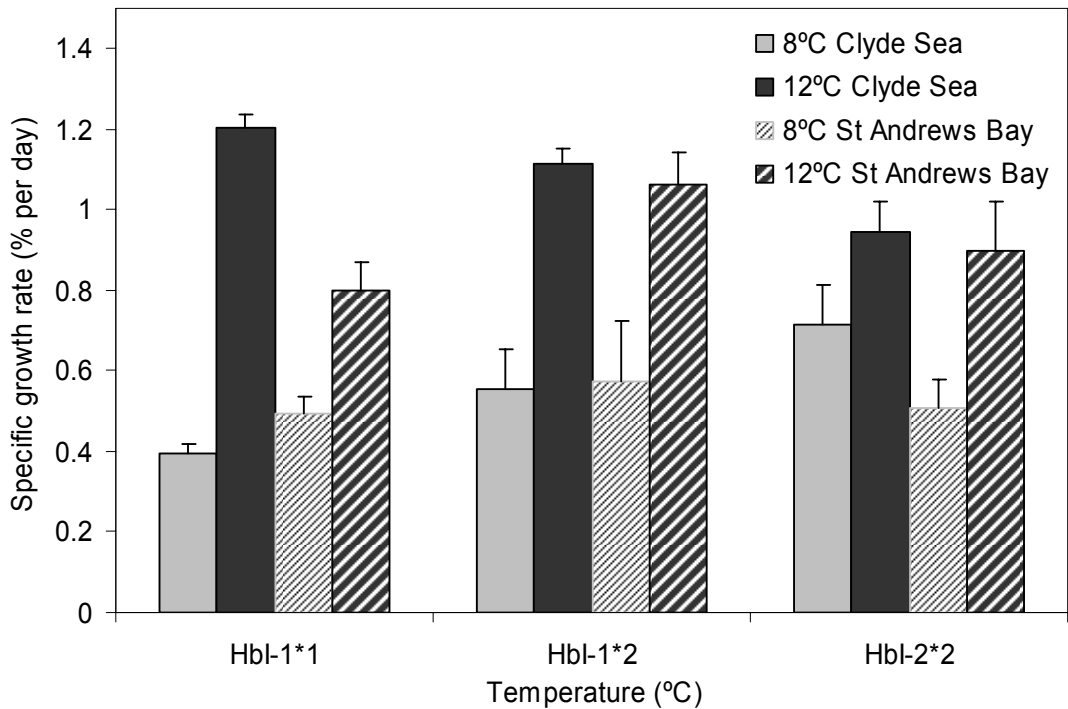
Figure 3.8. Mean G_s (% day^{-1}) with 95 % C.I. for haemoglobin genotypes (HbI-1*1, HbI-1*2 and HbI-2*2), by population and temperature for experiment B only.

5   DISCUSSION

Many studies have demonstrated local adaptation in marine species across their environmental ranges (Conover and Schultz, 1995). Growth rate of cod in the present study was parabolic over the range of temperatures between 7 and 13 °C, peaking at 9 °C in those from the Clyde Sea and 11 °C in those from St Andrews Bay. This demonstrated that there may be a small but significant difference in the thermal optima for growth between wild-caught juvenile cod from the two geographically distinct areas when reared under controlled environment conditions. Results from the second experiment (conducted at temperatures between 8 and 12 ºC) suggested that the Clyde Sea cod had a significantly higher overall specific growth rate (mean difference of 0.2 % day^{-1} across the range of temperatures) than the St Andrews Bay cod. Growth rate increased with respect to temperature, increasing at a similar rate in fish from both areas in the experiments conducted between 8 and 12 ºC. Taken together (and given the absence of a haemoglobin genotype effect), there appears to be significant population differences in growth with temperature having a significant effect on both populations.

A positive effect of temperature on growth rate is well documented (Brander, 1995; Otterlei *et al.*, 1999; Purchase and Brown, 2001) and a parabolic (dome-shaped) pattern in growth is commonly found in relation to temperature, with an initial rapid increase, progressing through an optimum and decreasing at temperatures beyond (Jobling, 1988; Imsland *et al.*, 1996; Jonassen *et al.*, 1999; Otterlei *et al.*, 1999; Peck *et al.*, 2003). A similar such relationship has been shown in Peck *et al.*, (2003) in laboratory reared, cultured, 0-group cod from Canada when feeding at their maximum rate, in which growth peaked (at 2 % day^{-1}) at 12 °C. The finding of a weakly significant difference in optimal growth rate should be viewed with caution as this experiment was not replicated and differences could be due to tank effects, although all conditions were kept identical between experimental tanks. Had the cod from the Clyde Sea reduced feeding, growth rates could have dropped. A similar finding is reported in Peck *et al.* (2003) in which cod growth is investigated in relation to food intake. However, the differences in the peaks in growth rate seen in this study do reflect their respective environmental regimes. In the Clyde Sea, summer temperatures reach a mean of 12.3 ºC, and thus the low growth rate found at 13 ºC, may be because it is over their thermal range. The St Andrews Bay cod, however, do experience summer temperatures of 13 °C, which is perhaps why their growth rate still remains high at this temperature. The exact optimal temperature for St Andrews Bay is questionable, however. As there was no significant difference between growth rate at 9, 11 or 13 °C, the peak could be considered within this 4 °C range.

The comparison of growth rates between 8 and 12 °C showed that in all cases there was a trend for higher growth rates in the Clyde Sea population. However, the degree to which the populations differed varied between experiments and in one out of the four cases the difference was not significant. Such variability suggests that controlled environmental conditions do not necessarily equate with a complete control of conditions that influence growth and highlights the need to undertake multiple replicates in such studies. There are a number of possible explanations for the observed differences in growth rate, which could have also influenced growth in the first study. Firstly, despite the study being conducted on wild cod captured at an early stage, differences in post-hatching experience in fish caught at the two sites could have generated differences in one or more of the factors that influence growth rate (Johnston, 1993; Johnston *et al.*, 1998). Secondly the observed differences in growth rates might

have been the results of maternal effects. Maternal age, size and condition have been found to influence size of eggs and larvae in Atlantic cod (Kjesbu, 1989; Marteinsdottir and Steinarsson, 1998), although to the best of my knowledge there have been no studies reporting maternal effects in fish older than the larval stage. Thirdly, social interactions are an inherent source of unpredictable variation in communal tank experiments and may mediate population effects. Juvenile cod reared in tanks are known to be aggressive (Folkvord, 1991; Hatlen *et al.*, 2006; Chapter 2), particularly in low densities (Lambert and Dutil, 2001). Whilst fish were fed to excess in order to mitigate such effects, aggressive interactions may have accounted for some of the variation in size, although there was no evidence of a skewed distribution in this experiment associated with a few successful individuals. Finally the result might reflect genetic differences between the stocks, although it is worth pointing out here that there is no evidence from this study, or in the adult growth rate (Chapter 4), to suggest that it was due to haemoglobin genotype. To distinguish fully between these various possibilities would require common garden studies of fish held in standard conditions using fish of a known parentage, accounting for family effects and maternal size. However, the markedly differing environments of cultured cod to those experienced in the wild, can create fish with a higher condition and earlier age of maturity than they would in the wild (Thorsen *et al.*, 2003), and may result in differences in behaviour in only one generation (Huntingford, 2004).

The population effect on growth seen in the second study is for the most part, not consistent with other studies on cod, also reared under common environment conditions. Purchase and Brown (2000) documented a higher capacity for growth in a cooler, more northerly population (from the Grand Banks) than a warmer, more southerly population (from the Gulf of Maine), consistent with the countergradient hypothesis. Likewise, in the northeast Atlantic, Salvanes *et al*., (2004) report higher growth rates in a more northerly population along the Norwegian coast. There was no population by temperature interaction in the second study, which implies that there was no difference in plasticity to temperature between populations. The results therefore suggest that Clyde Sea cod may simply have a higher intrinsic capacity for growth than St Andrews Bay cod, similar to findings of Otterlei *et al.* (1999), in which larvae and juveniles of the more southerly Norwegian coastal cod had a higher growth rate under common-garden conditions than Northeast Arctic cod over a range of temperatures. However, the

finding that the peak in growth rate is at a lower temperature in the Clyde Sea cod suggests that they may not be as plastic to higher temperatures as the St Andrews Bay cod. Since summer temperatures on the northeast coast are higher in the summer and annual temperature is more variable than in the Clyde, the St Andrews Bay cod may have a higher capacity to cope with thermal changes that the Clyde Sea cod.

The trend in growth rate in the second study is consistent with a linear increase with temperature, and indicates that the range of temperatures in which the experiment was conducted was still in the phase of linear increase and thus 12 ºC is less than or at their optimal temperature for growth. However, the step-wise temperature experiment would suggest that 12 ºC is already super-optimal. This slight discrepancy could have arisen due to differences in experimental design or time of year at which the experiment took place. The step-wise increases of 2 °C every two weeks may reflect a more natural, seasonal increase than a rapid 4 °C rise. The 12 °C treatments also took place slightly later in the year than the higher temperature treatment in the step-wise experiment (April and May compared to April in the step-wise experiment) and it is possible that the cod are more adapted to higher temperatures approaching summer than earlier in the year.

The coastal distance between the two areas (in the order of 900 km) is far greater than the spatial scale upon which local adaptation has been confirmed in the past for other species (Imsland *et al.*, 2001; Conover *et al.*, 2006). Whilst there is currently no genetic evidence for reproductive isolation between Scottish east and west coast cod, simulations of larval transport (Heath and Gallego, 1997), and investigations of juvenile and adult fidelity from otolith microchemistry and tagging studies indicates there is no exchange between the study sites (Gibb *et al.*, 2007; Wright *et al.*, 2006a and b). Fish from the two study areas also differ with respect to reproductive traits (Yoneda and Wright, 2004). Therefore limited gene flow is likely, bringing with it the opportunity for genetic adaptation to the local environment.

For local adaptation to occur there must also be a selective advantage of the trait in question in addition to genetic variance. A higher growth rate may be adaptive for reasons such as reducing the risk of predation, a competitive advantage against other conspecifics (Folkvord and Ottera, 1993) and a reduction in age at maturation (Godø

and Haug, 1999). Such advantages may have led the cod from the Clyde Sea to select for a higher rate of growth, but there must also be constraining factors that have limited the growth rate of the St Andrews Bay fish. It may be that there is a trade-off in a higher growth rate with other factors, such as timing of maturity and energy allocation to reproduction (Wootton, 1998; Rijnsdorp, 1994). Yoneda and Wright (2004) reported a higher fecundity in cod of a given length and age using field data from the inshore region of the northern North Sea cod compared to the west coast, thus perhaps the slower juvenile growth rate in the St Andrews Bay cod enables a greater reproductive output. It is also suggested that the higher growth rate in the Clyde Sea population may be due to a greater food intake in the Clyde Sea cod than those from St Andrews Bay (Chapter 2). Likewise, Salvanes *et al.* (2004), suggest that the higher growth rate of the more northerly group of Norwegian cod was due to more successful competition for food and a higher allocation of energy to storage tissues than the more southerly group. Irrespective of the cause of the variation, the present study suggests a pattern of diversifying selection in growth rate across the temperature gradient of the two study areas.

6   CONCLUSION

In conclusion, the finding of a difference in elevation of the growth responses in fish held in controlled conditions raises the possibility of population divergence and local adaptation. The ability to cope with and adapt to rising temperatures is especially pertinent in the light of the current predictions of climate change and sea temperature rise. The precise effect of temperature on growth of juvenile cod from each population is difficult to predict due to the discrepancies between studies. However, this work suggests that juvenile cod growth increases with temperature between 8 and 12 ºC. In winter and spring, recent rises in temperature are likely to increase somatic growth in both populations. The study also suggests that the St Andrews Bay cod are adapted to higher thermal extremes than the Clyde Sea cod. However, these studies have concentrated within their current range of temperatures. The step-wise temperature experiment suggests that growth rate would decline at temperatures exceeding 11 ºC and possibly lower in the Clyde Sea, but before we can reliably predict the effect of a rise in sea temperature on local cod populations, further research is needed into the

response of growth to the upper extremes of temperature. From the discrepancies in results illustrated in these studies, I would also recommend carrying out such an experiment in a manner as close as possible to the natural situation, with the thermal regime varying on the same time scale as that in the wild. It would also be important to understand any consequences that thermally enhanced juvenile growth may have for maturation.

CHAPTER 4

Population variation in reproductive potential and response to temperature in Atlantic cod from the British Isles

1   INTRODUCTION

1.1   Spatial variation in reproductive investment

Life history theory predicts that the optimal reproductive strategy is a compromise between current reproduction versus growth, future reproduction and survival (reviewed in Stearns, 1989). Life history traits are often highly influenced by environmental effects and as a consequence significant spatial and temporal variability in life history traits, such as fecundity, may arise. Atlantic cod can exhibit a remarkable level of variation in reproductive traits across their species range. Female size-specific fecundity, the total number of ova in the gonads in a given year, of a 60 cm cod ranged between an average of 210 000 oocytes on the Flemish cap and 2.25 million in the eastern Baltic (Lambert *et al.*, 2005). Spatial variation is thought to be environment related, but trends may be masked by intra-regional and annual variability. Mature females can range in size from as little as 27 cm to as much as 140 cm within the same stock (Lambert *et al.*, 2005). The variability can also differ between years. In some years a female may produce over 2 million eggs, while in others she may skip spawning altogether (Burton *et al.*, 1997; Marshall *et al.*, 1998; Rideout *et al.*, 2006) or greatly reduce her fecundity (Kjesbu *et al.*, 1991). Individual fecundity may be related to her age, size and body condition (Stearns and Crandall, 1984; Kjesbu and Norbeg, 1991; Marshall *et al.*, 1998, 1999; Marteinsdottir and Begg, 2002). As fecundity is related to size in fishes, allocation of energy to spawning rather than growth could influence an individual's reproductive potential in future years (Henderson *et al.*, 1996). The focus of this chapter is to understand the spatial variability in reproductive potential in male and female Atlantic cod. Throughout this chapter I make reference to the term reproductive potential at both an individual level and also scaled up to a population level. Reproductive potential of an individual is the number of eggs it is expected to produce

in a given reproductive event, whereas at the stock level it has been defined by Trippel (1999) as, "The annual variation in a stock's ability to produce viable eggs and larvae that may eventually recruit to the adult population or fishery."

1.2   Potential influences on variability

The causes of spatial variation in reproductive traits are due to complex interactions between genotype and environment, in addition to trade-offs between allocation of energy to growth or reproduction. One such environmental variability is food availability. For example, fecundity of the Northeast Arctic cod stock is thought to vary with capelin biomass (Marshall *et al.*, 2003), one of their prime food sources (Kjesbu, 1998). Genetic differences in a phenotypic character may arise from adaptive divergence to their environment (McIntyre and Hutchings, 2003) or may reflect an evolutionary response to fishing (Law, 2000). By selecting for larger individuals, fishing may induce genetic changes in a population. For example, selective fishing mortality may drive a shift towards smaller sizes and younger ages at maturity, as was found in cod from eastern Labrador and southern Newfoundland, and the findings are strongly suggestive of fisheries induced evolution (Olsen *et al.*, 2004). Few studies, however, have investigated whether the spatial variability in reproductive traits is due simply to environmental effects or whether it is due to intrinsic genetic differences between populations. This chapter attempts to answer these questions and the approach taken is based on the 'common environment' method which rests on the assumption that if extrinsic factors can be controlled for and remain equal, emergent variation in 2 putative populations can be attributed to intrinsic population factors (Chapter 1). This is a powerful way of understanding the nature of biological variation and of disentangling the environmental from the intrinsic population effects (Conover and Schultz, 1995; Conover *et al.*, 2006).

1.3   The maturation process

Atlantic cod are iteroparous, spring spawning fish (Hislop, 1984). They are periodic spawners and release batches of eggs at regular intervals during the spawning period, which lasts about 6 to 8 weeks (Kjesbu, 1989). Development of the gonads is under endocrine control and oocyte growth is controlled by the sequestration of vitellogenin

(Tyler and Sumpter, 1996). Synthesis of vitellogenin requires lipids, the major store of which is found in the liver and in the white muscle (Kjesbu *et al.*, 1991; Lambert and Dutil, 1997). Oocyte development in cod and in most other teleosts occurs in four main stages: primary growth, cortical alveoli (yolk vesicle) formation, true vitellogenesis and maturation (Wallace and Selman, 1981; Kjesbu and Kryvi, 1989). Vitellogenesis (also known as yolk deposition), the deposition of nutrients in the oocytes, can begin about 6 months prior to spawning (Kjesbu, 1994) and the physiological commitment to mature (for brevity sometimes referred to as a "decision" on the part of the fish concerned) can occur up to a year before spawning (Skjæraasen *et al.*, 2006). The maturation process in males is somewhat different with spermatogenesis occurring as early as May in the year prior to spawning (Dahle *et al.*, 2003). An illustration of the intrinsic and extrinsic factors which contribute towards somatic growth and gonad development is given in Figure 4.1.
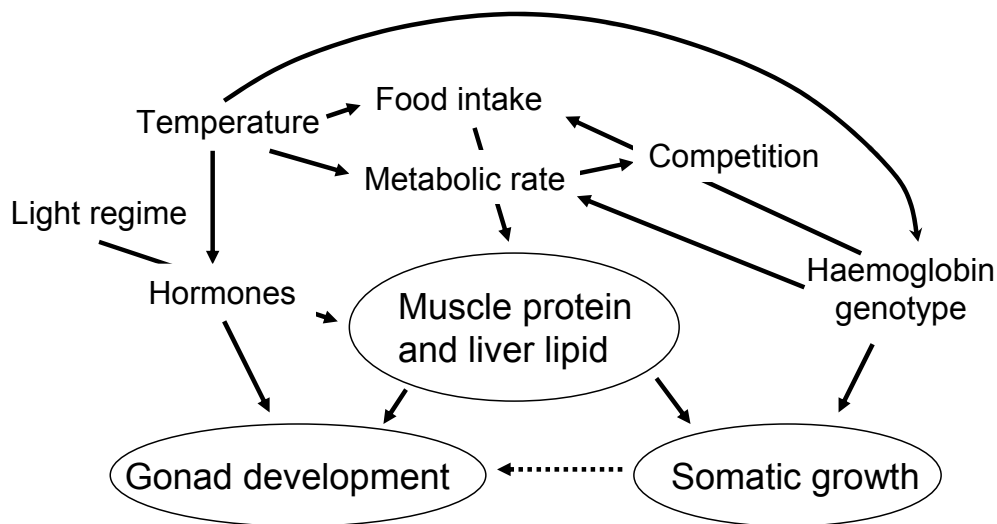
Figure 4.1. The contribution of intrinsic and extrinsic factors on gonad development and somatic growth.

1.4 Body size, condition and the "decision" to mature

Several studies have linked large size, fast growth and high lipid levels to reproductive potential in cod (e.g. Kjesbu and Holm, 1994; Rowe *et al.*, 1991; Silverstein and Shimma, 1994; Silverstein *et al.*, 1997). In the northeast Arctic, where cod store energy in their livers for subsequent migrations and spawning (Kjesbu, 1998), lipid energy has been shown to be a better proxy of egg production than spawning stock biomass

(Marshall *et al.*, 1999, 2000). In both cod (Yoneda and Wright, 2005a) and plaice (Holdway and Beamish, 1985; Rijnsdorp, 1990), it has been found that periods of fast growth are linked with a greater likelihood of maturing in any given year, although liver condition is also known to vary during the year (Skjæraasen *et al.*, 2006). Studies on cod (Kjesbu and Holm, 1994) and rainbow trout, *Oncorhynchus mykiss* (Bromage *et al.*, 1991), have found that fecundity was unaffected in fish that were fed on a low ration diet during the latter stages of vitellogenesis, while it was substantially reduced for fish fed on a small ration during early vitellogenesis. Evidence such as this suggests there may be critical windows in their development when they decide whether or not to mature and if so how much to invest in reproduction. For example, Skjæraasen *et al.* (2006) found that measurement of lipid energy three to four months before spawning was the most effective time for indicating prospective fecundity.

1.5   Influence of environment and temperature

Underlying the maturation process are endogenous, circannual rhythms that are entrained to environmental cycles. The effect of photoperiod on maturation has been well studied in cod (Davie *et al.*, 2007) and is thought to be a prime determinant of timing of reproduction (Bromage *et al.*, 2001; Skjæraasen *et al.*, 2006; Davie *et al.*, 2007). Over and above this, environmental factors, such as temperature, can greatly modify the process. Temperature is highly variable across the environmental range, but its effect is less well understood (Brander, 1995). Field studies, such as McIntyre and Hutchings (2003) and Lambert *et al.* (2005), have revealed spatial variability in fecundity in cod, which may be due to differences in water temperature. However, the effect is difficult to isolate from photoperiod, which can also vary over a temperature gradient (Bromage *et al.*, 2001). Temperature may influence growth and maturation through its effect on metabolism and surplus energy (Wootton, 1998). Temperature also directly affects synthesis and secretion of the hormones that control gametogenesis (van der Kraak and Pankhurst, 1997) and thus affects the phase of reproduction, particularly ovulation and spawning (Suquet *et al.*, 2005). It has also been found that a 1 ºC drop in temperature will delay spawning by 8 to 10 days in Norwegian coastal cod (Kjesbu, 1994). Thus, temperature may influence reproductive potential of cod and timing of both the decision to mature and spawning (Yoneda and Wright, 2005a).

Studies on the temperature effect on reproduction in cod and other fishes have found varying responses. A study of effects of temperature and food manipulation on first-time spawning cod has shown that low temperatures can result in a smaller proportion of both males and females maturing (Yoneda and Wright, 2005a and b). In Scottish west coast cod, relatively low winter temperatures may arrest vitellogenesis, at least under experimental conditions (Yoneda and Wright, 2005a). Likewise, observations on wild cod in Newfoundland suggested that disruptions to gametogenesis may be related to cold water temperatures (0-0.5 °C), although response to such extremes in temperature are unlikely to be experienced by temperate water cod (Rideout *et al.*, 2000). High temperatures have also been reported to have a negative effect both in cod and other species (Webb-Brewer *et al.*, 1999 in sturgeon, *Acipenser transmontanus*; Jobling *et al.*, 1995; Taranger and Hansen, 1993; Duncan *et al.*, 1999 in salmonids). A higher temperature treatment (12 °C compared to 8 °C) was reported to reduce the percentage of ovulating female cod (Johansen *et al.*, 1999) and the percentage of fertilised females was found to decease at temperatures higher than 10 °C (van der Meeren and Ivannikov, 2001). A study on the effect of temperature on pollack, *Pollachius pollachius* (a gadoid species), revealed that the number of spawning females, the duration of the spawning period and the total and viable egg output were all reduced at 12 °C compared with 8 and 10 °C (Suquet *et al.*, 2005). The differences in responses to temperature between such studies may be due to the magnitude in temperature differences between treatments. Given that populations may exhibit local adaptation to temperature, this may be relative to both the optimal species range and the population from where individuals were sampled.

1.6   Influence of genetics

In addition to environmental factors and prior condition, the maturation process may also be under genetic control (Aubin–Horth, 2005; Wright, 2007). In a common environment study on maturation in cod in relation to temperature and haemoglobin genotype, genotype was not found to affect maturation (Johansen *et al.*, 1999), but as the effects of haemoglobin genotype are often temperature dependent, the range of temperatures over which the study was carried out (8, 12 and 15 ºC) may not have been sufficiently wide to find an effect. Since maturation has been linked to growth rate (Skjæraasen *et al.*, 2004) and both haemoglobin genotype (Nævdal e*t al.*, 1992; Imsland

et al., 2004), and pantophysin, a nuclear RFLP locus (Case *et al.*, 2006), have been found to affect scope for growth, we might expect to find an effect of genetics on maturation.

1.7   Experimental approach and aims

The combination of traits which determine reproductive potential may be expected to vary with environmental conditions such as photoperiod and temperature over the species range, which ultimately may lead to population differentiation. A replicated common environment study, on wild caught cod from four locations along the Norwegian coast, found that spawning time differed by up to a month between groups, indicating a genetic difference in onset of spawning (Ottera *et al.*, 2006). The present study was designed primarily to examine spatial variation in reproductive potential in cod from geographically separated localities in response to temperature. Appreciation of the extent of variability in growth and reproductive potential in cod stocks and temperature driven shifts in these traits will enable conservation of the phenotypic diversity of the species in response to climate change.

In this study a common environment approach was used on wild-caught first-time spawning (recruit spawning) cod from three different populations around the British Isles, which differ in their natural thermal regimes (Appendix 1, Figures 1 and 2). Two experiments were conducted over two years. One compared cod from two regions; Lowestoft from the southern North Sea and St Andrews Bay from the northern North Sea on the Scottish east coast. The second compared cod from the Clyde Sea on the Scottish west coast to cod from St Andrews Bay and also investigated the response to a 2 °C rise in temperature. Since these cod originally came from differing thermal environments (Chapter 1, Appendix 1) and the geographic scale of this study corresponds to statistically significant differences between the populations (Chapters 1, 2 and 3), we might expect cod to exhibit local adaptation in reproductive traits in response to temperature. As previous studies have shown the importance of growth and condition, prior to spawning, in affecting reproductive potential (Skjæraasen *et al.*, 2006), I took account of individual variability in growth and condition over periods of 10 and 11 months prior to spawning. The questions I investigated were as follows: -

- Are there population differences in the reproductive development under controlled environment conditions?

- Is there a relationship between growth and/or body condition and reproductive potential?

- What is the effect of temperature on reproductive development in these populations?

2   METHODS

2.1   Fish collection and husbandry

Juvenile 0-group cod were collected from the wild from two locations from the east coast of Scotland in the North Sea and from the west coast of Scotland in the northeast Atlantic in October of 2004 and 2005 (as in Chapter 2). The Lowestoft cod were caught using gill nets in January and February 2005 (centre point at 52°27.46'N 1°44.36'E). Cod were housed at the FRS Marine Laboratory and maintained as described in Chapter 2.

2.2   Experimental set up

In both experiments a randomly selected sub-sample of fish was transferred into the two experimental tanks (7 x 3 m, 1 m depth, each tank was split in half using a plastic mesh separator to provide a total of 4 compartments) one month prior to starting the experiment for acclimation and PIT tagged according to methods in Chapter 2. Details of photoperiod, feeding and husbandry are the same as described in Chapter 2.

For the Lowestoft – St Andrews Bay experiment, 60 fish from each population were transferred in March 2006. They were separated into four single population groups and thus 30 fish were put in each compartment. Although it was desirable to obtain an even size distribution in each group, this was not possible in the Lowestoft – St Andrews Bay comparison, as the Lowestoft fish were larger at age 1 than the St Andrews Bay fish.

Both tanks were maintained at ambient for the Outer Firth of Forth region (Figure 4.2). After every monthly measurement, each group was transferred into a different compartment. This minimized any tank effects, including any small temperature discrepancies between the two tanks. Upon sacrifice, a number of fish were found to have an unidentifiable parasitic infection. The effect of presence or absence of the disease was analysed on reproductive potential, liver condition and growth parameters. No effect was found and so diseased individuals were not excluded from the analysis.
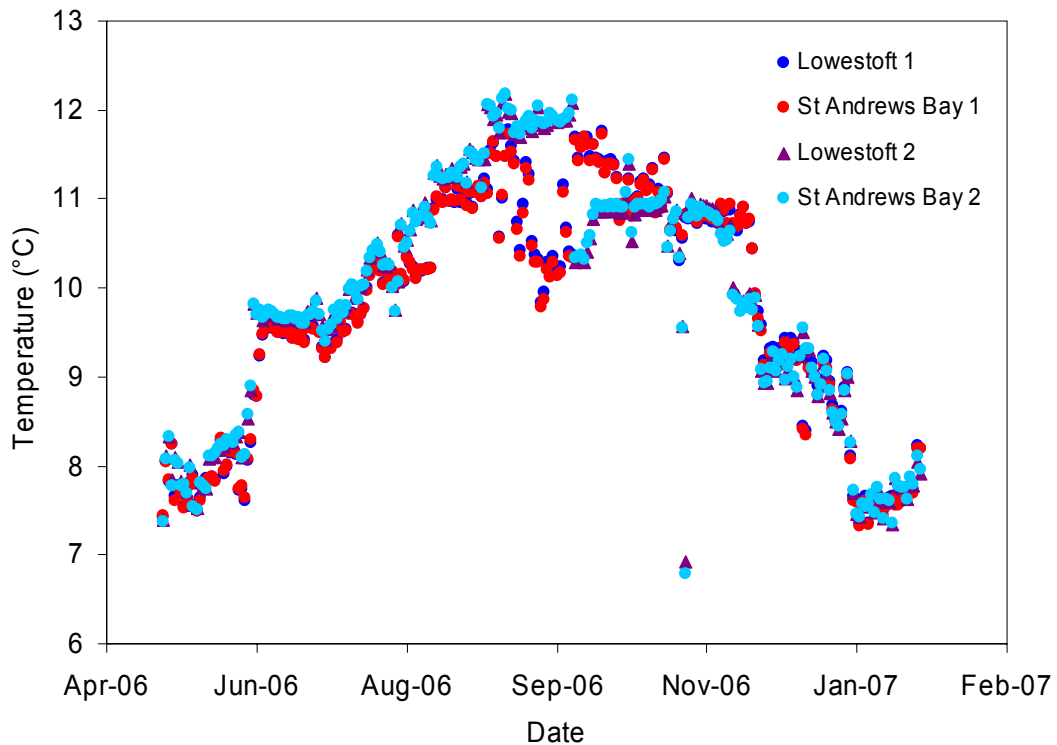
Figure 4.2. Temperature regime for each group of cod in the Lowestoft – St Andrews Bay experiment, during the experimental period between (26th April 2006 and 24th January 2007). The tanks were maintained at ambient sea surface temperature for the Outer Firth of Forth region. The sporadic points in October are due to a chiller malfunction in one of the tanks over two days.

In the Clyde Sea – St Andrews Bay experiment 40 fish from each population were moved into the experimental tanks in March 2007 and were separated into four groups of 20 fish of the same population. One tank was maintained at the ambient temperature, as in the previous experiment, while the other tank was at ambient plus 2 °C (Figure 4.3). In each tank, one half contained 20 St Andrews Bay cod and the other half contained 20 from the Clyde Sea.
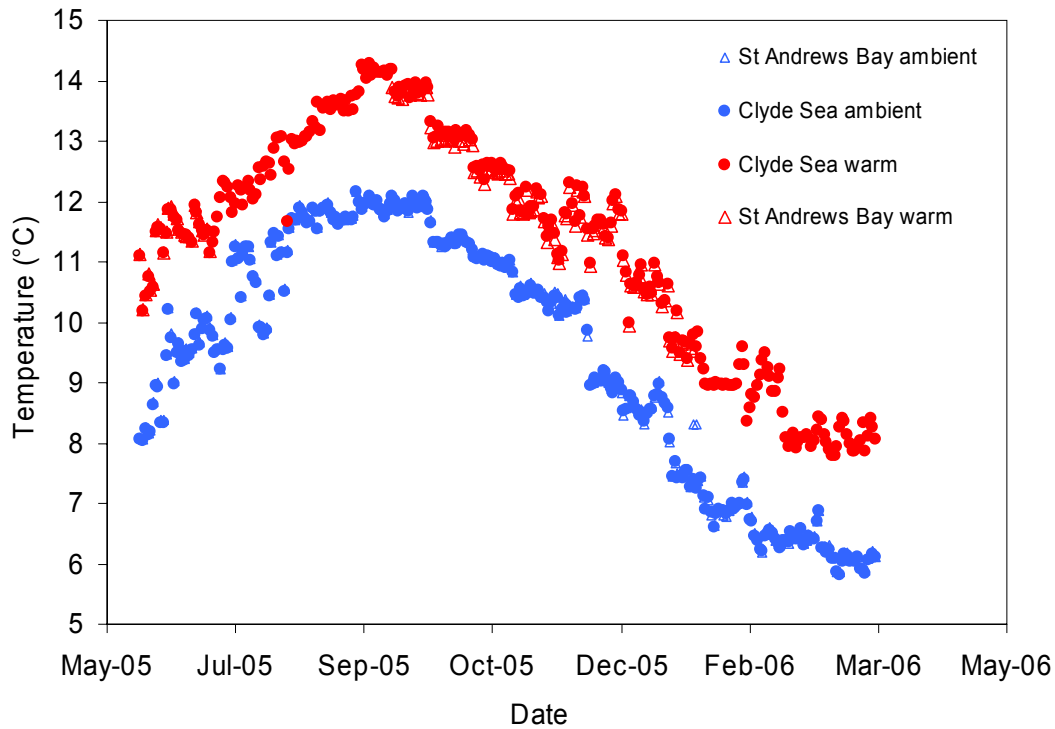
Figure 4.3. Temperature regime for Clyde Sea – St Andrews Bay experiment for the experimental period between 26th May 2005 and 21st March 2006. One tank was maintained at ambient for the Outer Firth of Forth region (blue), while the other tank was maintained at ambient plus 2 °C (red). Each tank contained two groups, one of each population (Clyde Sea: circles, St Andrews Bay: triangles).

2.3   Growth rate

The specific growth rate (G_s) and condition factor (K) (formulae in Chapter 2) was measured over the whole course of the experiment for each individual. Weight ($\pm$ 0.1 g) and length ($\pm$ 0.1 cm) measurements were taken monthly. In the Clyde Sea – St Andrews Bay experiment cod were measured fortnightly in the three months prior to spawning. Condition factor (Fulton's K) was also calculated monthly:

2.4   Fecundity and testicular development

In the Lowestoft – St Andrews Bay experiment, fish were sacrificed in January (prior to spawning time between January and March) and their gonads removed for analysis of fecundity and gonadosomatic index (GSI). In the Clyde Sea - St Andrews Bay experiment, fish were sampled fortnightly to assess their level of maturation prior to sacrifice in the three months before the predicted spawning period (January to March).

Initially slight pressure was applied along the abdominal cavity towards the genital pore and sperm or oocytes collected. Failing that, the catheterisation method was used in which a catheter was inserted into the vent and either oocytes or sperm were extruded if the fish was mature (McEvoy, 1984). The catheter was made out of a 20 ml disposable syringe barrel attached and glued to some flexible rubber tubing (3 mm internal diameter). The end of the tubing was burned to form an obliquely shaped, smooth end for entry into the vent. Males were sacrificed immediately prior to running. Testes were weighed ($\pm$ 0.1 g) and assessed for their maturity using the classification developed by Tomkiewicz *et al.* (2002) for Baltic cod. The GSI was calculated as:

$$GSI = 100(TW/W),$$

where TW is testis weight and W is total weight.

In females, oocytes were sampled from the specimens before spawning took place. The extracted oocytes were placed in physiological saline (1.07 % NaCl). Fecundity, defined as the number of vitellogenic oocytes in the ovary prior to spawning (Kjesbu and Holm, 1994), was derived using the auto-diametric fecundity method (Thorsen and Kjesbu, 2001). Predicted potential fecundity (F_{PP}) was expressed using the regression for cod developed by Thorsen and Kjesbu (2001) and supported by Yoneda (unpubl. data): F_{PP} = 2.139 x 10^{11} M_O $D_O^{-2.70}$ ($300 \leq D_O \leq 850$), where D_O is oocyte diameter. The oocytes were separated from the matrix by adding 2 ml of enzyme solution (Collagenase D and 5 mM $CaCl_2$ buffer) and shaking for 120 minutes on a shaker table at room temperature. The oocytes were then transferred into sera solution (ethanol: formalin: acetic acid ¼ 6: 3: 1) for examination under the microscope. Diameter measurements (D_O; μm) from a minimum of 100 oocytes were taken using Image Analysis. Those fish that exhibited late stage vitellogenesis were sacrificed.

In deterministic spawners, fish usually commit to spawning when the primary oocytes develop into vitellogenic oocytes (Kjesbu, 1994; Okuzawa, 2002). Mature fish were recognized as either having vitellogenic or hydrated stage oocytes. Vitellogenic ovaries were classified as either early (< 520 μm) or late stage oocytes (> 520 μm). Immature fish were recognized as having oocytes with pre-vitellogenic (pre-nucleolus or cortical alveoli stage) oocytes (Kjesbu, 1994).

Pre-ovulatory atresia can considerably reduce fecundity. To account for atresia, the percentage of pre-ovulatory alpha stage atretic oocytes relative to the total number of vitellogenic ooctyes (known as the intensity of atresia (A_O), Hunter and Macewicz, 1985; Witthames and Greer Walker, 1995) was calculated. Alpha stage atresia can be recognized under a binocular microscope as being of an irregular shape, with an uneven transparency (Oskarsson *et al.*, 2002). Yoneda and Wright (2005a) found no significant difference in the level of atresia between different sections of the ovary, thus oocytes were not selected from any particular part of the ovary.

Fecundity was estimated from specimens having ovaries with the late vitellogenesis. The potential fecundity (F_P) was then recalculated from the F_{PP} correcting for intensity of atresia using the following equation:

$$F_P = F_{PP} (100 - A_O),$$

where A_O is the intensity of atresia in the ovary.

Atresia was only accounted for in the Lowestoft – St Andrews Bay comparison, since some measurements were omitted in the Clyde Sea – St Andrews Bay experiment. Therefore F_P was used in the Lowestoft – St Andrews Bay experiment and F_{PP} in the Clyde Sea – St Andrews Bay experiment. A comparison of the two measurements in the Lowestoft – St Andrews Bay experiment suggests they are highly correlated ($P < 0.001$, $r^2 = 0.985$) and no difference in intensity of atresia was found between populations (t-test, t = -0.48, d.f. = 23, $P = 0.635$).

2.5   Somatic growth and condition

Fish were sacrificed immediately prior to spawning. The following measurements were taken upon sacrifice: total length (TL), total weight (W), ovarian weight (OW) or testis weight (TW) and liver weight (LW), all measured to 0.1 g. The following calculations were made (Lambert and Dutil, 1997): -

Somatic weight (SW) = W – (OW or TW),

Hepato-somatic index (IH) = 100 LW SW $^{-1}$,

Somatic condition factor (K) = 100 SW TL^{-3}.

2.6   Analysis

Analysis of fecundity, testis weight and liver weight were carried out using analysis of covariance (ANCOVA) with population as a factor and somatic weight or total weight less liver weight as the covariate. Interactions were tested but are only given where significant. Data were tested for normality and homogeneity of variance and a log transformation was carried out when appropriate. Growth of the cod up until the maturity measurement (January) was analysed using linear mixed effects models to account for repeated measures on the same fish over the 9 or 11 time points and differences between populations. Individual fish and time were fitted as a random effect with population, temperature (in the St Andrews Bay – Clyde Sea comparison only) and time as a factor and as a variate and their interaction as fixed effects. Time was modelled as both a factor and as a variate to account for divergence from a common trajectory between the groups over time and to account for variation between all time points. The model was fitted by first inputting all parameters and then simplified by removing parameters that were non-significant at the 5 % level as estimated by the Wald test. The AI algorithm was used and an antedependence structure of order 2 was selected as it produced the lowest Akaike Information Criterion (AIC) score (Chapter 3) in all models.

The relationship between growth parameters (natural log (ln) of weight and ln G_s) and condition factor (ln Fulton's K) at specific time points in the experimental period prior to spawning was analyzed using linear regression, with population and temperature (only in the Clyde Sea – St Andrews Bay experiment) as factors. These time points were chosen to minimize autocorrelation between them. In assessing the same individual at different time points there is always going to be some degree of autocorrelation. To reduce this effect, however, autocorrelation regression analysis was performed for each individual for ln weight, ln growth and ln condition for both males and females. Results suggested that in 78 % of cases there was no correlation between

ln weight after 3 time lags and no correlation in 100 % of cases after 4 time lags. In the G_s there was no correlation in 71.4 % after 3 time lags and none in 97.1 % after 4. In the condition (Fulton's K) there was no correlation after 2 time lags in 64.7 % of cases and none in 94.1 % of cases after 3. Time periods of 3 time lags apart (periods 1, 4 and 7) were therefore chosen whereby the autocorrelation is non-significant in the majority of cases.

In order to assess differences between populations in the proportions of fish reaching maturity for a given length, a maturity ogive could be plotted. However, this was not possible due to insufficient sample size and an overlapping size range of mature and immature cod (Table 4.1).

3   RESULTS

3.1   Population variation in northern and southern North Sea cod

3.1.1 Growth trajectories

The growth trajectories of the cod in the nine months preceding spawning in terms of their length, weight and condition factor (Fulton' K) for males and females are illustrated in Figure 4.4. Initially there was a small but significant size difference between populations. The Lowestoft males were longer initially (mean difference and SED, 6.3 $\pm$ 0.9, t = 7.36, d.f. = 34, $P < 0.001$), but had a lower condition factor (difference of 0.1 $\pm$ 0.0, t = -3.54, d.f. = 34, $P = 0.001$). Those females from the Lowestoft population were longer initially (median difference of 4.05, Mann-Whitney U, U = 95, n = 47, $P < 0.001$), but there were no differences in condition factor (t = -0.64, d.f. = 30.4, $P = 0.527$). Despite these initial differences, the variation between the two populations further increased throughout the experiment. A distinct bimodality in weight and length developed in the Lowestoft population. In those fish that grew well, the Lowestoft cod were larger than those from St Andrews Bay throughout the sampling period, but their condition factor was consistently lower. At the other end of the scale, the fish that grew more poorly generally came from the Lowestoft population.
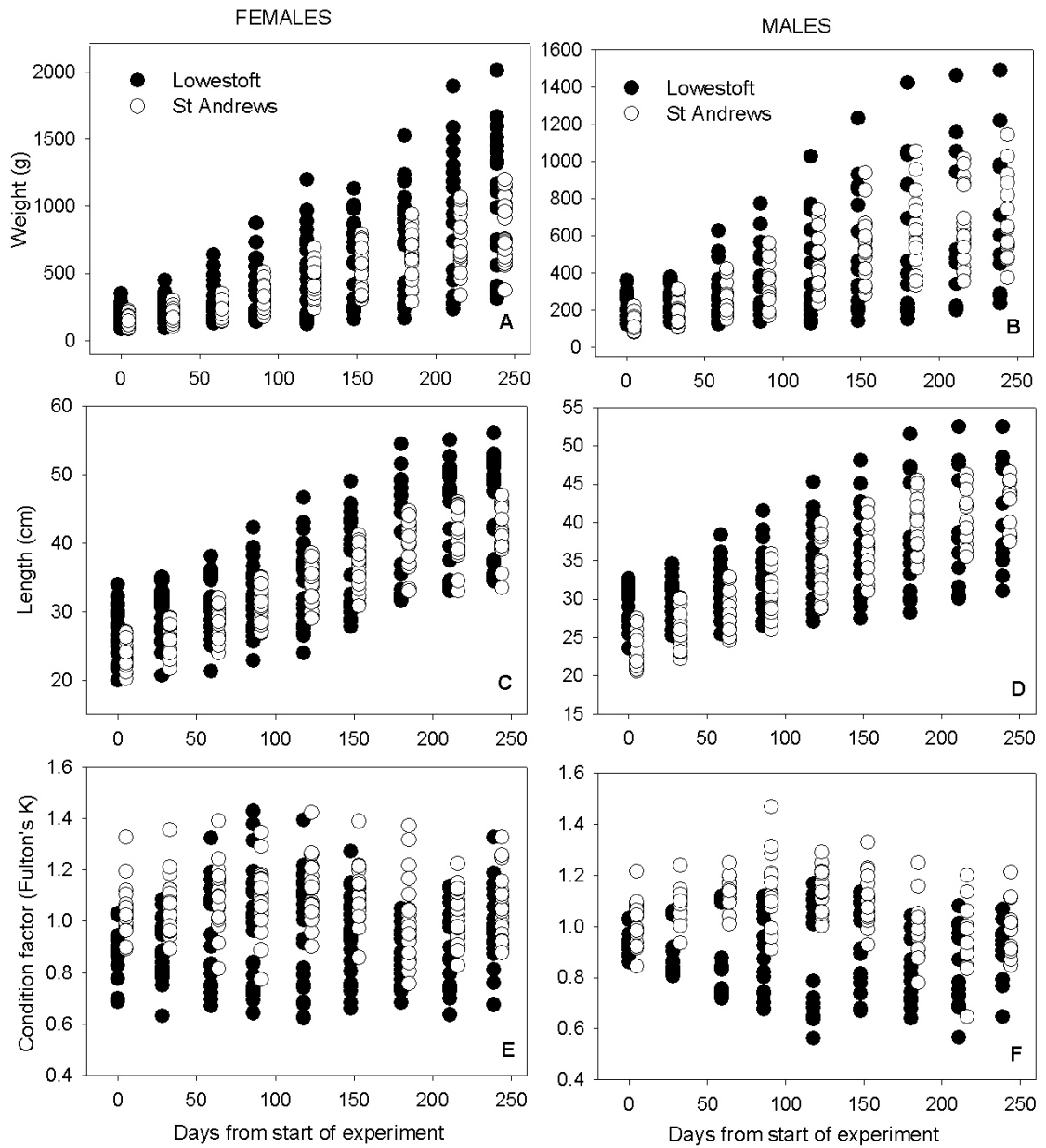
Figure 4.4. Growth trajectories by length, weight and condition factor (Fulton's K) for females (A, C and E) and males (B, D and F) for Lowestoft (solid circles) and St Andrews Bay (open circles) between April 2006 and December 2006.

3.1.2 Numbers maturing

The number of cod reaching maturity for each population and the length range of mature and immature cod from each population is given in Table 4.1 for males and females. More males than females matured and more of the St Andrews Bay cod matured than the Lowestoft cod.

Table 4.1. Stage of maturity of sacrificed females (by development of oocyte) and males (by staging of testes), proportion mature and length at time of sacrifice for mature and immature cod from Lowestoft and St Andrews Bay cod.

Sex	Population	Numbers at stage of maturity					Proportion mature	Length range (cm)	
		1	2	3	4	Not staged		Immature	Mature
Female	Lowestoft	11	2	5	3	5	0.48	34.5 - 51.5	35.5 - 56.0
	St Andrews	6	3	10	5	1	0.75	33.5 - 47.0	35.5 - 47.0
Male	Lowestoft	5	1	1	2	4	0.58	31.0 - 37.0	36.0 - 52.5
	St Andrews	0	3	0	8	4	1.00	n/a	37.5 - 46.5

In order to work with the bimodality in the dataset (Figure 4.4), immature fish were excluded from the analysis of growth. These included six males from Lowestoft, 10 females from Lowestoft and one female from St Andrews Bay. The linear mixed effects models used to model growth of the males and females accounting for population and haemoglobin genotype was run on the dataset excluding the immature individuals, but a very similar result was found when analysing the full dataset. Results of the model are given in Table 4.2 and Figure 4.5 and 4.6. In the females growth was approximately linear with a significant population effect but no population x time interaction. In the males, the interaction term between time and population demonstrates that the two populations were growing in a non-parallel profile, although the interaction between time (factor) and population indicates that some of the variation through time is also random. In both sexes, the Lowestoft cod were heavier throughout the experiment.
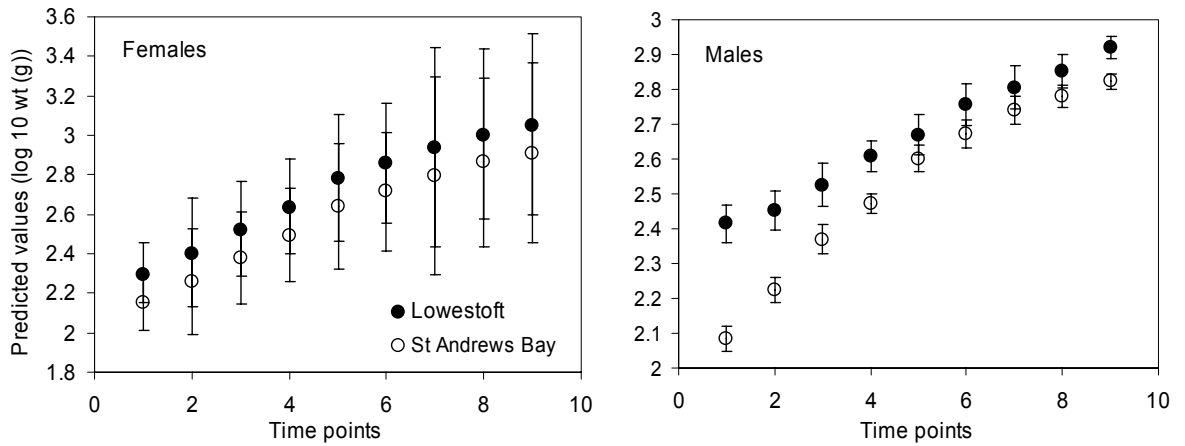
Figure 4.5. Predicted values of the log $_{10}$ weight (g) from the mixed effects models for the mature females and males between April 2006 and December 2006, displaying the mean and SEM for each population and temperature; solid circles: Lowestoft, open circles: St Andrews Bay cod. Both populations were measured at the same time intervals. Significance given in Table 4.2.
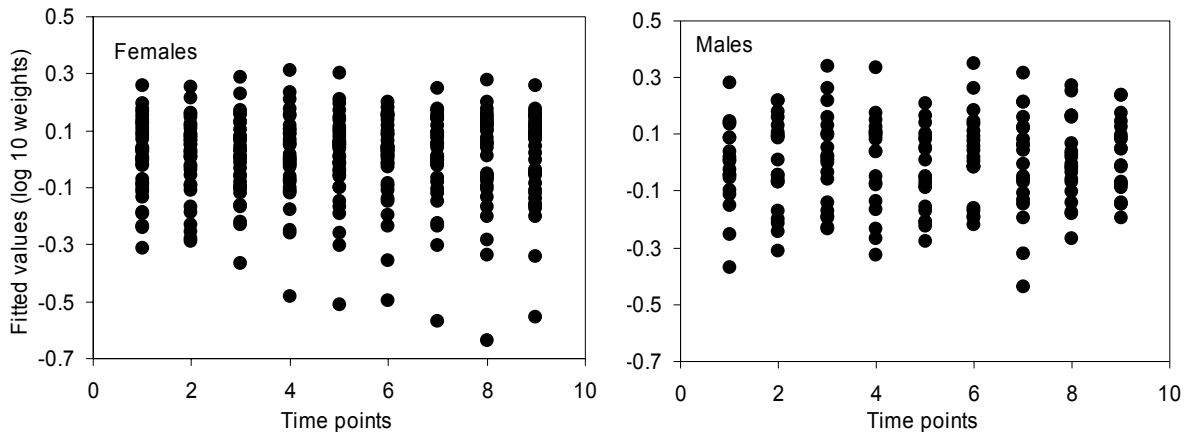
Figure 4.6. Residuals over time of the mixed effects models on log $_{10}$ weights (g) for females and males.

Table 4.2. Modelling the weight change of the mature males and females through time using a linear mixed effects model on log $_{10}$ weight (g) using an antedependence Order 2 model. Random and fixed effects are given in the table and the Wald statistic with significance.

Sex	Fixed effects	Random effects	Factor	Wald statistic	d.f	chi pr
Females of maturity stage 2+	constant + time + time (factor) + population	individual + individual.time (factor)	time	872	1,34	<0.001
			time (factor)	10.8	7,34	<0.001
			population	12.8	1,34	0.001
Males of maturity stage 2+	constant + time + time (factor) + population + population.time + population.time (factor)	individual + individual.time (factor)	time	609	1,18	<0.001
			time (factor)	6.06	7,18	<0.001
			population	30.5	1,18	<0.001
			time.population	24.7	1,18	<0.001
			time(factor). population	3.61	7,18	0.0132

3.1.3 Reproductive potential and liver condition

Population differences in potential fecundity or testis weight and liver size (presented separately for males and females) are illustrated in Figure 4.7, parts A to D and in Table 4.3. Potential fecundity and GSI varied more in the Lowestoft population than in those from St Andrews Bay, reflecting the bimodality in the Lowestoft population. Potential fecundity varied between 522 000 and 1.18 million in the Lowestoft cod and 173 000 and 814 000 in St Andrews Bay. Male GSI varied between 0.02 and 14.2 in the Lowestoft cod and between 1.0 and 13.0 in the St Andrews Bay cod. Figure 7, parts E and F illustrate the relationship between relative liver weight and fecundity or testis size for females and males respectively. No difference in potential fecundity was found between populations. The Lowestoft cod did have a greater absolute fecundity (F_{1-26} = 0.23, P = 0.633), but they were also larger and heavier females in all populations were found to have a higher fecundity (F_{1-26} = 15.9, P = 0.001).

The females of the St Andrews Bay population had significantly larger livers for a given weight than did those from Lowestoft (F_{1-43} = 204, P < 0.001), but there was no relationship between liver size and fecundity (P = 0.638). In the males, the St Andrews Bay cod had significantly higher testis weight than those from Lowestoft for a given weight (F_{1-21} = 6.08, P = 0.023) and weight was positively correlated with testis size (F_{1-21} = 17.9, P < 0.001). There was also a significant positive relationship between relative liver size and relative testis size for each population when accounting for size (F_{3-26} = 8.77, P < 0.001). There was no evidence for an effect of haemoglobin genotype on reproductive potential, liver condition or growth in either sex and thus it was dropped from the models.
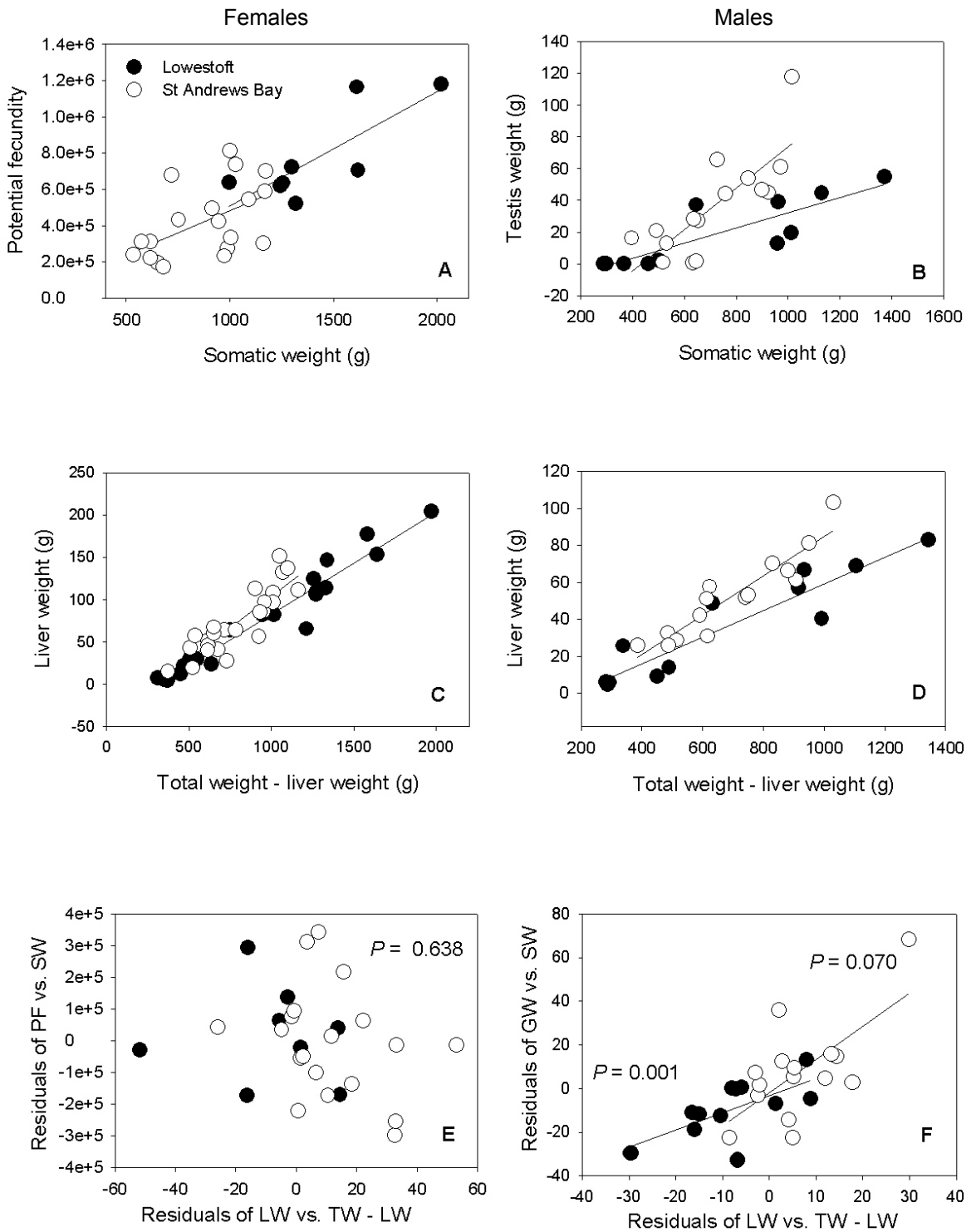
Figure 4.7. Population differences in reproductive traits for females (A, C and E) and males (B, D and F) between St Andrews Bay (open circles) and Lowestoft cod (closed circles). Plots A – D illustrate differences in potential fecundity (A), testis weight (B) and liver weight (C and D) for a given weight of fish and are analysed using ANCOVAs given in Table 4.3 In E and F regressions are given between potential fecundity (E) or male gonad weight (F) against liver size. In E this is fitted as Residuals of potential fecundity (PF) vs. SW = residuals of LW vs. TW – LW. In F this is fitted as Residuals of testis weight (TW) vs. somatic weight (SW) = residuals of liver weight (LW) vs. total weight (TW) - LW + population (significance given in the figures).

Table 4.3. Analysis of covariance (ANCOVA) of the potential fecundity or testis weight and liver weight, with population as the factor and with somatic weight or total weight less liver weight as the covariate.

Sex	Response	Factor	F	*P*
Female	potential fecundity	Population	$F_{1\text{-}26} = 0.23$	0.633
		somatic weight	$F_{1\text{-}26} = 15.9$	0.001
	liver weight	Population	$F_{1\text{-}43} = 204$	<0.001
		total weight – liver weight	$F_{1\text{-}43} = 5.56$	0.230
Male	testis weight	Population	$F_{1\text{-}21} = 6.08$	0.023
		somatic weight	$F_{1\text{-}21} = 17.9$	<0.001
	liver weight	Population	$F_{1\text{-}21} = 4.08$	<0.059
		total weight – liver weight	$F_{1\text{-}21} = 63.6$	<0.001
		population.total weight – liver weight	$F_{1\text{-}21} = 9.39$	0.007

3.1.4 Relationship between reproductive potential and weight, growth and condition factor preceding spawning

The relationship between potential fecundity or gonadosomatic index in the males and weight, G_s and condition factor (Fulton's K) in the time preceding spawning (April, July and October) for Lowestoft and St Andrews Bay cod are given in Figure 4.8 (females) and Figure 4.9 (males). In females, weight predicted fecundity at all time periods, but this relationship was strongest in July. Growth and condition were poorer predictors of fecundity. There was a significant negative relationship of potential fecundity with growth in July in the St Andrews Bay population and a marginal relationship in the Lowestoft population. Condition was only a significant predictor in the Lowestoft population in the first time period (April), at which point there was a negative relationship with condition and fecundity. In the males, weight was only a significant predictor of GSI in the St Andrews Bay population, the trend being for larger fish to have larger testes. There was no relationship with growth, but again condition only had a positive relationship with GSI in the St Andrews Bay population, the strongest relationship being in October.
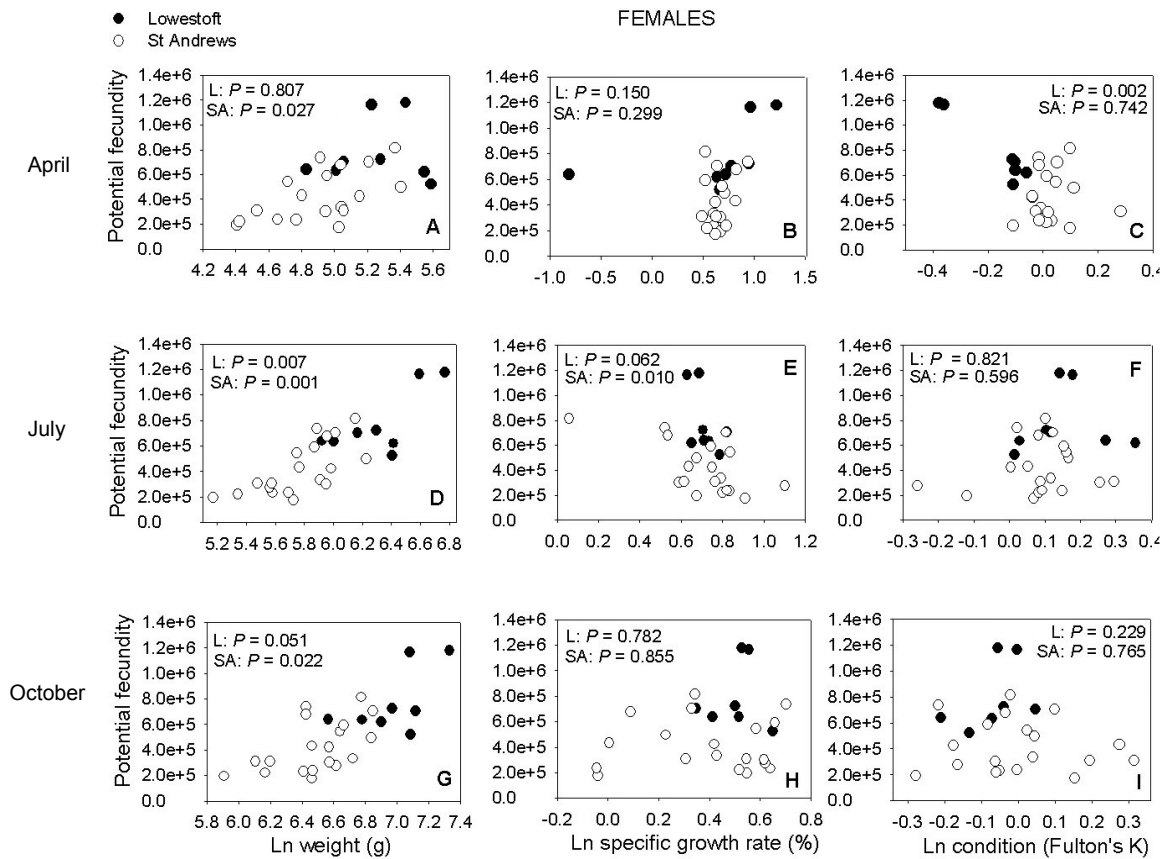
Figure 4.8. Relationships between ln (natural log) total weight, ln G_s (% day^{-1}) and ln condition factor (Fulton's K) and potential fecundity at time points 1 (April 06) (plots A, B and C), 4 (July 06) (plots D, E and F) and 7 (October 06) (plots G, H and I) for the Lowestoft (black filled circles) and St Andrews Bay (open circles) cod. *P*-values of regressions given in plots for Lowestoft (L) and St Andrews Bay (SA).
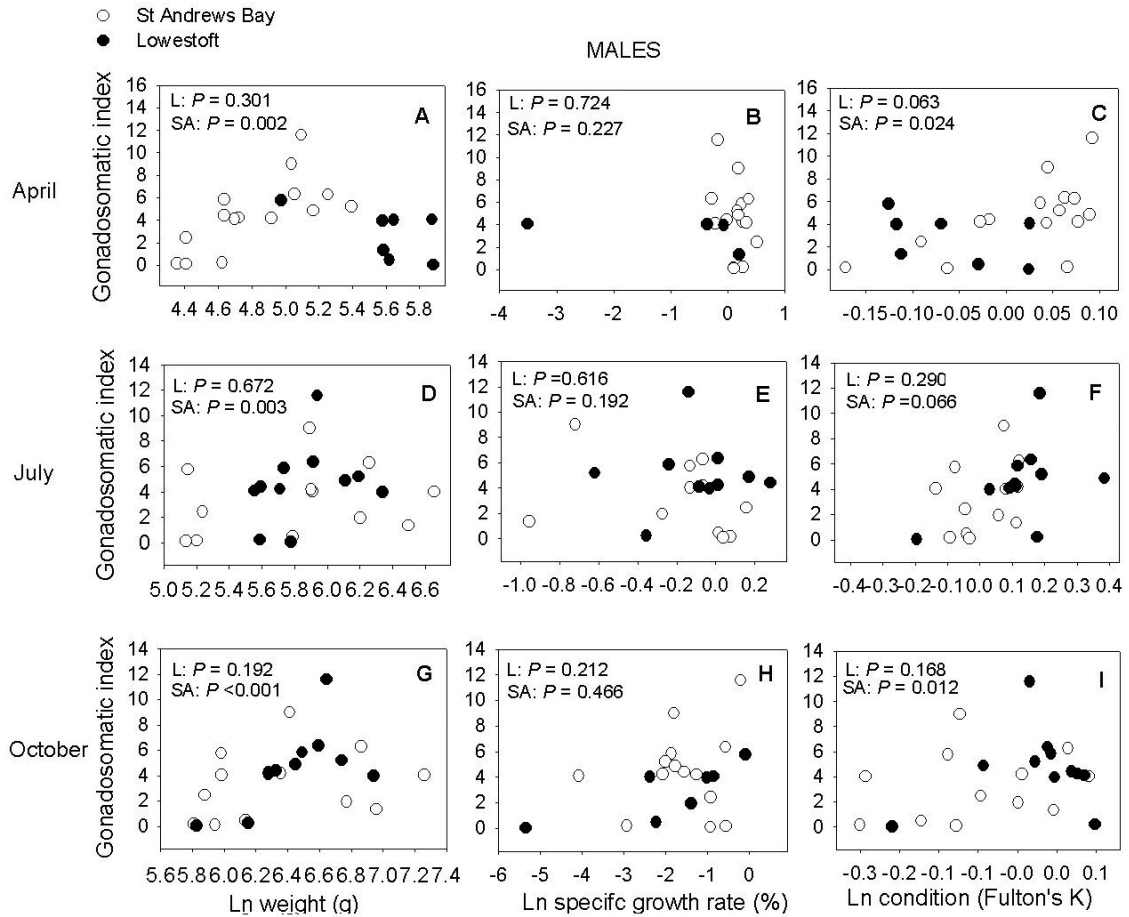
Figure 4.9. Relationships between ln total weight, ln G_s (% day^{-1}) and ln condition factor (Fulton's K) with male GSI at time points 1 (April 06) (plots A, B and C), 4 (July 06) (plots D, E and F) and 7 (October 06) (plots G, H and I) for the Lowestoft (black filled circles) and St Andrews Bay (open circles). *P*-values of regressions given in plots for Lowestoft (L) and St Andrews Bay (SA).

3.2 Population variation and response to temperature in Scottish east and west coast cod

3.2.1 Growth trajectories

The growth trajectories, in terms of weight, length and condition factor, for all individuals are illustrated in Figure 4.10 for females and Figure 4.11 for males. The majority of cod grew well, but there was great variation in the rate of growth and in condition. Cod were size-matched initially for length and allocated to tanks randomly with respect to gender. No tank effects were found for length ($F_{3-55} = 2.26$, $P = 0.092$) or weight ($F_{3-55} = 1.75$, $P = 0.169$). There were, however, significant differences in condition (Fulton's K) ($F_{3-55} = 3.28$, $P = 0.028$). Condition of the St Andrews Bay cod

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was on average 0.057 ± 0.021 higher than those from the Clyde Sea. There were no differences in weight ($F_{3-22} = 1.69$, $P = 0.281$) or condition ($F_{3-22} = 1.30$, $P = 0.303$) between groups (replicates). After the initial period, there were no significant differences in length, weight or condition in the females between temperature treatments or populations. There was however, bimodality in weights and lengths, which developed from time period 3 in the Clyde Sea population in the warm treatment. The distribution in the ambient treatment and for the St Andrews Bay population in both was more continuous. Condition was initially low and uniform (mean and 95 % C.I.: 0.95 ± 0.40), but became highly variable over time ranging between 0.8 and 1.5 in period 11.

In males there were no initial differences in length or weight, but there was a difference in condition between groups, with those from St Andrews Bay in the ambient treatment having a significantly higher condition factor than the other groups ($F_{3-32} = 1.05$, $P = 0.008$). In general, not including those fish that did not mature, the Clyde Sea cod were heavier and longer throughout than those from St Andrews Bay, but there was also greater heterogeneity in those from the Clyde Sea, especially in the ambient treatment. There was a significant difference in length in period 2 and weight in periods 2 and 3. In period 2, those in the warm treatment were significantly longer than those in the ambient treatment (mean difference of 2.6 ± 1.1, $F_{1-32} = 6.08$, $P = 0.019$) and a similar pattern was found for weight. Condition was variable throughout and, as in the females, initially had a much lower range (0.8 to 1.2) than in the final period (0.9 to 1.4). In the ambient treatment the St Andrews Bay cod generally maintained a higher condition. In periods 3 and 4 there were significant differences in condition factor. Those from St Andrews Bay in the ambient treatment had the highest condition and those in the warm treatment had the lowest ($F_{1-31} = 7.77$, $P = 0.009$). This was also evident in period 11 in a population x temperature interaction ($F_{1-23} = 1.53$, $P = 0.230$).
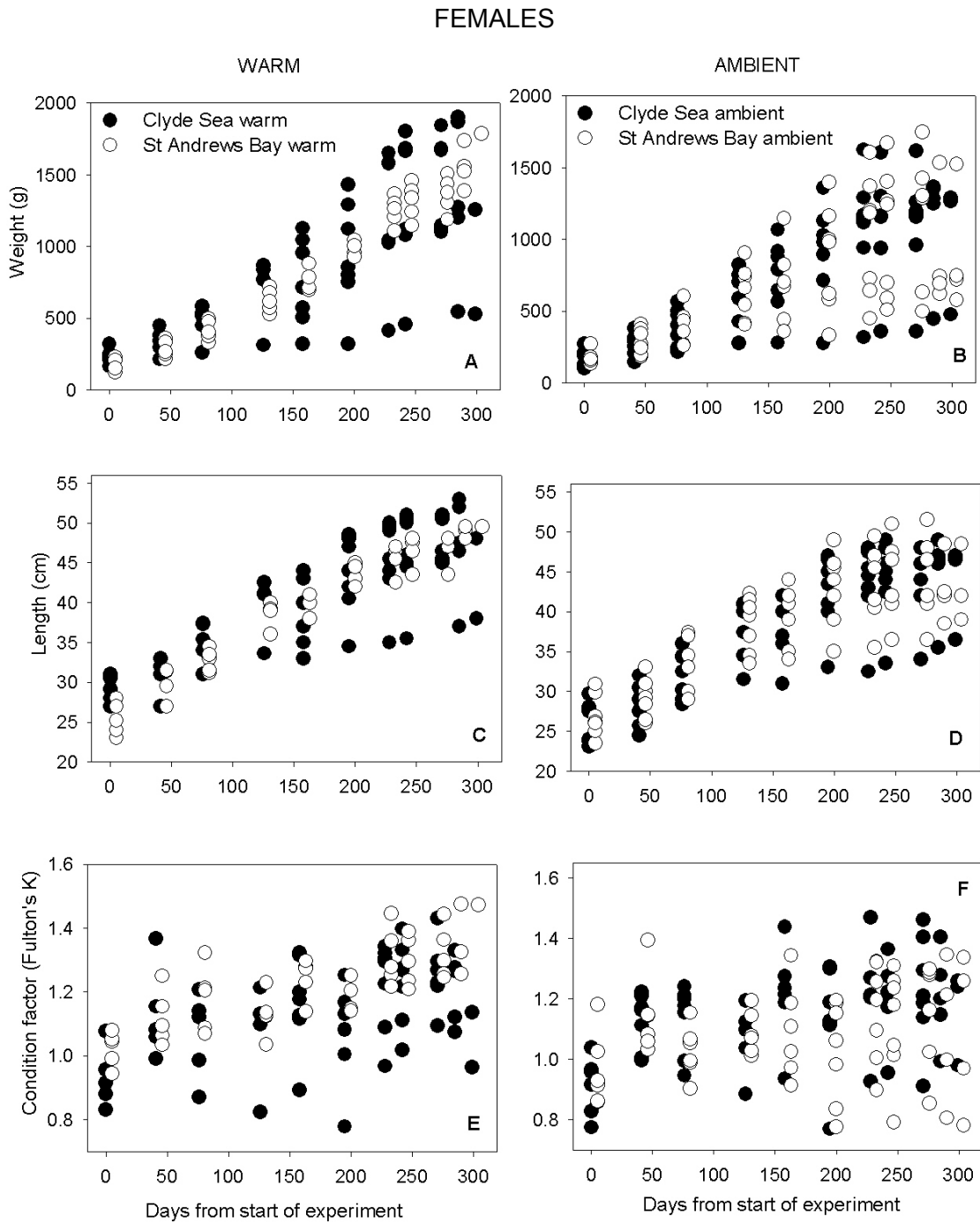
Figure 4.10. Growth trajectories by length, weight and condition factor (Fulton's K) for females in the warm treatment (A, C and E) and ambient treatment (B, D and F) from the Clyde Sea (solid black circles) and St Andrews Bay (open circles) between May 2005 and March 2006.
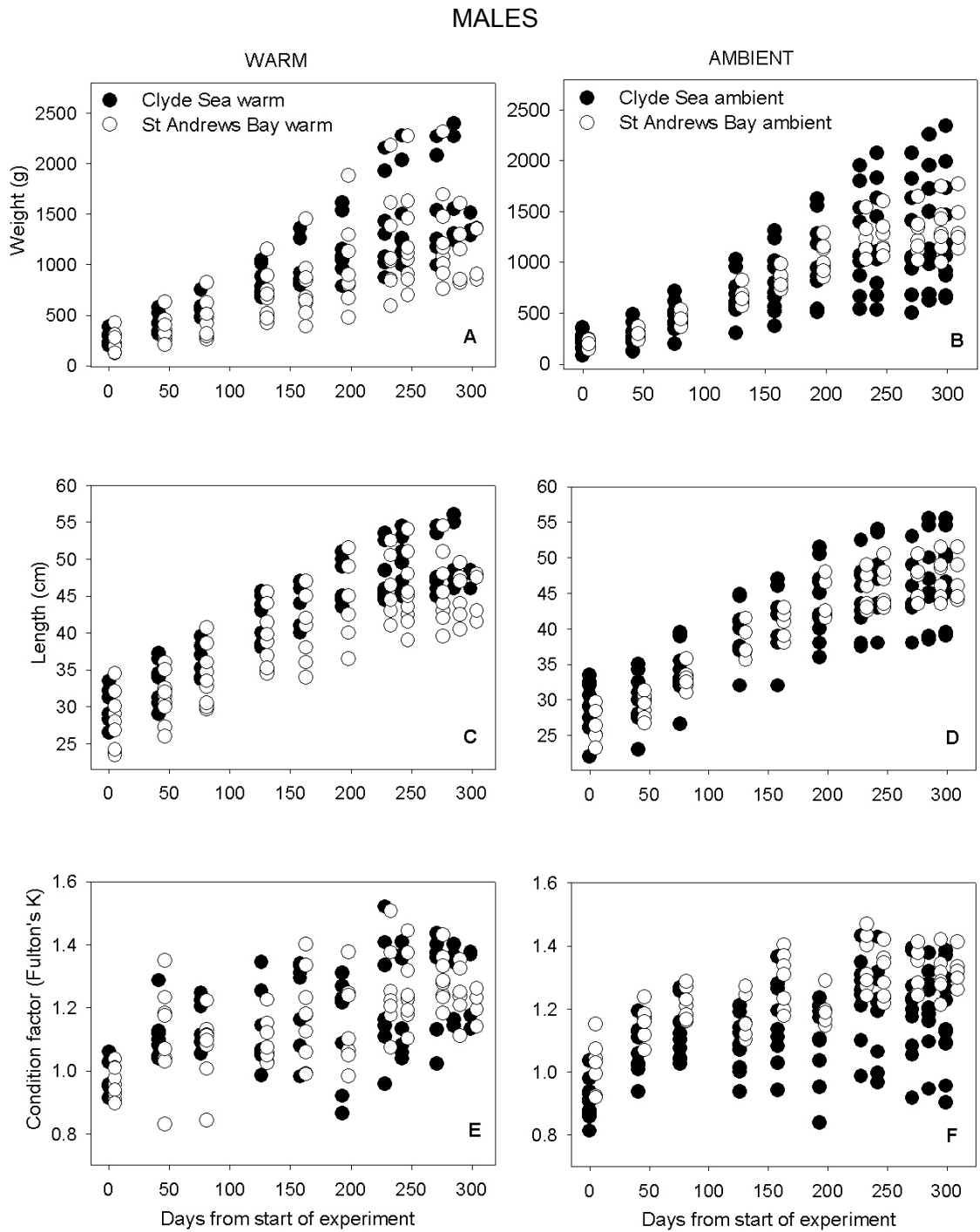
Figure 4.11. Growth trajectories by length, weight and condition factor (Fulton's K) for males in the warm treatment (A, C and E) and ambient treatment (B, D and F) for the Clyde Sea (solid black circles) and St Andrews Bay (open circles) between May 2005 and March 2006.

3.2.2 Numbers maturing

Numbers of individuals reaching maturity and the stage of maturity are given in Table 4.4, including the size range of immature and mature cod from each group. Differences in length between mature and immature fish could not be assessed statistically using a logistic regression method, due to an insufficient number of immature individuals. Only three out of 59 individuals did not mature by the time they were sacrificed in March. These were all females from the Clyde Sea, two from the ambient treatment and one from the warm. The three that did not mature were all the shortest or second shortest of the females in their group. The size range of the mature individuals was between 39 and 53 cm in the females and between 39 and 56 cm in the males.

Table 4.4. The number and proportion of males and females reaching maturity when sacrificed (February to March 06), the stage of maturity and the length range for immature and mature cod from each group.

Sex	Population	Temperature	Numbers at stage of maturity					Proportion matured	Length range	
									Immature	Mature
			1	2	3	4	5			
Female	Clyde Sea	Warm	1	5				0.8	38.0	46.0 - 53.0
	St Andrews	Warm		5				1.0		43.5 - 49.5
	Clyde Sea	Ambient	2	5				0.8	36.5 – 38.0	43.0 – 49.0
	St Andrews	Ambient		7				1.0		39.0 - 51.5
Male	Clyde Sea	Warm		4	3			1.0		46.0 – 56.0
	St Andrews	Warm		6	3			1.0		41.5 - 54.0
	Clyde Sea	Ambient		9	2			1.0		39.0 – 55.5
	St Andrews	Ambient		6		1		1.0		43.5 - 51.5

3.2.3 Modelling growth trajectories

Those individuals that did not reach maturity were excluded from the analysis of growth trajectories, since there are known to be physiological differences in the allocation of energy to growth between maturing and non-maturing individuals (Yoneda and Wright, 2005a) and there were insufficient numbers of immature individuals to be included as a separate group. Mixed effects models were used on remaining individuals separately for females and males, given in Table 4.5 and predicted values from the models in Figure 4.12. No pattern in the residuals over time was found (Figure 4.13). In both males and females, cod exhibited a seasonal parabolic growth curve with growth rate initially high, but falling to zero or less than zero at maturity. In females growth varied with respect to time and temperature at certain time points, as indicated by the time x temperature interaction term. These temperature-related differences appeared to be most apparent towards the beginning and towards maturation. In the males there was a significant effect of temperature, time and a marginal effect of temperature x population. The Clyde Sea cod in the warm treatment maintained a higher weight while those in the ambient treatment usually had the lowest mean weight. The interactions between time (factor) with temperature and population indicates that the effects of temperature and population through time were variable but with no particular pattern.

Table 4.5. Mixed effects models of the growth of the mature females and males through time (May 06 to March 07). The effect of population, temperature and time on weight was modelled using an ante-dependence structure of order 2. Random and fixed effects along with significance of the model fit are given in the table.

Sex	Fixed effects	Random effects	Factor	Wald statistic	d.f.	chi pr
Females excluding maturity stage 1	constant + time + time (factor) + temperature + time (factor). temperature	individual + individual. time (factor)	time	64.8	10,10	<0.001
			time (factor)	180	10,10	<0.001
			temperature	0.37	1,10	0.557
			time (factor).temperature	4.33	10,10	0.015
Males (all matured)	constant + time + time (factor) + population + time. population + time (factor). temperature + time (factor). population	individual + individual. time (factor)	time	1320	1,26	<0.001
			time (factor)	203	9,26	<0.001
			population	0.27	1,26	0.608
			temperature	6.33	1,26	0.018
			time.population	4.42	1,26	0.0454
			time (factor).temperature	5.83	10,26	<0.001
			time (factor).population	3.09	9,26	0.0120

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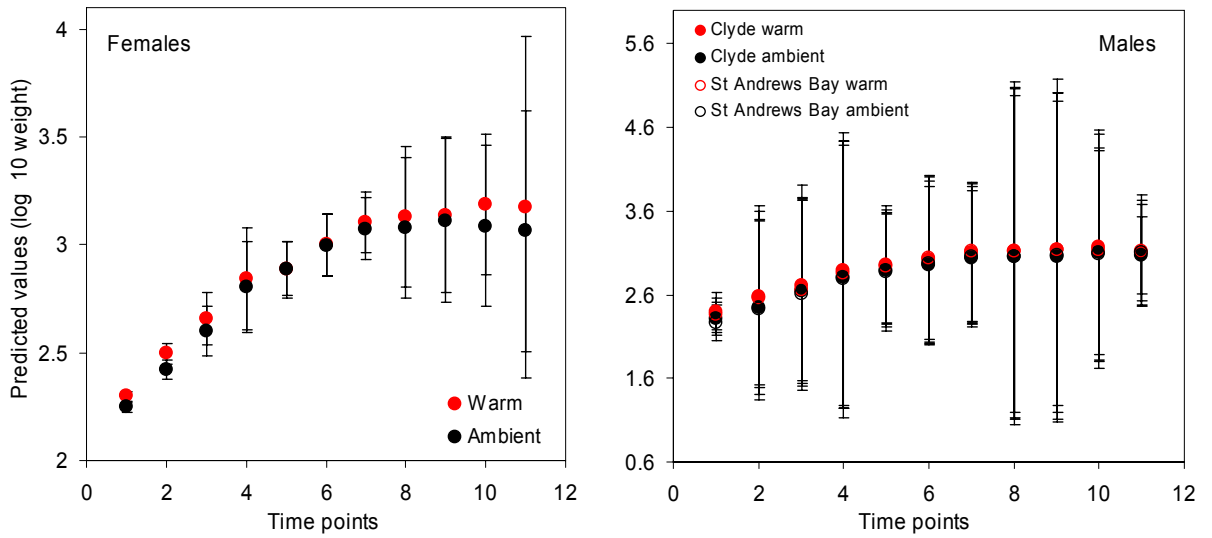
Figure 4.12. Predicted values of the log $_{10}$ weight (g) from the mixed effect models for the mature females (A) and males (B), displaying the mean and SEM for each population and temperature; solid red circles: Clyde Sea warm, solid black circles: Clyde Sea ambient, open red circles: St Andrews Bay warm, open black circles: St Andrews Bay ambient. Both populations were measured at the same time points from May 05 to March 06.
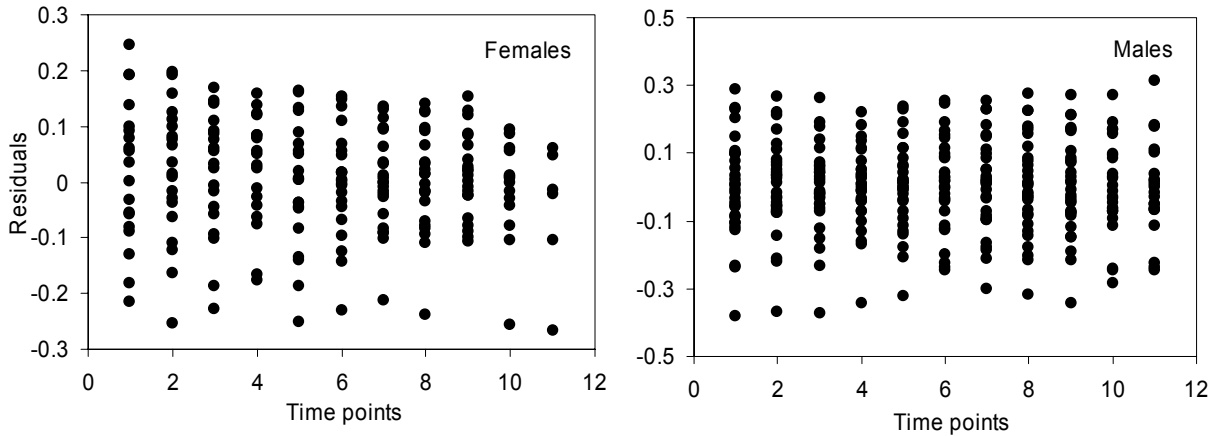
Figure 4.13. Residuals over time from the mixed effects models on log $_{10}$ weights (g) for females and males.

3.2.4 Reproductive potential and liver condition

Differences in potential fecundity or testis weight and liver size between groups are given in Table 4.6 and Figures 4.14 and 4.15 (A to D). A significant positive effect of

somatic weight was found on potential fecundity ($P < 0.001$) and the Clyde Sea cod had a marginally significant higher fecundity ($P = 0.062$). In males there was a similar pattern with weight, but the St Andrews Bay cod had a significantly higher testis weight ($P = 0.001$). In females the St Andrews Bay cod had a significantly higher liver weight ($P = 0.025$) and males in the ambient temperature treatment had a marginally greater liver size ($P = 0.054$). The relationship between the residuals of liver weight and size and between potential fecundity or testis weight and size was assessed to examine the effect of liver condition on reproductive output (Figures 4.14 and 4.15, E and F). No significant relationship was found.

Table 4.6. Analysis of covariance (ANCOVA) on potential fecundity or testis weight and liver weight by population with somatic weight and total weight less liver weight as covariates.

Sex	Response	Factor	F	P
Female	potential fecundity	population	$F_{1-21} = 3.94$	0.062
		Somatic weight	$F_{1-21} = 16.4$	<0.001
	liver weight	population	$F_{1-25} = 5.79$	0.025
		total weight – liver weight	$F_{1-25} = 98.9$	<0.001
Male	$\log_{10}$ testis weight	population	$F_{1-32} = 13.3$	0.001
		Somatic weight	$F_{1-32} = 63.6$	<0.001
	liver weight	temperature	$F_{1-33} = 4.01$	0.054
		total weight – liver weight	$F_{1-33} = 166$	<0.001
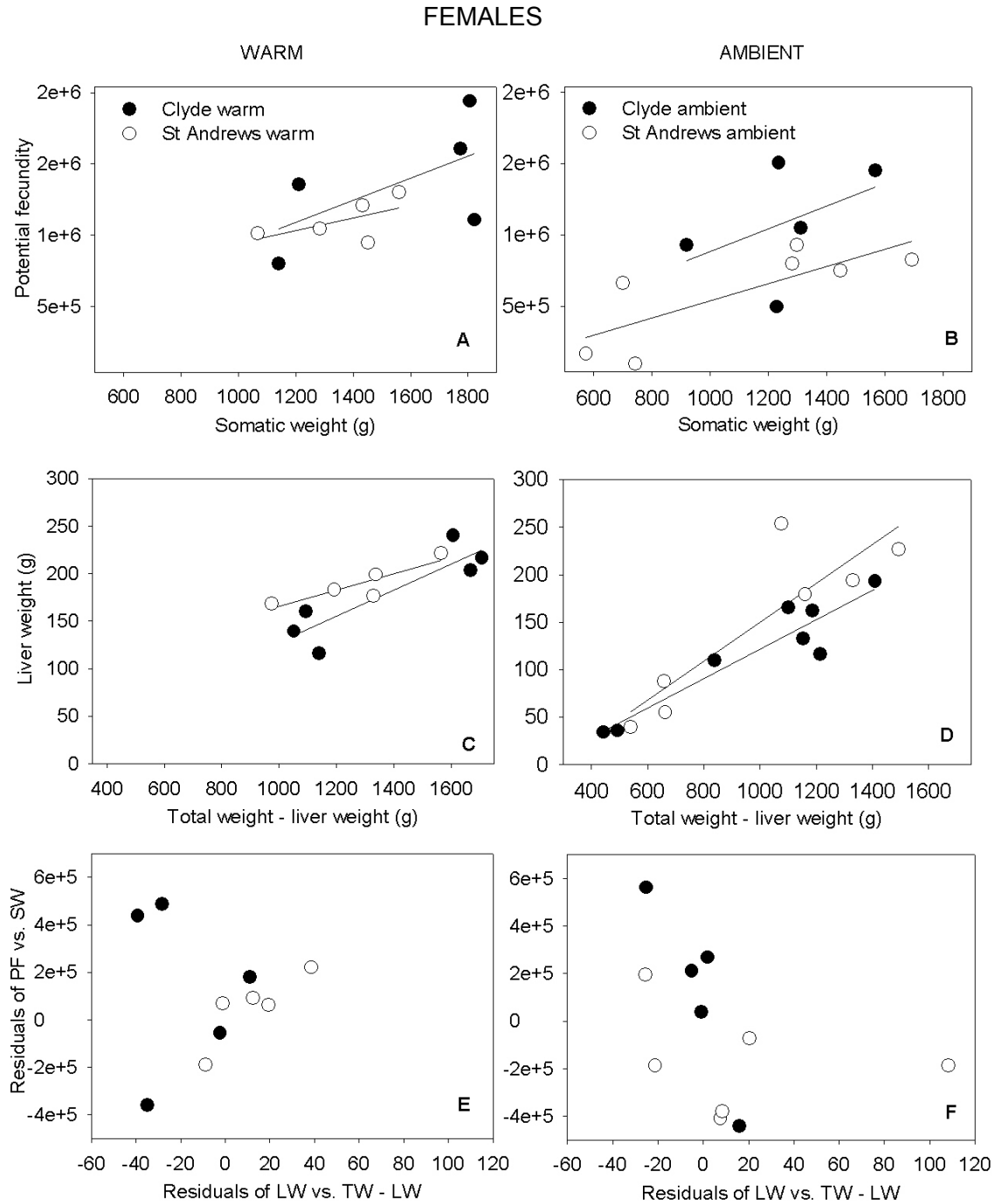
Figure 4.14. Population differences in females for potential fecundity (warm: A and ambient: B), liver weight (warm: C, ambient: D) and for the relationship between liver weight and potential fecundity. A regression was fitted as: Residuals of F_{PP} vs. SW = 0.0102 - 0.00316 residuals of LW vs. TW – LW (non-significant).
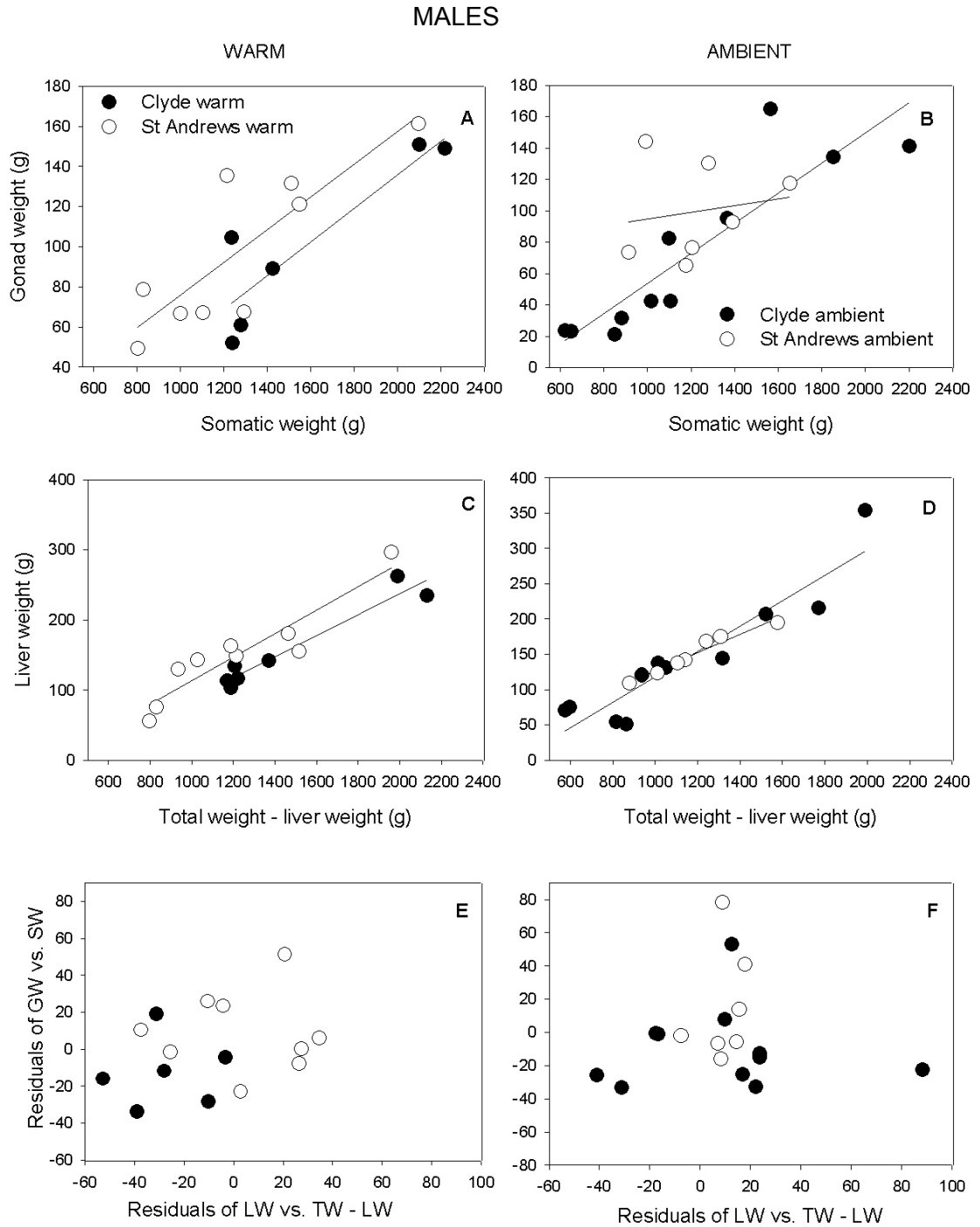
Figure 4.15. Population differences in males for testis weight (warm: A and ambient: B), liver weight (warm: C, ambient: D) and for the relationship between liver weight and potential fecundity. A regression was fitted as: Residuals of GW vs. SW = 0.0102 - 0.00316 residuals of LW vs. TW – LW (non-significant).

3.2.5 Predictors of fecundity

Total weight, length and condition factor were used to predict fecundity and testis size at periods spaced to avoid autocorrelation between time points, as in the Lowestoft – St

Andrews Bay experiment. Weight and condition factor were good predictors of fecundity from the beginning of the experiment in May (Figure 4.16, Table 4.7). In May, cod from the Clyde Sea had a higher fecundity for a given weight and a higher condition factor than cod from St Andrews Bay. In October those in the warmer treatment were heavier and had a higher condition factor, but condition was less related to fecundity than those in the ambient treatment (Figure 4.16F, Table 4.8C). This pattern was also apparent in the condition - fecundity relationship in January (Figure 4.16I). The pattern of growth rate with fecundity was less consistent, but generally those in the warm treatment grew better.
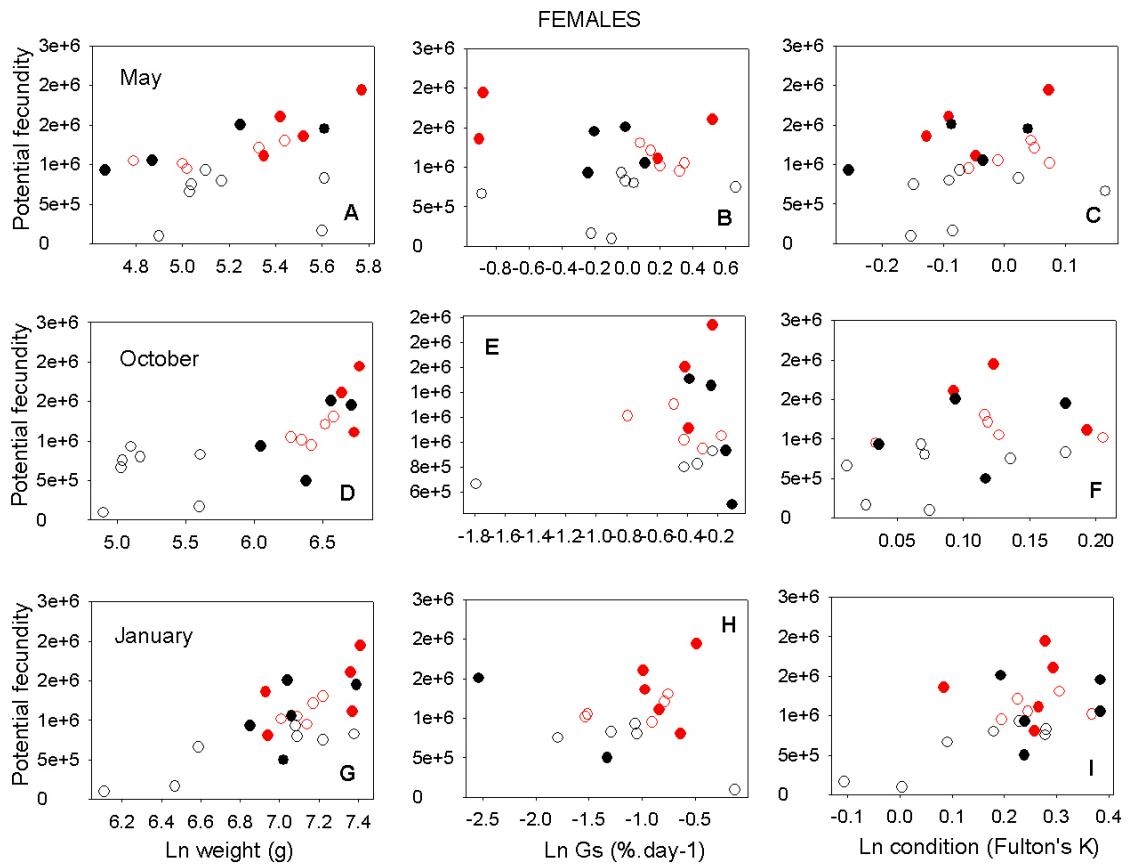
Figure 4.16. Relationships between potential fecundity and ln (natural log) total weight, ln G_s, % day[-1] and ln condition factor (Fulton's K) at time points 1 (May 05) (plots A, B and C), 4 (October 05) (plots D, E and F) and 7 (January 06) (plots G, H and I) for the Clyde Sea and St Andrews Bay; solid red circles: Clyde Sea warm, solid black circles: Clyde Sea ambient, open red circles: St Andrews Bay warm, open black circles: St Andrews Bay ambient. Significance given by statistics in Table 4.7 and 4.8.

In the males, there was a significant positive regression in all periods between GSI and both weight and condition, but this was only true for G_s in October (Figure 4.17, Table

4.7). Males from St Andrews Bay had a higher GSI for a given weight than those from the Clyde Sea and a similar pattern was found for condition factor. There was a positive relationship between G_s and GSI in January and this relationship was stronger in the cod from the Clyde Sea than those from St Andrews Bay (Figure 4.16H, Table 4.8B), despite those from St Andrews Bay having a higher GSI.
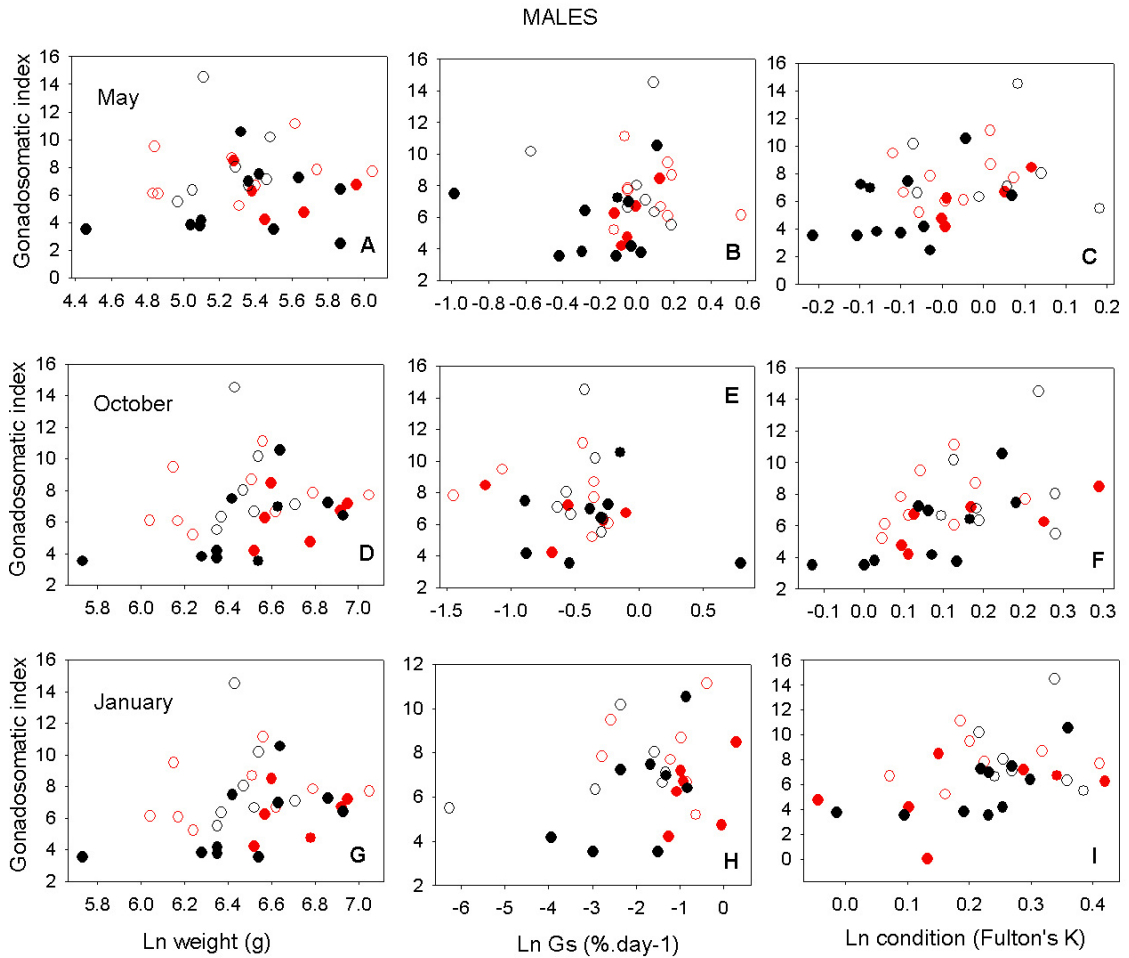
Figure 4.17. Relationships between GSI in males and ln (natural log) total weight, ln G_s, % day^{-1} and ln condition factor (Fulton's K) at time points 1 (May) (plots A, B and C), 4 (October) (plots D, E and F) and 7 (January) (plots G, H and I) for the Clyde Sea and St Andrews Bay; solid red circles: Clyde Sea warm, solid black circles: Clyde Sea ambient, open red circles: St Andrews Bay warm, open black circles: St Andrews Bay ambient. Significance given by statistics in Table 4.7 and 4.8.

Table 4.7. Regression analysis of effect of ln (natural log) weight, ln specific growth rate and ln condition on gonadosomatic index in males and fecundity in females for periods 1 (May 05), 4 (October 05) and 7 (January 06).

Sex	Effect	Period 1 (May)	Period 4 (October)	Period 7 (January)
Female	ln weight (g)	$F_{3\text{-}19} = 9.91$, $P = 0.001$	$F_{2\text{-}18} = 6.15$, $P < 0.010$	$F_{2\text{-}21} = 15.38$, $P < 0.001$
	ln specific growth rate (% day^{-1})	$F_{3\text{-}19} = 8.48$, $P = 0.001$	$F_{3\text{-}15} = 3.20$, $P = 0.062$	$F_{2\text{-}16} = 4.93$, $P = 0.024$
	ln condition (Fulton's K)	$F_{3\text{-}19} = 12.3$, $P < 0.001$	$F_{2\text{-}18} = 3.84$, $P = 0.004$	$F_{2\text{-}21} = 8.60$, $P = 0.002$
Male	ln weight (g)	$F_{2\text{-}31} = 4.30$, $P = 0.023$	$F_{2\text{-}31} = 5.27$, $P = 0.011$	$F_{2\text{-}30} = 5.34$, $P = 0.011$
	ln specific growth rate (% day^{-1})	$F_{2\text{-}30} = 3.28$, $P = 0.53$	$F_{2\text{-}26} = 2.03$, $P = 0.154$	$F_{2\text{-}26} = 4.04$, $P = 0.031$
	ln condition (Fulton's K)	$F_{2\text{-}31} = 5.11$, $P = 0.013$	$F_{2\text{-}31} = 8.62$, $P = 0.001$	$F_{2\text{-}29} = 5.56$, $P = 0.009$

Table 4.8 A, B and C. Regression analyses of weight (A), specific growth rate (B) and condition factor (C) on fecundity in females or GSI in males. In cases where there were insufficient numbers to perform a regression or where the overall regression was not significant it is labelled n/a. If an effect was non-significant and subsequently removed from the model it is labelled ns.

A

Sex	Factor	Period 1 (May)	Period 4 (October)	Period 7 (January)
Female	ln weight	ns	T = 2.05, d.f. = 19, P = 0.057	T = 4.48, d.f. = 21, $P < 0.001$
	temperature	ns	T = -2.25, d.f. = 19, P = 0.039	ns
	population	T = -3.62, d.f. = 19, P = 0.002	n/a	ns
Male	ln weight	ns	ns	ns
	temperature	ns	ns	ns
	population	T = 2.90, d.f. = 31, P = 0.007	T = 2.92, d.f. =31, P = 0.007	T = 2.86, d.f. = 30, P = 0.008

B

Sex	Factor	Period 1	Period 4	Period 7
Female	ln specific growth rate	ns	n/a	ns
	temperature	$T = -2.98$, d.f. = 19, $P = 0.009$	n/a	$T = -3.06$, d.f. = 16, $P = 0.008$
	population	$T = -3.65$, d.f. = 19, $P = 0.002$	n/a	ns
Male	ln specific growth rate	n/a	n/a	$T = 1.89$, d.f. = 26, $P = 0.071$
	temperature	n/a	n/a	ns
	population	n/a	n/a	$T = 2.48$, d.f. = 26, $P = 0.021$

C

Sex	Factor	Period 1	Period 4	Period 7
Female	ln condition	$T = 2.10$, d.f. = 19, $P < 0.001$	ns	$T = 2.87$, d.f. = 21, $P = 0.010$
	temperature	$T = -2.59$, d.f. = 19, $P < 0.020$	$T = -2.16$, d.f. = 18, $P = 0.047$	$T = -2.26$, d.f. = 21, $P = 0.035$
	population	$T = -4.68$, d.f. = 19 $P < 0.001$	n/a	ns
Male	ln condition	ns	$T = 3.02$, d.f. = 31, $P = 0.005$	$T = 1.75$, d.f. = 29, $P = 0.092$
	temperature	ns	ns	ns
	population	$T = 2.13$, d.f. = 31, $P = 0.042$	ns	$T = 2.41$, d.f. = 29, $P = 0.023$

3.3 Comparison of potential fecundity and testis weight for all study populations

3.3.1 Comparison in females

Potential fecundity and testis weight of all populations from both experiments were compared, separately for females (Figure 4.18) and males (Figure 4.19). Statistical analysis of the data was not possible as there were slight differences in the experimental procedures. In the Scottish east and west coast experiment the fish were sacrificed as they became mature between late January and March, whereas in the southern and northern North Sea comparison they were all sacrificed in January. In order to compare the two experiments, potential fecundity against weight in January was plotted for all

populations and treatments. The observation that the St Andrews Bay cod from both experiments had a similar fecundity-weight relationship suggests that both experiments can be compared. The relationship between fecundity and weight in southern North Sea cod was similar to that of the St Andrews Bay cod, but the Clyde Sea cod had a greater fecundity for a given weight. Temperature appeared to have a small effect on the fecundity-weight relationship in the St Andrews Bay cod, but not the Clyde Sea cod, although there is much variation in the Clyde Sea data points. The effect of temperature is questionable due to lack of replication and high variation, but the trend in response to the warm temperature treatment was to increase fecundity in the St Andrews Bay cod to approximately the level of the Clyde Sea cod. There was no such effect in the Clyde Sea population; those in the warm treatment had a similar fecundity for a given weight than those in the ambient treatment.
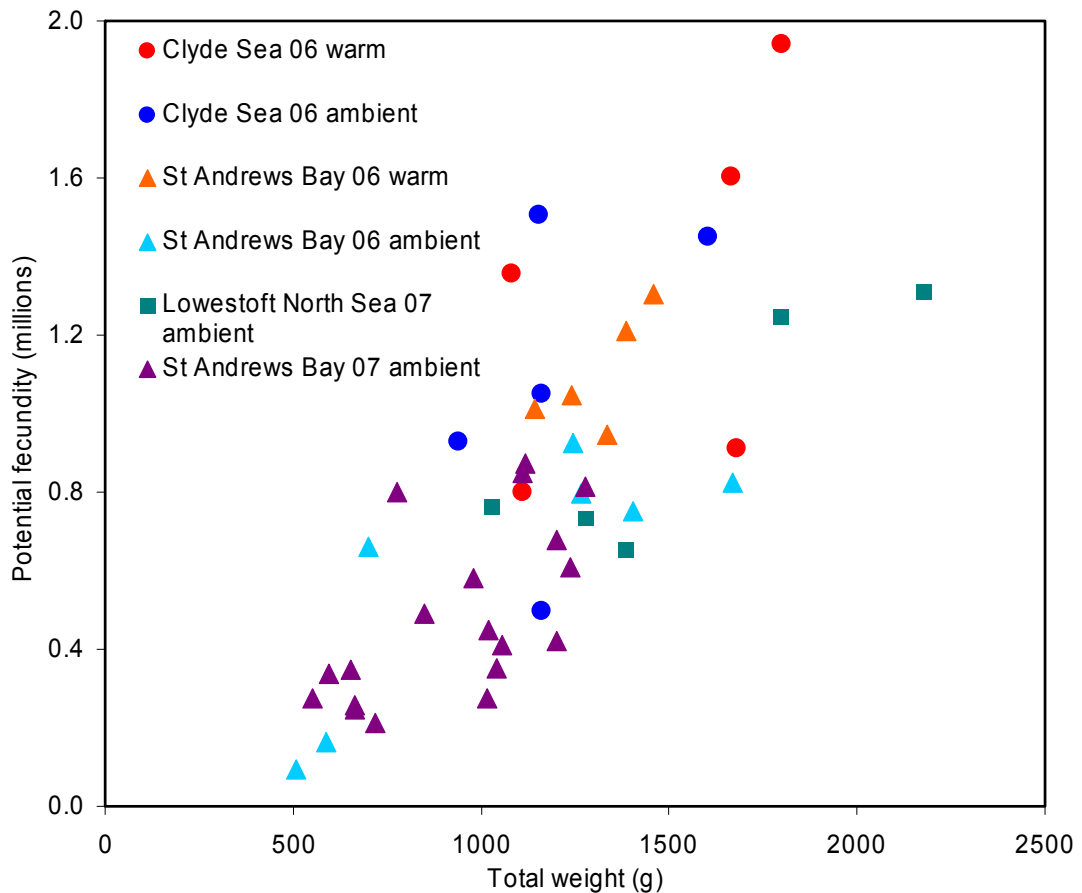
Figure 4.18. Weight-specific fecundity relationship for all study populations and from both temperature treatments. Total weight of fish in January is given rather than immediately prior to spawning.

3.3.2 Comparison in males

In the males, the results from the two experiments were less comparable, since the testis weight vs. total weight relationship in the St Andrews Bay 2006 experiment was slightly higher than it was in the 2007 experiment (Figure 4.19). Allowing for this discrepancy between years, the most striking difference was that the Lowestoft cod tended to have a lower testis weight for a given total weight than the other groups, while the St Andrews Bay cod in the 2006 experiment had the largest relative testis weights. The cod in the warm water treatment from the Clyde Sea were slightly heavier than those in the ambient treatment but they did not have a higher testis size for their respective weight.
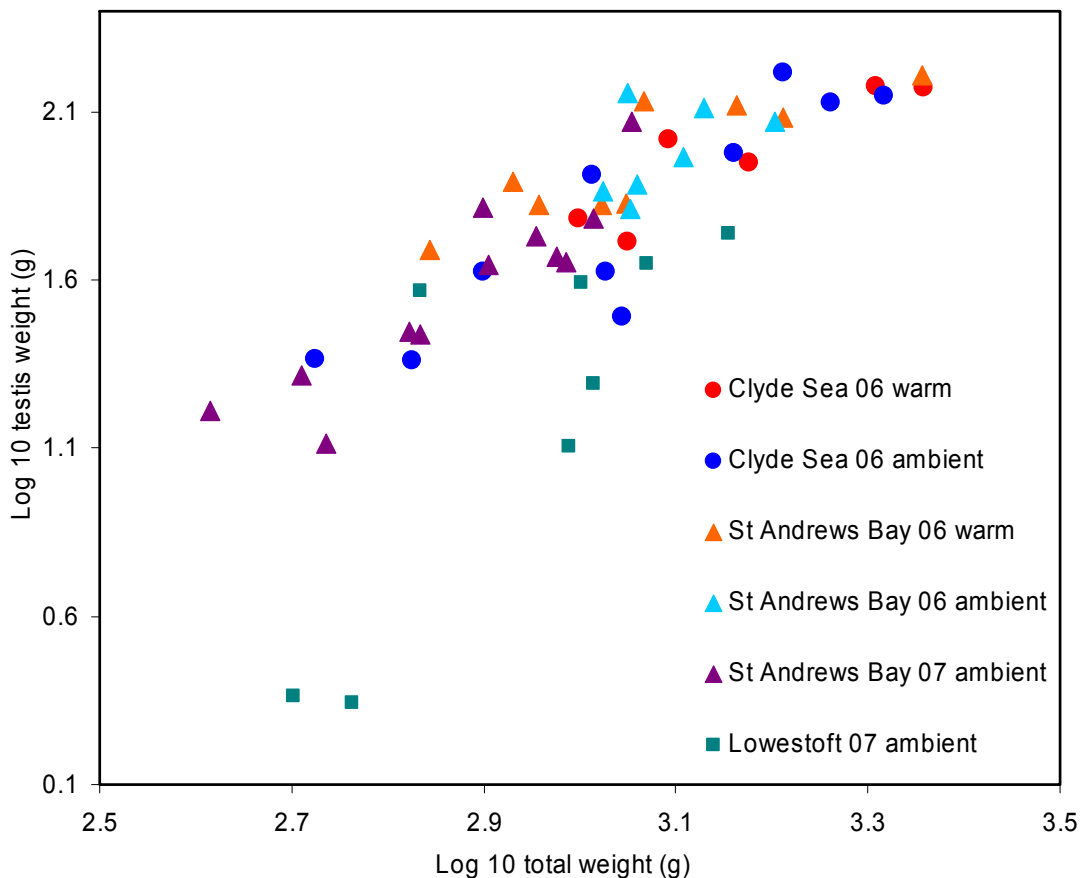
Figure 4.19. Weight-specific testis weight ($\log_{10}$) relationship for all populations and from both temperature treatments. Total weight in January is given rather than immediately prior to spawning.

4   DISCUSSION

The variation in reproductive potential shown in this common environment study adds to the accumulating evidence of spatial variation in reproductive traits (Yoneda and Wright, 2004; Lambert *et al.*, 2005). The variation in adult growth also follows similar patterns to that of juveniles from the same Scottish populations (Perutz *et al.* submitted; Chapter 3). Moreover, field observations have also identified spatial diversity in life history traits throughout the species range, in Scottish waters (Yoneda and Wright, 2004) and off eastern Canada (McIntyre and Hutchings, 2003). Reproductive potential appeared to be strongly related to growth rate and condition in the period leading up to spawning and varied with both population and temperature, as has been witnessed in similar such studies (Yoneda and Wright, 2005a and b; Skjæraasen *et al.*, 2006), although there were sex related differences as to the relative importance of each.

4.1   Differences in potential fecundity by population and temperature

Females from both the northern and southern North Sea populations had a similar weight-specific fecundity. The Clyde Sea cod had considerably higher fecundities (approximately 50 % higher for a 1 kg fish) than both of the North Sea populations, although the difference was only marginally significant. There are a number of possible explanations for this result. Firstly, sample sizes were small and tank effects cannot be ruled out as the Clyde Sea − St Andrews Bay experiment was not replicated. However, the St Andrews Bay population was replicated, by year and within one of the experiments, and no differences were found between replicates. The variation could also reflect differences in numbers reaching maturity. This analysis was based on only those fish that matured. A larger proportion of females from the St Andrews Bay population matured, some of which had a relatively low fecundity, whereas a lower proportion of those from the Clyde Sea and Lowestoft matured. Those that did, however, had higher fecundities. This difference in the proportion maturing reflects the greater bimodality in the size of the Lowestoft and Clyde Sea fish than those from St Andrews Bay. Although the fish were fed to excess and each tank was fed equal amounts, there was clearly a difference in food intake between individuals. Size at the time of sacrifice varied between 476 and 2666 g and growth rates during the course of the experiment varied between -0.6 and 2.4 % day^{-1}. Results from Chapter 2 suggested that competitive

interactions in juvenile cod could result in suppression of growth in the subordinates and Yoneda and Wright (2005a) found that fewer first-time spawning cod matured when deprived of food.

Lastly the variation reported could be a result of genetic differences between the North Sea and Clyde Sea populations. However, since this study used wild-caught rather than cultured cod, effects of early environment (Johnston, 1993, 1998) and maternal effects (Chambers and Leggett, 1996; Marteinsdottir and Steinarsson, 1998) cannot be accounted for and thus genetic effects cannot be confirmed. However, no known studies have related either early environment or environmental effects to fecundity in the F_1 generation. In the field, population variation in weight-specific fecundity has been reported (McIntyre and Hutchings, 2003; Yoneda and Wright, 2004). Contrary to this experiment, Yoneda and Wright (2004) found that cod from the inshore North Sea had a higher relative fecundity than cod from the Scottish west coast. However, this was a field study and it was not limited to first-time spawning cod and differences could have been related to plasticity in the environment rather than to genetic differences. Contrary to Yoneda and Wright (2005a), the present study found no direct effect of temperature on fecundity. In Yoneda and Wright (2005a) lower temperatures were found to prevent the cod from under-going vitellogenesis. However, those cod experienced a lower minimum temperature than cod in the present study (3 ℃ compared to 6 ℃ respectively) and the difference between treatments was greater than in the present study (4 ℃ compared to 2 ℃ respectively). It is suggested that lower temperatures affected the biochemical processes and prevented the cod from under-going vitellogenesis (Yoneda and Wright, 2005a).

4.2   Differences in male gonad investment by population and temperature

In males the variation between populations was very different to that of the females. The males from St Andrews Bay had a higher gonad weight per unit body mass than those from the Clyde Sea and from Lowestoft. Similar to results of other temperature manipulation studies on cod (Cyr *et al.*, 1998) and sea bass *Dicentrarchus labrax* (Pawson *et al.*, 2000), there was no effect of temperature on testicular weight. However, Yoneda and Wright (2005b) reported that lower temperatures reduced the proportion of males that matured in first time spawning cod. This difference in findings is again likely

due to the lower temperatures experienced by the cod in Yoneda and Wright (2005b) than in the present study. In comparison to females, all of the males in the Clyde Sea and St Andrews Bay populations matured. A large proportion (42 %) of males from Lowestoft did not mature but as in the females, a high bimodality in size had developed in this group and therefore those individuals that did not mature were likely too small or in too poor a condition to spawn. The length at maturity is comparable to that of wild age 2 cod from areas nearby. Mature females from St Andrews Bay in this study were a mean length of 43.2 cm $\pm$ 0.8 (mean and SEM) similar to those from the Inshore North Sea (41 cm $\pm$ 2.6), although some matured at as little as 35 cm from Lowestoft and St Andrews Bay. Those from the Clyde Sea in this study were a mean length of 47.2 cm $\pm$ 0.4, comparable to those from the West coast in the wild in 2002 and 2003 (48.0 cm $\pm$ 1.2). The finding that more males matured than females has also been reported in other species (Thorpe, 1994; Jacobsen and Ajiad, 1999). This could be a result of the lower energy requirements in production of sperm than oocytes (Wootton, 1998) and so males may be able to mature at a smaller size or in a poorer condition than females. Experimental studies have confirmed this suggestion that females tend to utilise more energy in gonad maturation than do males (Jonsson *et al.*, 1991).

4.3   Effect of body size on females and males

Although there was an overlap in size between those individuals that did and those that did not mature, in general, non-maturing fish were at the lower end of the size range. In addition to those that did not mature at all, there were others that matured with a very low fecundity or testis weight, but these again tended to be smaller fish. The relationship between reproductive potential and weight is well recognised (Wootton, 1998; Skjæraasen *et al.*, 2006; Yoneda and Wright, 2005b) and weight in this study was almost always a highly significant covariate in analyses of reproductive potential. In fish such as cod (Yoneda and Wright, 2005a) and plaice (Holdway and Beamish, 1985; Rijnsdorp, 1990) it has been reported that the fish that mature are those that are the fastest growing and it has been found that fish with fast growth tend to have a higher reproductive potential (e.g. Rowe *et al.*, 1991; Kjesbu and Holm, 1994; Silverstein and Shimma, 1994; Silverstein *et al.*, 1997). While in this experiment growth per se did not have a consistent relationship with fecundity or GSI in males, those fish that grew well attained a higher body weight, which in turn resulted in a higher fecundity or GSI.

In the St Andrews Bay - Lowestoft experiment, variation in weights was apparent early on in the experiment. The Lowestoft fish were brought in from the wild larger than the St Andrews Bay fish, despite being the same age. These fish retained a size advantage throughout the experiment, although the size difference did decline over time. Similarly in the Clyde Sea – St Andrews Bay experiment, while there was no overall difference in length between groups, there was an initial difference when accounting for sex. The Clyde Sea females were initially longer than the other groups. This difference actually declined in time but overall those in the warmer temperature treatment grew better than the other groups, as did the males from the Clyde Sea. The higher growth rate of the Clyde Sea males as adults of age 1 and 2 followed the same trend as in the juveniles (Perutz *et al.*, submitted; Chapter 3) and the higher growth of the Lowestoft cod reflects the trend proposed in Perutz *et al.* (submitted) and Chapter 3. One explanation for the pattern of growth reflects the cogradient variation hypothesis, in which the environment serves to accentuate a physiological effect (Conover, 1998). In this experiment temperature enhanced growth rate and the populations with the higher growth rates, came from the warmest environments. For all but the winter months, temperature in the southern North Sea far exceeds that of the northern North Sea (Appendix 1). Equally, the Scottish west coast has higher mean annual temperatures than the east coast (Slesser and Turrell, 2005).

Weight was also highly variable within groups and a large bimodality developed in the Lowestoft cod and to a smaller extent in the Clyde Sea cod. This variability is likely related to differential access to food affecting energy levels. Similarly, Metcalfe *et al.* (1989) have shown that dominance and feeding efficiency in Atlantic salmon can lead to bimodality in size distribution with the larger individuals subsequently achieving higher rates of growth. Although the fish in this study were fed to excess, and food scattered evenly throughout the tank, it is likely that consumption varied between individuals. The differences in the development of the bimodality in the size distribution between populations, suggests that there is relatively more competition occurring in the Lowestoft and Clyde Sea individuals than in the St Andrews Bay cod. In studies of first time spawning in fish it has been reported that poor growth and inadequate food consumption at the time of vitellogenesis may prevent the onset of maturity as in the case of Atlantic cod (Yoneda and Wright, 2005a) or result in lower fecundities in

rainbow trout (Bromage and Jones, 1991). Therefore insufficient feeding, perhaps driven by competition, may have resulted in some individuals not maturing.

4.4 Linking weight, growth and condition to gonad investment

The effect of body size on reproductive traits was apparent in both sexes but the effect was slightly stronger in the females. In the females, those fish that attained the greatest size, the Lowestoft and Clyde Sea females and those in the warmer treatments, had the highest fecundities. In the males, however, liver condition appeared relatively more important than weight. Previous studies have suggested that liver size might be a cause of differences in reproductive potential (Marshall *et al.*, 1999; Rowe *et al.*, 1991, Lambert and Dutil, 2000), liver being the storage organ for vitellogenin in the females and lipids. The St Andrews Bay cod attained a greater testis weight despite being the same weight or slightly smaller than the Lowestoft and Clyde Sea populations. This concurs with the findings of Yoneda and Wright (2005a and b) in which they suggest that in females energy is allocated to reproduction according to their size, whereas in males, the liver condition is relatively more important in determining gonad investment. Liver condition could predict testis weight for a given size fish in the males of the St Andrews Bay – Lowestoft comparison but not in the females. This sex difference is surprising as reproduction in females is more costly than in males and females might be expected to rely more on energy storage in the liver to produce vitellogenin. However, Skjæraasen *et al.* (2006) suggest that the need for large liver reserves in females happens earlier than in males, about three to four months before spawning during the time of vitellogenesis, whereas liver measurements in these experiments were taken at or up to two months prior to spawning.

This finding of weight being relatively more important in females and liver condition in males is also evident in the time prior to spawning. Liver size could not be measured but condition factor (Fulton's K) gives an indication of fatness of the fish (Lambert and Dutil, 2001). Weight was an important indicator of reproductive potential from the first measurement in both males and females, in agreement with Skjæraasen *et al.* (2006). For example, in the St Andrews Bay – Lowestoft comparison, weight in July was the most reliable indicator of prospective fecundity, but weights in July reflect those in the period before in April. In the males condition factor appears to be an equally or in some

cases slightly better predictor of prospective GSI than weight itself, in July (Lowestoft – St Andrews Bay comparison) and in October (in the St Andrews Bay cod in the Clyde Sea – St Andrews Bay comparison). Growth itself is generally a poorer indicator, but the finding of a negative correlation between growth and fecundity in the July period may indicate that energy is being allocated towards vitellogenesis rather than to growth at this time. Since an individual's size and condition in one period are linked to its size and condition in the months preceding this, it is not possible to comment on the most critical window in which reproductive output is decided. Energy reserves are important prior to vitellogenesis but it may be that fish that start off well maintain these advantages throughout. However, the strongest predictors of reproductive output are found in July and October, and thus it is likely that this is the time when the "decision" of how much energy to allocate to reproduction is made.

The population differences in life history traits identified in this study; in adult growth rate, condition, testicular weight, and fecundity, suggest an adaptive response to their local environment. The St Andrews Bay and Clyde Sea populations have already been reported to have differences in juvenile growth rate in the same pattern as found here (Perutz *et al.*, submitted; Chapter 3). A similar but more extreme finding was reported in Newfoundland cod (McIntyre and Hutchings, 2003). While cod matured at a similar size (42 cm), the Southern Gulf cod took about three years longer to mature than the Georges Bank cod because of their slower growth rate. Likewise, Lowestoft cod and males from the Clyde Sea may reach maturity at a younger age than those from St Andrews Bay due to their relatively faster growth rate, although it has yet to be established whether they mature at different sizes and have differing spawning times.

In those fish that matured, the pattern of fecundity between populations in relation to their natural environment is surprising. The northern and southern North Sea cod come from environments with the most contrasting thermal regimes and yet they have a very similar fecundity-weight relationship. The thermal regime of the Clyde Sea and St Andrews Bay cod are more similar and yet the Clyde Sea population is the outlier. This suggests that temperature may not be the driving factor behind fecundity. Adaptation to other factors such as food availability (Yoneda and Wright, 2005a and b) or light regime (van der Meeren and Jørstad, 2001; Hansen *et al.*, 2001) may be relatively more important. Alternatively the fecundity–weight relationship may be fixed for cod within

these areas of the North Sea. While genetic studies have found weak genetic differentiation between regions in close proximity to St Andrews Bay and the Lowestoft populations (Hutchinson *et al.*, 2001), this is not necessarily reflective of their phenotypic traits. Certainly growth and condition factor appear to be critical determinants of reproductive potential and as previously demonstrated, growth rate is strongly influenced by the prevailing environment.

The North Sea and Scottish west coast has already been rising in temperature since 1970 (ICES, 2006) and annual sea surface temperatures in the North Sea are predicted to rise by 1 to 2.5 °C in the next 50 years (Clark *et al.*, 2003). This study has demonstrated that a temperature rise of this order would increase growth rates in both St Andrews Bay and Clyde Sea cod. Even in the summer when the additional 2 °C would have put the fish in excess of their normal range of temperatures, the fish still grew better. Therefore, the enhanced growth would lead to larger fish, with higher fecundities. However, Cook and Heath (2005) highlighted that rising temperature may not be favourable to recruitment dynamics in North Sea cod. Other processes such as egg and larval development (Chapter 5) may be weakened by adverse temperature or low food availability. A different scenario occurred in the males, however. The liver condition appeared to be an equally important predictor of testis size and liver condition was slightly lower at higher temperatures, perhaps due to the increased metabolic demand at higher temperatures. Therefore, a rise in temperature may serve to reduce testicular development. To understand the full effect of temperature on growth and reproductive potential it would be necessary to maintain the fish over their full range of temperatures and beyond, as has been done for the egg and larval development (Chapter 5; Jordaan and Kling, 2003) and juvenile growth rate (Chapter 3). This would confirm whether or not the suggestion in this study, that the Clyde Sea fish are already at their upper thermal tolerance limit but the St Andrews Bay fish are not, is actually the case.

5   CONCLUSION

This common environment study has established population and sex differences in life history patterns in cod from different regions around the UK. Lowestoft cod appeared to have higher growth rates relative to the Scottish populations but had a similar fecundity for a given weight relative to the St Andrews Bay population. The Clyde Sea males had higher growth rates than those from St Andrews Bay and may also have had a higher GSI. The St Andrews Bay cod had a relatively greater testicular mass than both the Clyde Sea and Lowestoft populations. The most important factor in determining reproductive potential appears to be the weight of the females and weight and condition of the males, both of which are affected by temperature. Therefore, it may be most critical to establish population differences and thermal responses in growth and condition as a pre-cursor to understanding reproductive investment. This identification of spatial diversity in cod maturity patterns and improved understanding of growth provides us with baseline population data that may facilitate future management of phenotypic diversity. In addition, an understanding of population responses to temperature will enable predictions of the changes in life history traits in response to climate change.

CHAPTER 5

Thermal responses of egg and larval development in Atlantic cod

1 INTRODUCTION

The development rates of early life history stages of Atlantic cod are highly variable across their geographic range. The incubation period (the time between when an egg is spawned until hatching) can vary between 39 days around Newfoundland in seas where temperature can be as low as −1 ˚C (Pepin *et al.*, 1997) and 11 days in the southern North Sea at 7 ˚C (Hall *et al.*, 2004). Seasonal temperature varies throughout the range of cod during the spawning period (Geffen *et al.*, 2006). As temperature is a prime determinant of vital rates (Brander, 1995), it is likely to have a large influence on the development of the embryo (Dannevig, 1895; Zhao *et al.*, 2003; Geffen *et al.*, 2006). These studies have reported that incubation time decreases with increasing temperature, but at the expense of larval size. In most studies, a larger larva is hatched out at lower temperatures (Zhao *et al.*, 2003; Peterson *et al.*, 2004). Both large larval size and short incubation time are likely to be advantageous for survival, but as temperature drives these two processes in opposite directions, they are likely to trade-off against each other with the optimal balance depending on the particular early life history ecology of the species. One advantage of a shorter hatching time is less time for predation upon the egg, but equally, hatching out at a larger size could also be advantageous in terms of better feeding success and swimming speed, which in turn would reduce their risk of predation (Hutchings, 2002). While cod may be adapted to the thermal conditions of their specific environment, there may be limits in the extent of their plasticity in response to varying temperature. Small variations in driving parameters, such as temperature, may have large consequences for the development of the eggs and larvae (Zhao *et al.*, 2003). Inter-annual fluctuations in survival of cod eggs and larvae may greatly influence recruitment to the stocks and year-class strength (Hunt von Herbing *et al.*, 1996; Zhao *et al.*, 2003). A negative association between recruitment and temperature has been documented in cod (Beaugrand *et al.*, 2003). However, this is

thought to be an indirect consequence of the associated change in plankton communities that has resulted in a 'mismatch' (Cushing, 1975) in timing between hatching of cod larvae and production of their food.

Cod are serial batch spawners, spawning during the late winter and early spring. Each female produces 17 to 20 batches over the season and each batch contains up to 300 000 eggs (Kjesbu, 1989; Hall *et al.*, 2004). The development of cod eggs has been described as a series of stages (Thompson and Riley, 1981; Hall *et al.*, 2004). Stages I to V follow the development from fertilisation, through successive cell cleavages, to the blastula and gastrulation stages, the growth of the tail, development of the eye and establishment of pigmentation. Hatching occurs when the tail has grown around the head and pigmentation becomes prominent (Thompson and Riley, 1981). The three main factors reported to affect embryo development during the period between fertilisation and hatch are temperature, oxygen levels (Rombough, 1988; Barrionuevo *et al.*, 1999) and egg size (Blaxter, 1969; Kamler, 1992). The effect of temperature on cod embryo development has been well studied but results are often contradictory. Pepin *et al.* (1997) found embryo mortality in Newfoundland cod increased with decreasing temperatures, while other studies report that survival is reduced at higher temperatures (Pepin, 1991; Geffen *et al.*, 2006). All studies on the relationship between temperature and hatching time have reported a decrease in hatching time with temperature. The causes of these temperature responses are unknown, but a plausible suggestion is that they are linked to metabolic rate (Zhao *et al.*, 2003), which is also strongly related to temperature. Thus shorter hatching times at higher temperatures might arise from a higher basal metabolism and increased development rate. In the zebrafish, *Danio rerio,* higher heart rate and rate of oxygen consumption as a function of development time were reported at three rearing temperatures (Barrionuevo *et al.*, 1999). Studying metabolic rate in embryonic or larval fishes is difficult due to their small size and low rates of oxygen uptake and requires lengthy and sophisticated techniques carried out on many samples (Hoegh-Guldberg and Manahan, 1995; Bang *et al.*, 2004). An alternative is to measure cardiac rate, which can easily be recorded at the embryo stage, and to calibrate this to metabolic rate (Armstrong, 1986), although cardiac rate is only generally reflective of respiration rate (Barrionuevo *et al.*, 1999). Finally, egg size can also affect egg development and egg size can vary with maternal condition (Ouellet *et al.*, 2001; Marteinsdottir and Steinarsson, 1998). Egg size varies with fish age, size,

experience and spawning condition (Trippel, 1998). In general, repeat spawners have larger eggs than first time spawners and egg size may decrease during the spawning period (Trippel, 1998). A larger larva is usually hatched out from a larger egg (Zhao *et al.*, 2001, 2003).

This chapter examines the thermal responses in egg development of cod from the Shetland Isles, from the northeast Atlantic, while accounting for eggs from different batches and egg size. The effect of temperature on cardiac rate is also examined as a first step to investigating basal metabolic rate. This will illustrate the extent of thermal plasticity within egg and larval development in cod from this region and the direction of the relationships between temperature, incubation time and egg and larval size. The results are compared to other published studies in British waters and the northwestern Atlantic. The aims of the study were:

- To examine the effect of temperature, batch and egg size on survival to hatch.

- To examine the effect of temperature, batch and egg size on time to 50 % hatch, and test the hypothesis that eggs incubated at a higher temperature have a shorter hatching time.

- To examine the effect of temperature and batch on the larval size for a given egg size, and test the hypothesis that larger larvae hatch out at lower temperatures when accounting for egg size.

- To examine the effect of temperature and batch on cardiac rate, under the prediction that cardiac rate increases with temperature.

2   MATERIALS AND METHODS

2.1   Maintenance of broodstocks

Eggs were obtained from local Shetland broodstock held at the North Atlantic Fisheries College hatchery in Scalloway, in the Shetland Isles (60º13'N 1º18'W). The broodstock consisted of wild caught, 3-year-old cod (8 females and 4 males) held in a 4 m circular tank, of 1 m depth, in a flow through system with water being replaced at 60 litres min^{-1}. Naturally spawned eggs were used in the experiments rather than artificial fertilisation through stripping, as the broodstock were sensitive to handling during spawning. The cod spawned naturally between 2nd February and 28th April 2005. Viable fertilised eggs floated to the surface and were collected each morning using a custom-made 50 μm mesh filter from the outflow of the surface drain of the tank.

2.2   Egg selection and incubation

For the purpose of this study, a batch is defined as eggs collected after one night's spawning. There was no indication of staggered stages, thus it was unlikely that more than 1 female spawned in a night (unless two fish spawned simultaneously). Eggs were of a similar size within a batch and differed between batches (F_{6-211} = 8.16, P < 0.001). Therefore by collecting eggs daily, it is likely that each batch came from a different female.

A total of 7 batches of eggs were taken between a third and a half way through their spawning period on successive spawning days (27th, 28th February, 1st, 2nd, 5th and 7th March 2005). They were each incubated at six temperatures (5.9 $\pm$ 0.8, 6.6 $\pm$ 1.2, 8.1 $\pm$ 1.7, 9.3 $\pm$ 1.7, 10.7 $\pm$ 2.7 (a) and 10.7 $\pm$ 1.1 (b) ºC) to reflect the natural annual range of temperatures experienced by local cod. After collection, the batches of eggs were disinfected in peracetic acid (Vetroxide - Pharmaq) at a concentration of 4000 ppm in sterile seawater for 45 seconds before being transferred to sterile seawater. Each egg was pipetted on to a microscope slide for examination using a sawn off pipette in a droplet of 0.75 ml of seawater, before transferral to an individual compartment of a well-plate. Handling of eggs was minimised to avoid damage.

Eggs were placed in 3 ml 24 hole well-plates, inside plastic boxes and submerged in controlled water baths (90 L Paxton chilled with ICES TAE-08 chiller for 5.9 °C, Techne Junior TE-8J for 6.6, 8.1, 9.3 °C, Griffin & George BJL-400-110F for 10.7 °C (a), LMS Ltd 305 refrigerator for 10.7 °C (b)). A temperature-logging device inside the plastic boxes recorded temperature every 5 minutes (Lotek LTD1110) and every hour (Vemco minilogger). Eggs were kept in 24 hour darkness, except when under examination, during which they were placed under a microscope (Zeiss Semi 2000C) on a microscope slide. The microscope was calibrated to the height of the water droplet on the slide. Each egg was measured (mm) and then poured into a well on the well-plate already filled with 1.25 ml of seawater. Seawater was filtered through 1 μm mesh and UV sterilised. 24 eggs were incubated per batch per temperature. The eggs were examined daily under a microscope in a dark room kept at 6 ºC until one day post-hatch. Stage of development was assigned using Thompson and Riley's (1981) index to development stages of cod eggs.

2.3 Cardiac rate

The cardiac rate of each individual was recorded at stage V using the classification of Thompson and Riley (1981) under a light microscope at 5 x magnification. The following sampling protocol was used to estimate cardiac rate. The number of beats in 15 seconds was counted five times to obtain a mean value of beats per minute. Observations were carried out on the embryo while still in the well-plate under a light microscope (Zeiss Semi 2000C) at a temperature of 5 ºC no more than 5 minutes after removal from the water bath. The room was kept silent to reduce disturbance to the larvae. An external rather than a transmitted light source was used so as not to warm the water in the well-plate. In this way the water temperature in the well-plate did not change significantly in the time after removal from the water bath. In the first trial the temperature was measured in an empty well-plate on the same tray before measurement and no change from the incubation temperature was observed.

2.4 Size and morphology at hatching

One day post-hatch, the larvae were again measured and photographed at 3.2 x magnification (Nikon Coolpix 4500) under a light microscope (Zeiss 2000-C).

Photographs were analysed for skeletal malformations (Fitzsimmons and Perutz, 2006; Appendix 3).

2.5   Analysis

Logistic regressions were used to describe the relationship between survival, egg diameter and batch. Incubation period was analysed using analysis of covariance (ANCOVA), with batch as a random effect and temperature as a co-variate. Linear regression was used to investigate the relationship between temperature and length at hatch and incubation time and length at hatch, while first accounting for egg size. An ANCOVA was used to examine the effect of temperature and batch on cardiac rate. Interactions are given where significant. The temperature coefficient (Q_{10}) was calculated to quantify the temperature effect. The Q_{10} of mean cardiac rate was calculated using the equation: -

$$Q_{10} = (K_1/K_2)^{10/t_1-t_2},$$

where K_1 and K_2 are the cardiac rate at temperatures t_1 and t_2 respectively (Peterson *et al.*, 2004).

Normality and homogeneity of variance assumptions were checked prior to analysis and transformations made when possible. Parametric or non-parametric tests were chosen as appropriate.

3   RESULTS

3.1   Survival to hatch

Out of the 864 larvae incubated, a total of 249 (28.8 %) hatched in all batches and across all incubation temperatures (Table 5.1). The survival of eggs through to hatching was analysed using a logistic regression, maximal model: survival = egg diameter * temperature * batch, using a binomial distribution and a logit link function. Survival between different temperatures varied from 24 to 35 % but there was no significant

difference in survival between incubation temperatures, nor was there an effect of egg diameter on survival. There were, however, differences in survival between batches ($F_{6-906} = 29.4$, $P < 0.001$), varying from 15 to 65 %. In some cases no eggs in a batch survived at certain temperatures. No batch * temperature interaction was found and no trend in survival rate of different batches over time was apparent.

Table 5.1. Summary data for each temperature treatment including: number of eggs incubated, % survival to hatch, numbers examined and % of these which were malformed, incubation time (days to 50 % hatch), length at hatch and cardiac rate (beats minute^{-1}) taken at stage V.

Temperature (°C)	5.9	6.6	8.1	9.3	10.7 a	10.7 b
Eggs incubated	144	144	144	144	144	144
Survival to hatch (%)	34	24	35	26	21	34
Number examined for malformations	49	34	50	37	30	49
% normal (no malformations)	59	62	64	57	66	58
Incubation time (mean days and 95 % C.I.)	15.7 + 0.3	18.9 + 0.3	12.2 + 0.4	13.6 + 0.3	10.7 + 0.3	9.7 + 0.2
Length at hatch (mm) (mean and 95 % C.I.)	4.7 + 0.1	4.8 + 1.0	4.6 + 0.1	4.5 + 0.1	4.3 + 0.1	4.4 + 0.1
Cardiac rate (beats minute^{-1}) (mean and 95 % C.I.)	58.2 + 1.5	61.3 + 2.4	67.8 + 3.3	72.2 + 3.4	70.6 + 5.4	72.5 + 2.2

The malformed larvae identified from the photographs were excluded from the analysis, as they were significantly smaller at hatch than those in normal condition (median of 3.8 mm and 4.6 mm respectively, Mann Whitney test, T = 9.82, d.f. = 105, $P < 0.001$).

3.2   Incubation period

Incubation period (days to 50 % hatch) declined significantly with temperature (ANCOVA, $F_{5-211} = 579.5$, $P < 0.001$) (Figure 5.1). Incubation period varied between 10 days at 10.7 °C (b) and 19 days at 6.6 °C. Batch also had a significant effect on hatching time ($F_{6-211} = 7.53$, $P < 0.001$), the largest variation being at 9.3 °C in which hatch time varied between 11 and 14 days. Egg diameter had no effect on incubation period when accounting for temperature and batch.
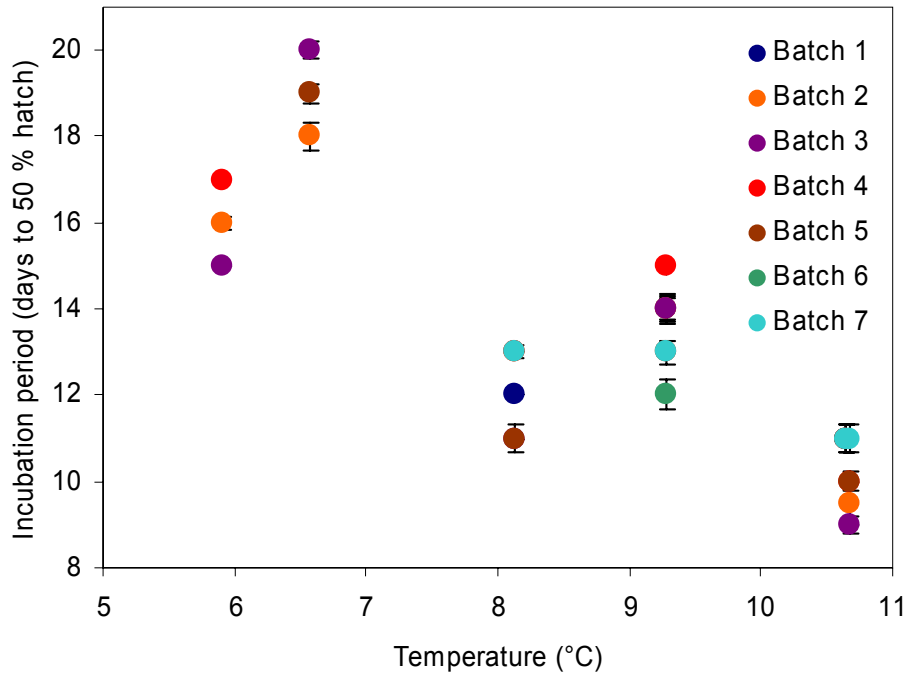
Figure 5.1. Relationship between temperature (ºC) and incubation time (days to 50 % hatch) (mean and 95 % C.I. for each temperature treatment and each batch).

3.3   Length at hatch

Total length at hatch varied between 3.2 mm and 5.5 mm. In determining effect of temperature on length at hatch, egg size was first accounted for (Figure 5.2). Egg diameter significantly affected length at hatch (regression: length at hatch = -335 + 449 egg diameter, $F_{1-211} = 15.4$, $P < 0.001$, $r^2 = 0.68$), although it accounted for considerably less of the variation (6.84 % compared to 20.05 %) in comparison to temperature.
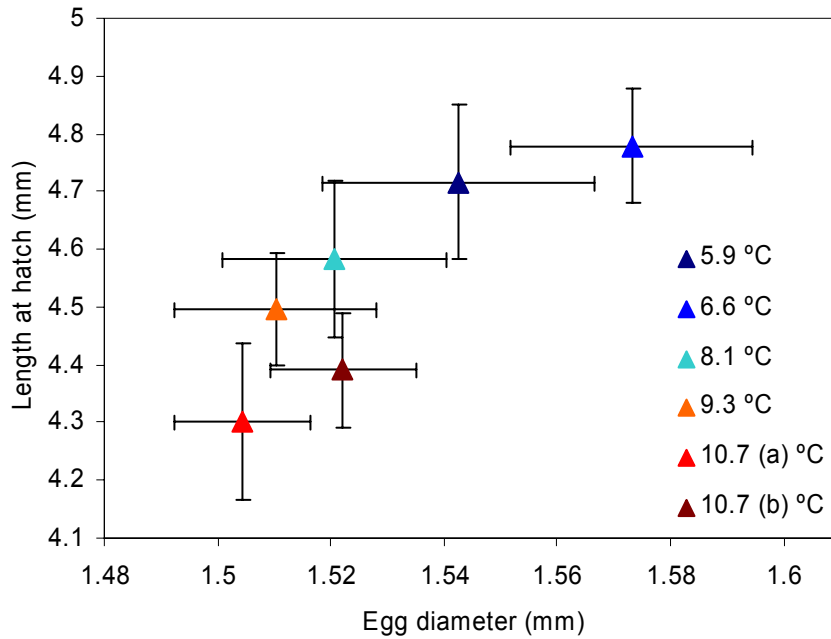
Figure 5.2. Relationship between egg diameter (mm) and length at hatch (mm) for each temperature treatment (mean and 95 % C.I.).

The residuals of length at hatch vs. egg diameter were plotted against temperature and a negative effect of temperature found (Figure 5.3, $F_{1-211} = 35.2$, $P < 0.001$). There was no effect of batch. Similarly, there was a marginally significant negative trend between the residuals of length at hatch vs. temperature against egg diameter (regression, $F_{1-211} = 3.53$, $P = 0.061$).
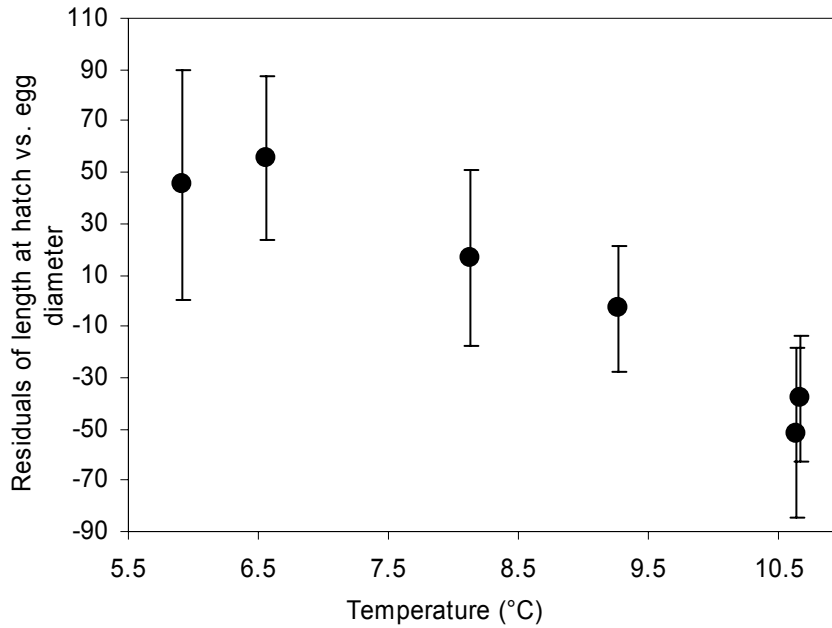
Figure 5.3. Relationship between temperature (ºC) and residuals of length at hatch (mm) vs. egg diameter (mm) (mean and 95 % C.I. plotted for each temperature treatment). Regression equation: residuals of length at hatch vs. egg diameter = 193 - 21.8 temperature.

Further to the relationship between temperature and incubation time and length at hatch and temperature, there was also a positive relationship between incubation time and length at hatch when accounting for egg size (Figure 5.4, regression, F_{1-211} = 39.16, $P <$ 0.001, r^2 = 0.16).
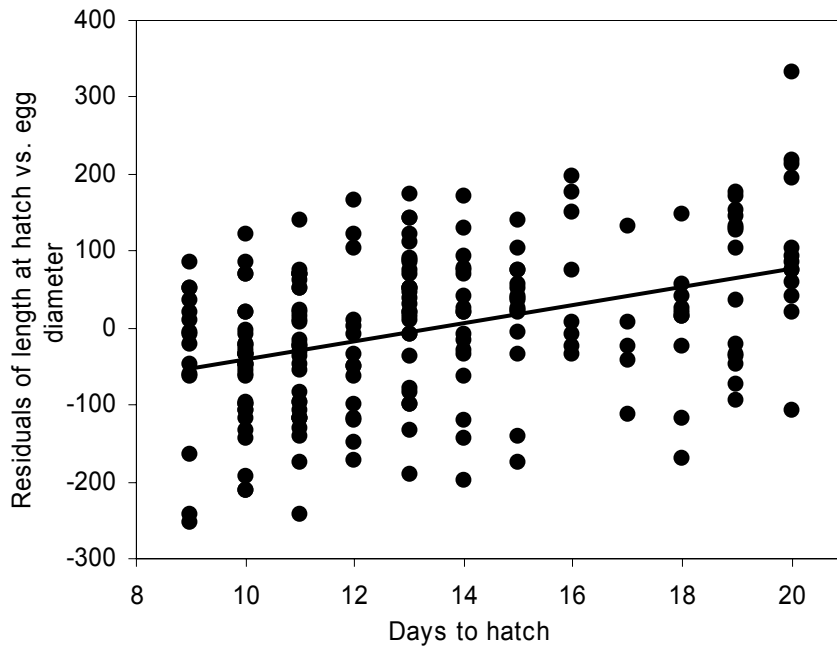
Figure 5.4. Relationship between residuals of length at hatch vs. egg diameter and incubation time (days to hatch). Regression: residuals of length at hatch vs. egg diameter = - 159 + 11.9 incubation time (days to hatch).

3.4 Cardiac rate

Cardiac rate varied between 58.2 ± 0.8 (mean and 95 % C.I.) beats minute^{-1} at 5.9 ºC to 70.6 ± 2.7 at 10.7 ºC (Figure 5.5). Cardiac rate also varied with batch, between 56.1 ± 5.3 in batch 7 and 73.8 ± 3.7 in batch 3 (Figure 5.6). It increased linearly with temperature, but started to plateau at 9.3 ºC. A significant positive effect of temperature was found on log $_{10}$ cardiac rate (beats minute^{-1}) (ANCOVA, $F_{1\text{-}184} = 73.0$, $P < 0.001$) and a significant effect of batch ($F_{6\text{-}184} = 15.2$, $P < 0.001$, $r^2 = 0.189$). The Q_{10}, calculated using mean cardiac rates for each temperature treatment in the linear part of the relationship (between 5.9 and 8.1 °C), was 1.98.
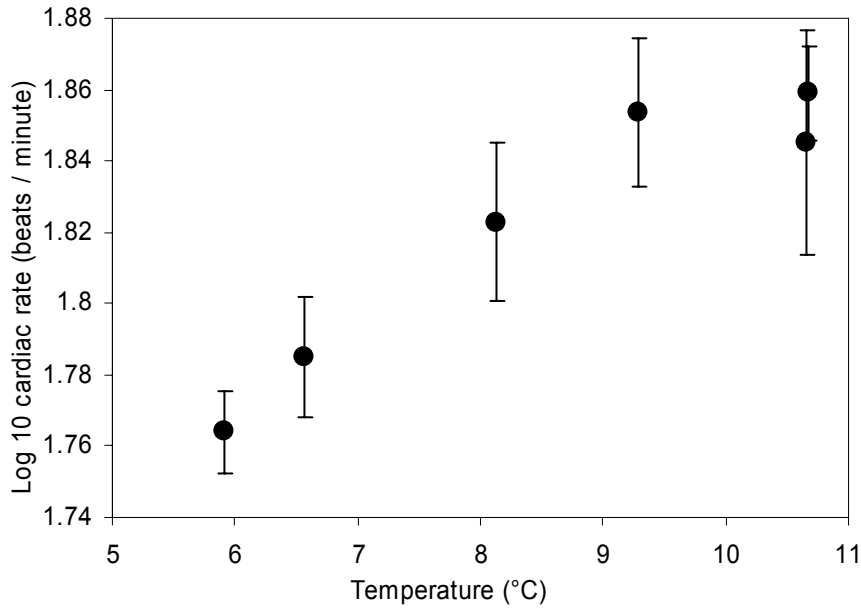
Figure 5.5. Relationship between $\log_{10}$ cardiac rate (beats minute^{-1}) and temperature (°C) (mean and 95 % C.I. plotted for each temperature treatment).
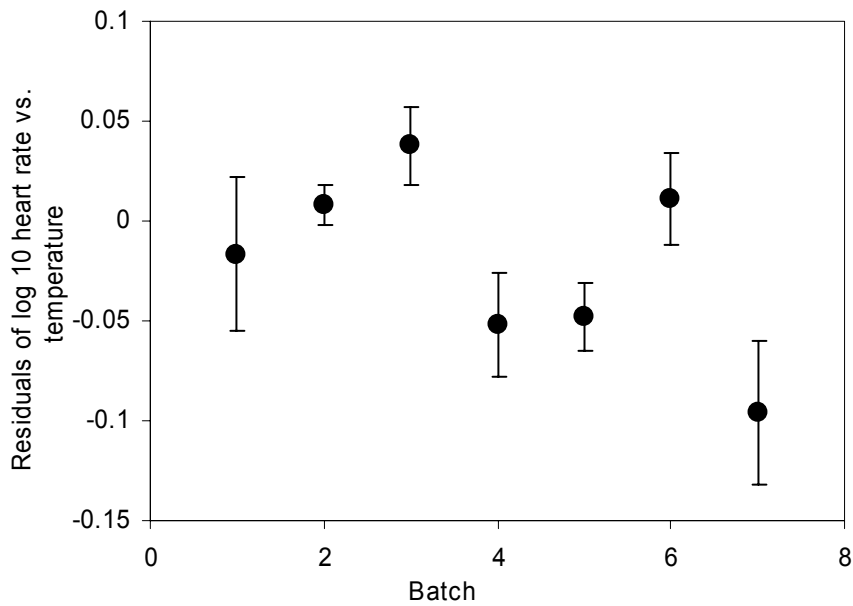
Figure 5.6. Residuals of $\log_{10}$ cardiac rate (beats minute^{-1}) vs. temperature (°C) against batch (mean and 95 % C.I. plotted for each temperature treatment).
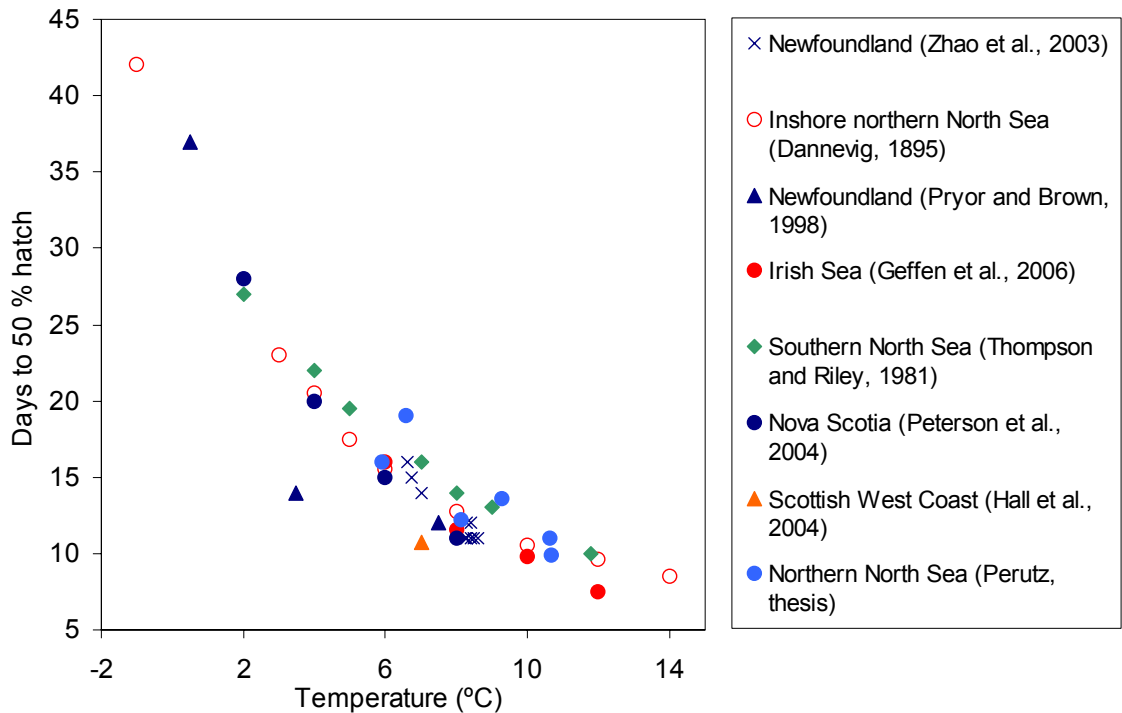
Figure 5.7. Relationship between temperature (ºC) and incubation time (days to 50 % hatch), plotted for published studies from across the species range.

4   DISCUSSION

This study has demonstrated a clear effect of temperature on many aspects of egg development including; incubation time, cardiac rate and the relationship between larval size and temperature. Similar findings have been reported in numerous other studies on cod embryo development for incubation time (e.g. Dannevig, 1895; Thompson and Riley, 1981; Zhao *et al.*, 2003) and larval hatch size (Zhao *et al.* 2003; Peterson *et al.* 2004), although the direction of such trends is not always consistent between studies and areas (Pepin *et al.*, 1997). An influence of batch on egg development was also found, as has been demonstrated previously (Knutsen and Tilseth, 1985; Zhao *et al.*, 2003) and points towards possible maternal effects (Marteinsdottir and Steinarsson, 1998), although it cannot be confirmed that different batches are entirely independent.

4.1 Egg development

Egg incubation time is strongly correlated with temperature, as being a poikilothermic process (body temperature varies directly with ambient temperature and thus development is subject to the laws of thermodynamics), the development rate is controlled by temperature to the point of hatch (Pauly and Pullin, 1988; Pryor and Brown, 1998). Egg development was also affected by batch, although this effect was small in comparison to the effect of temperature. Batch may therefore be a source of noise in the relationships between temperature and developmental processes, but batch was accounted for in the model and since all batches were used over all temperatures, the effect should have been equal between temperature treatments. If batch were indicative of family, this would provide evidence of incubation time being related to maternal (genetic) factors, although this study was not designed to be able to identify those factors.

The relationship between hatching time and temperature is similar across their environmental range from the northeast and northwest Atlantic (Figure 5.7 and references therein). In compiling these studies together, the hatching time–temperature relationship can be examined over a larger temperature range than in the present study, and the relationship appears to be quadratic rather than linear. All but two data points from this compilation of studies follow this trend, one from Newfoundland (Pryor and Brown, 1998) and one from the Scottish west coast (Hall *et al.*, 2004), both of which hatch out in less time than expected for that temperature. However, given that these studies were not standardised it is surprising that in general, there is such a similar temperature–hatch time relationship. The great variability in temperatures across the thermal range implies that incubation time is highly variable between populations. Eggs in Polar Regions, such as the northern Labrador Sea, develop in the surface layer at temperatures of -1 to 0 ºC and take 37 to 43 days to develop. In more temperate ecosystems, cod from the Shetland Isles (classed as temperate water cod) spawn at temperatures of 6.3 to 8.9 °C (min. and max. of February and March Scalloway temperatures from tagged fish, derived from Neat *et al.*, 2006) and thus would take between 12 and 20 days to develop, similar to other reports from the North Sea and the Baltic.

This and other studies have demonstrated the high plasticity in development of cod eggs, in that they are still able to hatch out at temperatures far higher than the norm for winter to spring temperatures. Eggs from the Shetland region would normally hatch at temperatures of between 6 and 9 °C, yet they are still able to hatch at temperatures of almost 11 °C. Thompson and Riley (1981) support this finding, and report that southern North Sea cod will hatch out between 1.5 and 12 °C, below which they do not hatch and above which they develop abnormally. Cod larvae from the northwest Atlantic are reported to hatch out at sub-zero temperatures (Pepin *et al.*, 1997) but it is likely that these populations are adapted to a different range of temperatures.

4.2   Larval size

Larvae were found to hatch at a larger size at lower temperatures and larger larvae hatched after a longer incubation time. Similar findings have been reported in studies of cod from Newfoundland (Pryor and Brown, 1998) and Nova Scotia (Peterson *et al.*, 2004). A plausible explanation for this is that in a longer incubation period, at lower temperatures, the larva is able to absorb more of the nutrients from the egg prior to hatching (Pryor and Brown, 1998). Larvae from the present study hatched out larger than that reported in other studies. However, not all studies have found such a response. A study on Newfoundland cod at temperatures between -1 and 4 °C found the opposite trend, however, in which larvae hatched out larger at higher temperatures (Pepin *et al.*, 1997). It was suggested, that this increase in length with temperature might be on the ascending limb of a dome-shaped relationship with temperature, as Miller *et al.* (1995) found the reverse trend at higher temperatures in cod from the same region. Alternatively there may be local differences in the relationship between incubation temperature and size at hatching. There is little pattern in the length of larvae hatched out with the environmental temperature the fish experience in the wild. Studies have suggested that a larger body size is advantageous in cooler environments (Atkinson, 1994; Neat *et al.*, 1995; Pepin, 1991). However, without controlling for egg size, this cannot be determined, since egg size has also been found to vary with environmental temperature (Ware and Lambert, 1985; Miller *et al.*, 1995). Larvae hatched out in the present study were at the larger end of the spectrum of sizes found in other studies (4.3 mm at 10.65 ºC and 4.78 mm at 6.57 ºC) and similar in size to those from Newfoundland (Zhao *et al.*, 2003) (between 4.3 mm at 8.3 ºC and 5.1 mm at 6.6 ºC).

However, other studies from the northwestern Atlantic report larvae as small as 2.9 mm at temperatures of -1 ºC (Pepin *et al.*, 1997).

4.3   Metabolic rate

Some authors suggest that the decrease in hatching time with temperature could be due to a greater metabolic rate at higher temperatures (Zhao *et al.*, 2003). The finding that cardiac rate increases proportionally ($Q_{10} = 1.98$) relative to temperature, suggests this might be the case. According to other studies, cardiac rate (Q_{10}) is exactly as expected for cod irrespective of age (Claireaux *et al.*, 1995; Keen and Farrell, 1994) and for other species (Armstrong, 1986; Barrioneuvo *et al.*, 1999). However, further work is required to calibrate cardiac rate to metabolic rate. Interestingly, there was an effect of batch on cardiac rate, which suggests that there may be variation between families, although there was no pattern to the variability (such as earlier batches having a different cardiac rate relative to later ones). One suggestion for this is that families differ in frequency of haemoglobin genotype, as metabolic rate has been related to haemoglobin genotype (Weber, 1990). A potentially confounding factor in measuring cardiac rate is disturbance. Larvae have been found to increase their cardiac rate during stress and with noise disturbance (Simpson *et al.*, 2005), although these possibilities were reduced by minimizing handling and carrying out the measurements in a silent room.

4.4   Survival

Contrary to other studies (Thompson and Riley, 1981; Pepin, 1990; Geffen *et al.*, 2006), results from this study did not demonstrate an effect of temperature on survival. It may be that the temperature range studied was too narrow to affect survival, although higher temperatures were found to increase severity of spinal malformations (Fitzsimmons and Perutz, 2006; Appendix 3). Thompson and Riley (1981) found that greater mortality occurred above and below the temperature range of this study. Also variation in survival between batches may have masked the effect of temperature. Differences in mortality rates between batches were up to 65 %, far higher than that between different temperatures (up to 35 %) and higher than that reported in other studies in which an effect of temperature on survival has been found. For example, in cod eggs from the Irish Sea a positive effect of temperature on mortality was found but mortality was far

lower than in the present study, less than 20 % (Geffen *et al.*, 2006). The variation in batch survival in the present study and between other studies indicates that females may lay eggs of varying quality, in agreement with Geffen *et al.* (2006). Ouellet *et al.* (2001) report high mortality at hatching in batches from females with a particularly low condition, which could potentially explain the low survival rate in one of the batches in this study. A further cause of mortality, which may also account for the discrepancies between studies, may have been handling. Handling the larvae has been found to increase mortality (Dannevig and Dannevig, 1950; Nordeng and Bratland, 1971; Thompson and Riley, 1981) and a similar response may exist in the eggs. Handling was minimized and was equal for each egg. However, other studies have reduced handling further by merely sampling batches of eggs without replacement (Dannevig, 1895). The ability to follow individuals, as in this study, provided a basis for a more powerful analysis, at the expense of increasing levels of disturbance.

4.5 Maternal effects

Maternal effects could not be unambiguously identified when using eggs from naturally spawning cod. Since we selected eggs of almost identical size for each batch, it is likely that all eggs allocated to a batch were from the same female. However, this is not certain, as it is possible that two or more fish could have spawned simultaneously and produced similar sized eggs. It is also possible that certain batches were from the same female, as one female could have spawned twice or more in a 9 day period. Had the cod been artificially fertilised, maternal and paternal effects could have been controlled. However, since the broodstock were all of the same age and similar spawning condition and had been maintained under identical conditions, it is likely that the females produced eggs of a similar quality. Indeed, the maximum mean difference in egg size between batches was only 0.094 mm (6.05 %). While studies have reported that initial egg size does affect larval length and that larval size can affect fitness of the early stages post-hatch (Marteinsdottir and Steinarsson, 1998), the link between maternal effects and larval fitness has been demonstrated through egg size rather than the actual female, and thus by accounting for egg size, it may be possible to control for maternal effects indirectly. However, this might not be the case if egg quality varies with females, which was not measured.

The importance of initial egg diameter, as found in this study, has been reported in many other studies (Knutsen and Tilseth, 1985; Miller *et al.*, 1988; Trippel, 1998, Zhao *et al.*, 2003). However, in agreement with Pepin *et al.*, (1995), it accounts for a lesser proportion of the variance than temperature. The dry matter of an egg is composed of proteins, lipids and carbohydrates and provides nutrition for the developing embryo and larva several days post-hatching (Brooks *et al.*, 1997). Therefore an egg of a greater dry weight would potentially be able to produce a larger larva than a smaller egg could. Egg size has been positively related to indicators of larval fitness, such as development of the swimbladder, feeding success and larval growth rate 15 days post-hatch (Marteinsdottir and Steinarsson, 1998). There are numerous fitness advantages of a higher growth rate and thereby a larger size at this early stage, such as predator avoidance both from cannibalistic conspecifics and other species and being able to catch and consume a wider range of prey sizes (Marteinsdottir and Steinarsson, 1998).

4.6 Climate change

The North Sea and Scottish west coast has already been rising in temperature (ICES, 2006) and annual sea surface temperatures in the North Sea are predicted to rise by a further 1 to 2.5 °C in the next 50 years (Clark *et al.*, 2003). Results from the present study have shown that a rise in temperature of 2 °C during spawning time would not exceed the thermal tolerance for cod egg development. No effect of temperature on survival was found under the range of experimental temperatures. However, there might be an increased prevalence of more severe larval deformities being hatched (Fitzsimmons and Perutz, 2006), which are unlikely to survive in the wild. The decrease in incubation time by 2 or 3 days with a 2 °C rise in temperature and the subsequent faster larval development, as has been demonstrated in laboratory experiments (Pepin *et al.*, 1991; Otterlei *et al.*, 1999), might actually increase survival in the wild. The stage duration hypothesis suggests that the likelihood of survival would be greater because the time to transit stages that are the most vulnerable to predators is reduced (Cushing, 1990).

However, the shorter time taken to hatch and the decrease in hatch size, by approximately 0.3 mm (6.3 %) with a 2 °C rise, may have consequences in the match with suitably sized prey. The larvae must feed very shortly after their yolk sac is

exhausted (Yin and Blaxter, 1987; Miller *et al.*, 1988; FSBI, 2007), 7 to 9 days post-hatching at a temperature of 7 °C (Marteinsdottir and Steinarsson, 1988). Moreover, they consume a specific size and species of food in the North Sea during the larval and early juvenile stages, calanoid copepods and euphausiids (Beaugrand *et al.*, 2003). If cod larvae hatch out smaller they may not be able to find sufficient food of the right size. Further adding to the potential for a mismatch in larval hatch time and prey availability is the changing plankton composition in the North Sea, which has been correlated to rising sea surface temperatures and an associated decrease in cod recruitment (Beaugrand *et al.*, 2003). Newly hatched cod in the North Sea would normally feed on the copepod, *Calanus finmarchicus*, which appear in the spring. In the summer, as early juveniles, they would feed mainly on euphausiids (Beaugrand *et al.*, 2003; FSBI, 2007). Since the mid-1980s, however, there has been a trend for a decrease in abundance of the larger bodied *Calanus finmarchicus* and an increase in the smaller *C. helgolandicus*. The latter species occurs too late, in the summer months, at a time when the early juveniles would normally be feeding off the euphausiids. Euphausiid numbers have also declined over the same period (Beaugrand *et al.*, 2003). In addition to this mismatch in food supply, a lower food intake also decreases the optimal temperature for growth due to the greater metabolic demands and energy costs associated with higher temperatures, thus exacerbating the impacts on larval recruitment success (Otterlei *et al.*, 1999; FSBI, 2007).

5   CONCLUSION

By using a controlled laboratory approach, following individual eggs through to larval hatch, this study revealed detailed thermal responses on the egg development and the trade-offs with larval fitness in cod from the northern North Sea. The study demonstrates that temperature decreases incubation time and increases cardiac rate. A lower temperature results in a larger larvae for a given egg size. A larger egg also results in a larger larva but has less of an effect than temperature. The importance of batch (and possibly family) is also evident in this study and can result in differences in egg size, cardiac rate, incubation time and survival. The relationship between cardiac rate and temperature and the differences in cardiac rate with batch have not been

reported previously in cod embryos and open up new avenues for potentially exploring how metabolic rate affects these interactions.

Comparison with other published studies using a similar methodology gives an indication of potential population variability in some aspects such as the variability of the thermal response on size at hatch, but also the lack of geographic differences in others. The similarity in the relationship between incubation time and temperature in cod from areas across their geographic range suggests that this aspect of the development is merely a function of temperature driving metabolic rate and there is no support for differing genetic effects between cod from differing areas. However, common-garden experiments are required to rigorously investigate this spatial diversity and to test for adaptive responses between populations. Variability due to geographic location demonstrates the need to examine these temperature patterns on all populations of interest. The information collected may be of value to the growing cod aquaculture industry in Shetland, in which such knowledge is required to optimize conditions for larval development. In the open sea, eggs would be subject to a range of interacting factors, such as predation, wave disturbance, pollutants and hypoxia in addition to temperature. Further studies are needed both to understand population variability in response to temperature and also to other such factors eggs would be exposed to in the wild. Such an understanding of present population variability is vital if we are to predict future changes in response to climate change.

CHAPTER 6

Discussion

1   Overview

The optimal life history strategy for an individual is a trade-off between traits such as growth, body condition and reproductive investment and between investment in current and future years. If these traits depend on environmental and social conditions, such as temperature, food availability and competition, then we might expect to see a high variation in life history traits across the range in which a species occurs. Atlantic cod is a good model system to explore the covariance between life history and environmental influences because of its broad geographic range and complex spatial population structuring.

The overall aims of this thesis were to examine whether Atlantic cod from geographically separated localities form distinct populations based on differences in their life history traits and thermal responses. A common environment method was selected to assess population divergence in cod from around the British Isles, an area that exhibits considerable variation in thermal regime. In an attempt to capture the full extent of life history variability, studies were made on the juvenile and adult growth rate, juvenile behaviour, adult condition and onset of maturation and egg development. While spatially significant differences have previously been found in the life history traits of cod in the field (Brander, 1995; Yoneda and Wright, 2004), studies of phenogeography are limited because the genetic contribution to the phenotype may be masked by environmental effects on the phenotype. By using a common environment method, however, it is possible to partition the phenotype into its environmental and genetic components (Purchase and Brown, 2000; Salvanes *et al.*, 2004; Marcil *et al.*, 2006a and b; Hutchings *et al.*, 2007). In this chapter I will assess firstly the overall evidence for population differentiation and thermal plasticity and then discuss the evidence for adaptation and its implication for conservation in the face of climate change.

2   Population differentiation

Traditionally broadcast spawning and widely distributed species such as cod were thought to have high dispersal capabilities and weak population structuring (Lage *et al.*, 2004). However, in recent years, a range of techniques including biophysical modelling, studies on migration and genetic variation in neutral markers, suggest that cod has finer population scale structuring than previously indicated by past genetic studies (Child, 1988). The present study provides new evidence for population differentiation between cod from the three study areas; St Andrews Bay on the Scottish east coast, the Clyde Sea on the Scottish west coast and Lowestoft in the southern North Sea. Significant differences in growth rates, body condition, reproductive parameters and behaviour were found between wild-caught cod from these areas.

As juveniles and adults, the Clyde Sea cod demonstrated higher growth rates than those from St Andrews Bay. As adults, the Lowestoft cod had a higher growth rate than cod from St Andrews Bay. The higher growth rate of the Clyde Sea cod may be due to a tendency for them to eat more than those from St Andrews Bay, as was inferred by the Clyde Sea cod being more successful in competing for food than those from St Andrews Bay (Chapter 2). This is similar to findings of Norwegian cod in which faster growing Northeast Arctic cod were found to have greater success in competition for food in a group situation than had slower growing Norwegian coastal cod (Salvanes *et al.*, 2004). In the present study, population differences were also found in levels of aggression. The slower growing St Andrews Bay cod were found to be more aggressive than those from the Clyde Sea. This could explain the suppression in growth rate by over 50 % in the cod from the Clyde Sea, when the two groups were reared together.

Beyond the juvenile stage, sex differences in reproductive potential became apparent. In the females, the cod from St Andrews Bay possessed a higher condition factor (Fulton's K) than those from the Clyde Sea, but had a marginally lower fecundity. This is perhaps indicative of a trade-off between condition and fecundity, resulting in a greater allocation of liver reserves being put into reproduction in the Clyde Sea females. Unlike the variation found in the growth rate, there was no difference in fecundity relative to weight between the two North Sea populations. The Lowestoft cod exhibited higher body weights and thus higher fecundities, but this fecundity was largely a result of a

higher growth rate in the Lowestoft cod. Similarly, males from St Andrews Bay also had a higher liver condition than both the Clyde Sea cod and those from Lowestoft, and exhibited a higher testis weight relative to their size. The relatively larger livers may be related to higher lipid reserves that enable the St Andrews Bay males to allocate more energy to reproduction.

Considering the effect of haemoglobin genotype on behaviour, it was perhaps surprising that no such effect was found on the growth or the reproductive parameters, especially because the effect of genotype on behaviour was found to override the influence of population. In agreement with Salvanes and Hart (2000), the HbI-2*2 genotype were found to be more competitive in the present study, but in terms of aggression rather than feeding as they had found. This greater level of aggression may restrict feeding in less aggressive individuals. Therefore growth differences amongst genotypes may be an indirect effect of the behavioural differences between them. Alternatively, effects of haemoglobin genotype might only be apparent under certain thermal or environmental conditions, an area for future investigation.

3   Plasticity of response to temperature

In the marine environment, temperature is a driver behind many physiological processes of poikilotherms (Brett, 1979; Brander, 1995). Phenotypic plasticity to temperature was demonstrated in a number of traits, but the thermal responses at the egg stage were particularly strong. Maternal effects could not be fully accounted for in this study, but the results were still consistent with other studies; hatching time decreasing with temperature by almost half between 7 and 11 °C. Cardiac rate increased proportionately to temperature. Larvae also hatched out larger for a given egg size and took longer to incubate at lower temperatures, in agreement with findings of Pryor and Brown (1998) and Peterson *et al.* (2004).

A parabolic relationship between temperature and growth rate has often been reported (Jobling, 1988; Imsland *et al.*, 1996; Jonassen *et al.*, 1999; Peck *et al.*, 2003) and was apparent in the juvenile cod used in these studies over their normal range of temperatures (7 to 13 °C). Focussing in on a narrower thermal range between 8 and 12 °C, growth increased linearly, at a similar rate for cod from both St Andrews Bay and

the Clyde Sea. In the adults, an increase in growth was found at the higher temperature for the most part, although in the males the effect was limited to those from the Clyde Sea and the effect differed through time.

A marginal effect of temperature on liver condition was also detected. As adults, males in the ambient treatment had relatively larger livers than those in the warm treatment, possibly as a consequence of higher metabolic demands incurred by living in warmer water. The effect of temperature on maturity was found to act indirectly through an influence on growth. Temperature generally increased growth rate and thus body size, and larger body size led to a greater reproductive potential.

The influence of temperature on a given trait was often an indirect consequence of its effect on body size. For instance, larger larvae were found to hatch out from larger eggs. Analysis of individual growth trajectories demonstrated that those individuals that were larger initially, remained large throughout and were thus more likely to mature or have a greater reproductive potential. In the pairwise trials fish were size matched to eliminate body size effects within pairs but interestingly it was found that smaller sized pairs were more aggressive than larger pairs. To infer adaptation, it is necessary to demonstrate heritability in phenotypic traits, which has been shown in other studies in the growth rate of cod (Gjerde *et al.*, 2004).

A limitation throughout these experiments is that in using wild caught cod it was not possible to eliminate the potential for maternal or early environmental effects, as is discussed in Chapters 2, 3, 4 and 5. However, this concern is likely to be only relevant to the egg experiment (Chapter 5), as there have been very few studies that have demonstrated maternal effects in life history stages beyond the larval stage. A limitation identified in this study but relevant to all common environment / garden type techniques is that common environment conditions do not necessarily equate to common social conditions. Throughout this study within group and between replicate differences (Chapters 2, 3 and 4) were identified, in addition to the population differences in competitive abilities identified in Chapter 2. Therefore social conditions and behaviour need to be considered in interpretation of this kind of experiment.

4   Patterns of adaptive divergence

Many studies of cod have identified local adaptation, a process whereby performance of a given trait for an individual is optimal in its native habitat relative to conditions elsewhere (Purchase and Brown, 2000; Salvanes *et al.*, 2004; Marcil *et al.*, 2006a and b; Hutchings *et al.*, 2007). As discussed in Chapters 2, 3 and 4, the present studies may also have identified possible cases of adaptive divergence, although since this study only compared two populations at one time, it is not possible to determine whether the pattern is adaptive or merely due to chance. Adaptive divergence in cod has normally been demonstrated as countergradient, in which the genetic and environmental effects work in opposition to each other and create a similar phenotype over a gradation of environmental conditions. Examples include juvenile (Salvanes *et al.*, 2004) and larval growth rate (Purchase and Brown, 2000; Hutchings *et al.*, 2007). Growth patterns in the present studies were consistent with cogradient variation, in which the genetic and the environmental variation act in the same direction to enhance the effect on the phenotype. Those individuals from warmer environments (Lowestoft and the Clyde Sea) displayed higher growth rates than those from the cooler environment (St Andrews Bay). Although Lowestoft and the Clyde Sea cod were not compared directly, the difference in growth between Lowestoft and St Andrews Bay cod is greater than that between the Clyde Sea and St Andrews Bay cod. This potentially greater intrinsic capacity of the Clyde Sea and the Lowestoft cod relative to those from St Andrews Bay cod agrees with findings of Otterlei *et al.* (1999) in which larval and juvenile growth rate of Norwegian cod also followed a cogradient trend.

Different patterns were apparent, however, with respect to liver condition and temperature for optimal growth. The St Andrews Bay cod from the cooler environment had a higher liver condition than those from the warmer environments. This pattern is also seen in the male gonad investment. Consistent with these results, Salvanes *et al.* (2004) also found a higher liver condition in cod from the cooler Northeast Arctic area than those from the warmer Norwegian coast. The finding of a difference in optimal temperature for growth (9 ˚C in the Clyde Sea cod and 9 to 13 ˚C in those from St Andrews Bay), reflecting different environmental conditions in the wild, is consistent with the theory that the genotype is most effective in conditions of its origin. Hutchings *et al.* (2007) also found evidence for divergence in reaction norms in larval growth and

survival of cod; warm-water adapted cod from the east coast of Canada were more sensitive to food availability, whereas those adapted to cold water were more sensitive to temperature changes. However, results in the present study pertaining to the difference in thermal optima and the patterns in liver condition should be viewed with caution as they were not replicated.

5   Selective forces behind population differentiation

While temperature does appear to be an important factor behind population differentiation, there may be a host of other interacting factors, both intrinsic and extrinsic shaping the variability in cod populations. The common finding of countergradient variation in growth rate among fish populations (Conover and Present, 1990; Conover et al., 1997; Conover, 1998) including cod (Purchase and Brown, 2000; Salvanes et al., 2004), may be in part due to a confounding environmental factor, such as photoperiod or season. Countergradient variation is often recorded across latitudinal or altitudinal gradients, in which growth rates are faster at higher latitudes to compensate for the shorter growing season (Conover and Schultz, 1995; Purchase and Brown, 2000; Salvanes et al., 2004). While there may have been similar latitudinal effects when comparing the Scottish populations with the population from the southern North Sea, the difference in latitude between the two Scottish populations is comparatively negligible, being of less than 1° of latitude.

The finding of possible genetic effects could either be a result of a suite of interacting genes, known as a polygenic effect, or may be linked to a single gene polymorphism, such as haemoglobin genotype. No differences in haemoglobin genotype frequency between populations were found (Appendix 2) and thus any effect should have been equal between populations, but there may have been effects within groups. Although there was no evidence for an effect of haemoglobin genotype on the growth or maturation, a significant effect was found on aggression within pairs. Those fish of the HbI-2*2 genotype were always more aggressive than other genotypes. Consequently, if cod of this genotype intimidated their companions and restricted their feeding, it may have accounted for the high variability in traits such as growth and fecundity within populations. For example, cod from Lowestoft soon developed bimodality in their size structure, more so than in the cod from St Andrews Bay or the Clyde Sea. This may

have resulted in the difference in the proportions of cod maturing, in that the majority of Clyde Sea and St Andrews Bay fish matured but almost 50 % of those from Lowestoft did not.

Results of the present study suggest that a combination of interacting factors are responsible for the observed phenotypic variation. Even though the experiments were conducted under controlled environment conditions, there may still be confounding factors, such as behaviour or slight differences in body size. Looking at the project as a whole, it would appear that haemoglobin genotype drives aggression, which could affect feeding and subsequently growth and maturation. Similarly, temperature may drive growth, which in turn may influence maturation. Thus in order to understand population-level effects, it is also necessary to consider the influence of the individual.

Cod from Lowestoft appear to be of a genotype that confers the highest gonadal investment at first time spawning. As they have the fastest growth rate, they could reach age of maturation earlier and being a larger size, they would have a higher reproductive output. The question is thus why all cod are not of this genotype. This study points towards a number of possible trade-offs between growth and condition and between growth and aspects of the behaviour. Fish from St Andrews Bay may be slower growers but appear to allocate more energy to condition than fish from the other two areas. Perhaps the need to store energy is greater in St Andrews Bay than in other areas. It was found that the males from St Andrews Bay have a higher GSI than cod from the other areas, which could be due to a higher allocation of energy to reproduction rather than growth. Studies have also shown that aggression can trade-off with growth. In the present study the Clyde Sea cod had a higher capacity for growth and a greater food consumption but were less aggressive than cod from St Andrews Bay. Therefore, the balance of traits exhibited by individuals from each population may be optimised in relation to local selection pressures and no one population is superior overall.

6   Barriers to gene flow

Supposing that there were no constraints acting upon adaptive divergence and all groups were adapted to the environmental conditions in their specific area, we might expect to find even greater differences between populations (Conover *et al.*, 2006). However,

gene flow works against adaptive divergence to smooth these differences (Foster and Endler, 1999). The extent of adaptive divergence is therefore the result of the strength of the selection differential working against geneflow. Cod can be a highly migratory species (Robichaud and Rose, 2004) and have a high capacity for dispersal as larvae, as simulations of larval transport have shown (Heath and Gallego, 1997). Yet despite this, there is evidence for residency linked to high larval retention (Gallego and Heath, in prep), local recruitment of settled juveniles (Wright *et al.*, 2006b; Gibb *et al.*, 2007) and limited adult movements (Neat *et al.*, 2006; Wright *et al.*, 2006a). A further line of study would be to examine the interplay between gene flow and local adaptation. This has been examined in three parapatrically residing populations of three-spined stickleback, *Gasterosteus aculeatus* L., for which direct measures of gene flow were required, using molecular genetic techniques in combination with common garden experiments (Hendry *et al.*, 2002; Conover *et al.*, 2006).

7 Effects of climate change

The physiological capacity of cod to cope with a sea temperature rise in the order of 1 to 2.5 °C, as is predicted to occur within the next 50 years, is partially dependent on the plasticity of cod to temperature and on their ability to adapt. Studies on the juvenile growth rate of the St Andrews Bay and Clyde Sea cod (Chapter 3), suggest that growth would increase with temperature between 8 and 12 °C. Therefore, for all but the summer months and early autumn when temperatures might exceed 12 °C, somatic growth rate would increase with temperature. Similarly, in the adults of the St Andrews Bay and Clyde Sea populations, growth rate was comparatively higher in the treatment 2 °C above ambient year round. Since fecundity and GSI in males is related to body size, higher temperatures may indirectly increase fecundity and GSI through its effect on enhancing growth rate. The step-wise temperature experiment suggested that cod from St Andrews Bay may have higher thermal optima than those from the Clyde Sea and thus may be better able to cope with higher extremes of temperature, but before we can reliably predict the effect of a rise in sea temperature on local cod populations, further research is needed into the response of growth to the upper extremes of temperature.

A higher spawning stock biomass need not necessarily result in greater recruitment, as survival to hatching and larval development are also critically dependent on temperature. A rise in sea temperature of 2 °C might result in a decrease in egg incubation time by 2 or 3 days and size at hatch might decrease by approximately 0.3 mm. Temperature did not have an impact on survival at hatch (Chapter 5), but whether such changes affect subsequent larval survival is dependent on the timing and relative availability and size of their food supply (Beaugrand *et al.*, 2003; FSBI, 2007).

8 Improvements to design of common-garden (environment) experiments

Results of the present studies have highlighted a possible flaw in procedures in the design of common-garden experiments. A standard technique in such experimental designs is to raise stocks together in order to mitigate tank effects. However, this experiment has provided evidence that might suggest that by mixing stocks, the response of the trait in question may be mediated by behavioural interactions among the stocks. For example, in the study by Salvanes *et al.* (2004), the Norwegian coastal cod from 70 °N were found to have a higher growth rate than coastal cod from 60 °N. However, the two stocks were mixed and therefore the lower growth rate of the more southerly cod may have been partly a result of the greater competitive abilities of the Northeast Arctic cod, rather than an intrinsic capacity for higher growth rate.

Even though in Salvanes *et al.* (2004) the cod were fed to excess and all individuals should have had an opportunity to feed, the present studies have shown that unequal feeding occurs in a group situation, and individual feeding is a result of the behavioural dynamics within the tank (Chapter 2 and 4). A possible solution might be to maintain the cod at higher densities. Game Theory predicts that at very high densities there should be strong selection pressure for less aggressive individuals because the number of interactions with conspecifics makes it impossible for them to maintain territories (Ruzzante, 1994; Petersson and Järvi, 2000). In the light of these individual effects, the present study demonstrates the need to understand a combination of traits, not simply one in isolation. For example, the findings of behaviour may explain the variability in the growth patterns, while the growth preceding spawning may explain the variation in reproductive potential.

9   Implications for conservation

The present management units upon which the cod fishery is assessed do not adequately represent their population structure. Evidence for metapopulation structuring in cod (Wright *et al.*, 2006b) is contrary to the traditional assumptions of a unit "stock", defined as regionally interbreeding populations that are reproductively closed (Cushing, 1968). Assessments and forecasts using average weight-at-age of a stock may not reflect the potential variation in local population dynamics that are likely to result from the spatial variation in life history traits presented in this study. Currently cod in the study region are managed as a West Coast stock and a North Sea stock. The finding of a similar fecundity-weight relationship in the North Sea but a possible difference with that of the Scottish west coast does support the current management regime in which spawning stock biomass is managed separately in the two areas. However, current assessment methods do not consider the regional differences in growth, condition and GSI as has been revealed in this study. Information on temperature dependent growth variation and reproductive traits could be used to improve spatially resolved population models of the type described by Andrews *et al.* (2006).

Findings in this study and others demonstrate a capacity for phenotypic plasticity in cod in response to temperature and suggest that juveniles and adults may be able to withstand a temperature rise of 1 or 2 ˚C. However, climate change is not the only pressure. How cod respond to selection pressures induced by fisheries in addition to climate change remains unknown. Both higher temperatures and harvesting pressures would force selection in the same direction, both selecting for higher growth rates and earlier ages of maturation. The effect of earlier age at maturation on the ability to adapt is debatable. On the one hand, a faster generation time might speed up the rate of evolution and thus enable quick adaptation to new selection pressures. On the other, reduced longevity may cause local depletions and loss in genetic diversity and reduce the ability of cod to persist through environmental change. During periods of environmental change, such as global warming, by maintaining a proportion of long-lived individuals within the population, there is a greater chance they will survive through years of low recruitment success and reproduce successfully when conditions are suitable (FSBI, 2007). Sea temperature rise may result in a greater frequency of

years of higher than average sea temperatures and thus result in more years of low recruitment due to mismatches in timing of food availability (Beaugrand *et al.*, 2003).

By leaving a proportion of the spawning stocks unharvested, this may allow them to adapt to shifting temperatures over time, while maintaining their genetic diversity. Marine reserves and no-take zones are currently highly debated topics, but their benefit to the conservation of adaptive divergence and prevention of local depletions is underappreciated (Conover *et al.*, 2006). The present study suggests that cod from the Clyde Sea do exhibit local adaptation and studies on migration find that all of the spawning stock from the Clyde Sea have either returned or have stayed in their natal juvenile grounds (Wright *et al.*, 2006a and b; Gibb *et al.*, 2007). As such the closure on cod fishing in the Clyde dating from 2002 appears to be entirely appropriate from the viewpoint of conserving an evolutionary significant unit. The nature of metapopulations implies that although populations exist as semi-isolated reproductive units, there is some degree of straying and exchange of individuals between areas. Therefore a network of marine reserves may facilitate exchange between fished and non-fished areas and ultimately enhance stock productivity and maintain genetic diversity.

This study also has implications for the expanding aquaculture industry. At present costs are high but by adopting practices from studies such as these, it may be possible to make cultured cod production more viable. Choice of broodstock requires controlled environment experiments to find a stock with high growth potential for the ambient thermal and other environmental conditions, as was also suggested by Salvanes and Braithwaite (2006). Using a local broodstock would have the advantage that they are likely to be well-adapted to the ambient environmental conditions. Additionally, escapees that interbreed with the local wild population would not be a source of genetic pollution in mixing genotypes that would otherwise not come into contact. The suppression in growth rate reported in Chapter 2 as a result of mixing stocks, also suggests that different stocks should be separated to avoid competitive interactions that result in reduced growth rate, as has also been noted by Griffiths (2003).

Cod face an uncertain future in seas around Britain (FSBI, 2007), in an environment where climate-related shifts in plankton communities may lead to recruitment failure and where localised over-fishing may lead to declining populations and loss of genetic

diversity. Whilst it may not be possible to alter the declining recruitment brought on by climate change, it may be possible to maximise the chances of conserving those traits most related to fitness that might enable cod to adapt to shifting environmental conditions and persist through climate change.

10   CONCLUSION

By taking a common environment approach, results in this thesis provide experimental evidence for population structuring in Atlantic cod, which corroborates field based and genetic studies. Temperature has been demonstrated to have a strong effect on life history and it is likely that this has been a key factor underlying population divergence. While the experimental approach taken here does have drawbacks as have been discussed, the novel insight gained into the relative contributions of the effects of population and temperature in determining the phenotype, would only have been possible using such a method. Combining knowledge of population structure and thermal responses will be an important challenge for developing new approaches to sustainable management in the face of continued exploitation and climate change.

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APPENDIX 1

Thermal regime and geography of study areas

1 Thermal regimes

Field measurements of the thermal regimes for the three sample areas (St Andrews Bay, the Clyde Sea and Lowestoft, Chapter 1) were collated to provide a background against which to interpret the adaptive significance of any observed differences in the thermal responses of the fish (Figure A1.1 and A1.2). The Outer Firth of Forth was used as a proxy for St Andrews Bay, as it is the nearest area (less than 50 km away) comparable to St Andrews Bay for which data could be found over the same period as that given for the Clyde Sea. Bottom temperature data were derived from the period 1960 to 2005 based on long term averaged hydrographical data (Slesser and Turrell, 2005). The Outer Firth of Forth had a greater variability in bottom temperature than the Clyde Sea (range of 8.0 and 5.8 ºC respectively), with cooler winter temperatures on average than the Clyde Sea (minimum of 5.2 $\pm$ 0.2 and 6.5 $\pm$ 0.7 ºC respectively) and warmer summer temperatures (maximum of 13.2 $\pm$ 0.8 and 12.3 $\pm$ 0.8 ºC respectively) (Figure A1.1). Overall the Clyde Sea had higher mean annual bottom temperatures than the Outer Firth of Forth (9.1 and 8.8 ºC respectively). Sea surface temperatures (Figure A1.2) were derived from buoy data for the same period as the bottom temperatures for the Outer Firth of Forth and the Clyde Sea, and between 1966 and 1992 for the Southern North Sea. There was a similar pattern in temperature variability between the regions to that of the bottom temperature, but surface temperatures were slightly higher in summer and slightly lower in winter than bottom temperatures. Also the peak in surface temperature occurred one month earlier (in August) than the peak in bottom temperatures.
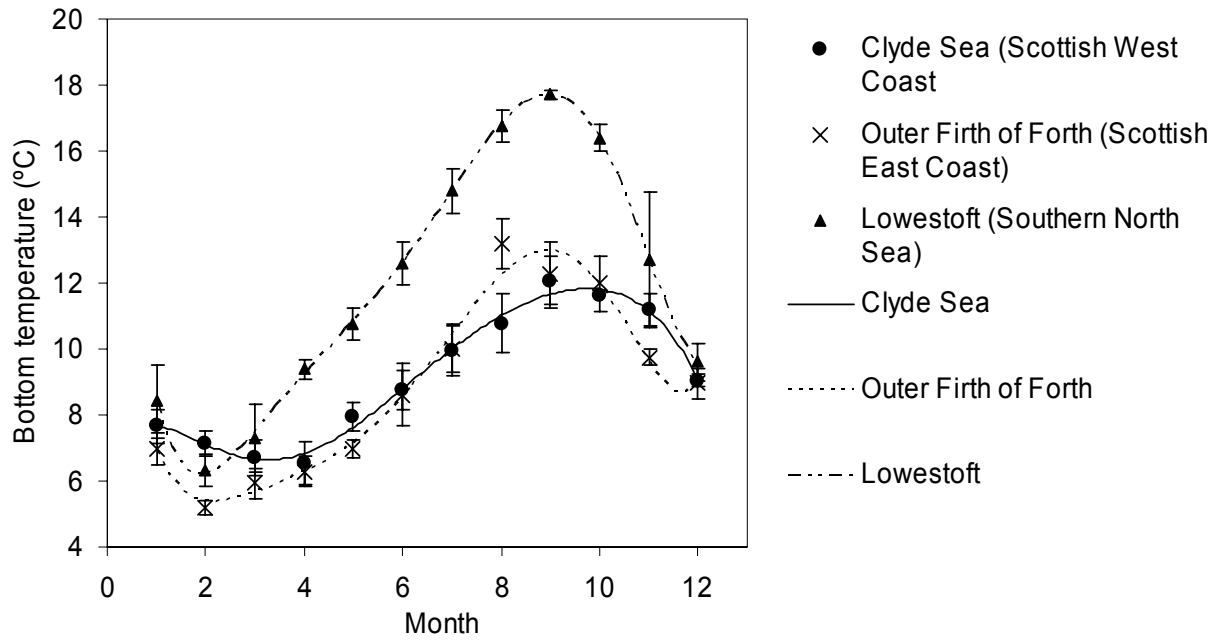
Figure A1.1. Bottom temperatures (mean and SD) from 30 to 40 metres averaged between 1960-2005 for the Outer Firth of Forth and the Clyde Sea, and data storage tag temperatures for Lowestoft cod.
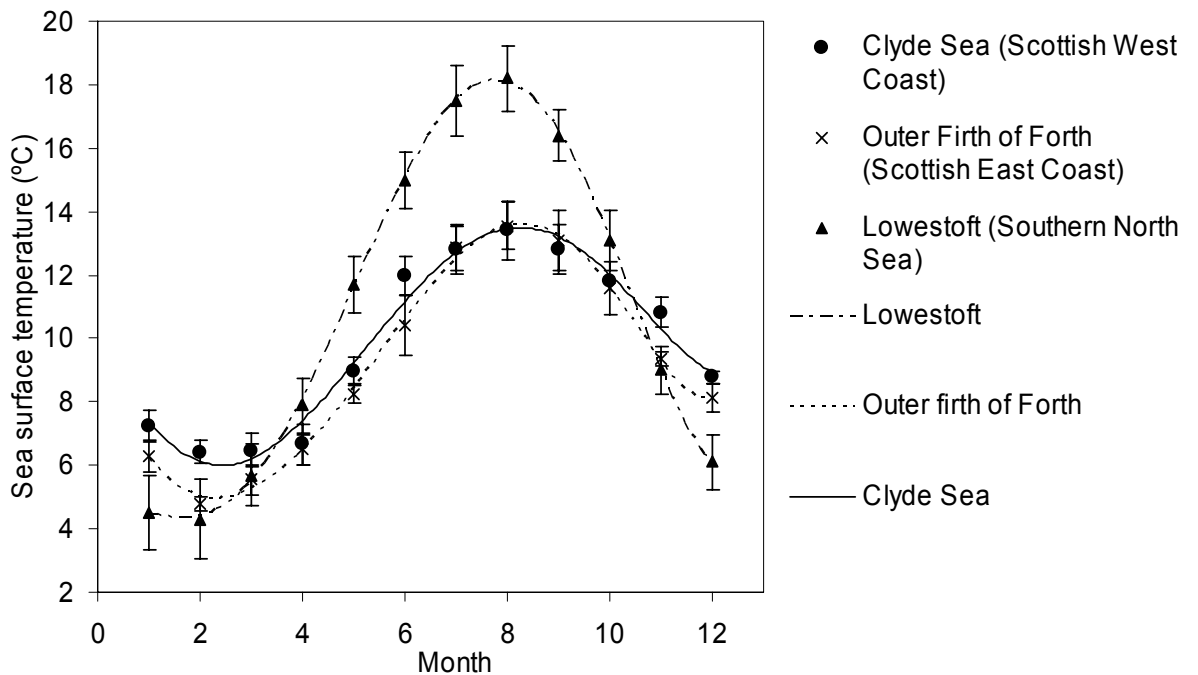
Figure A1.2. Sea surface temperatures (mean and SD) from < 10 metres averaged between 1960-2005 for the Outer Firth of Forth and the Clyde Sea and between 1966-1992 for Lowestoft.

2   Geography and habitat of regions

St Andrews Bay and the Clyde Sea were surveyed on SCUBA in June and July 2006 respectively, and habitat information collected from 4 sites within each (Figures 3 and 4), a minimum of 500 metres apart (5 transects, each of 12 m^2). These were selected to be at approximately 10 metres depth, two on soft substrate and two on hard. Juvenile 0-group cod (< 5 cm) were found on all sites surveyed, although some were found using baited cameras and baited fish traps rather than on SCUBA.

2.1   St Andrews Bay

St Andrews Bay is a shallow water bay, gradually sloping to a maximum depth of 20 metres, between the River Tay in the North (56°27'N 2°41'W) and the point at Fifeness in the south (56°17'N 2°35'W) (Figure A1.3). The seabed is composed predominantly of sand with the exception of the southerly coast of the bay, which is composed of rocky reef and boulders with areas of kelp forest (Figure A1.5). The seabed on the soft ground is composed of 100 % sand, but on the harder substrate there is a mixture of kelp forest (dense kelp plants, Laminariales) or kelp park (scattered kelp plants) (based on Hiscock, 1985), algae and faunal turf (sessile, animal dominated communities on hard substrata, Connor *et al.*, 1997).
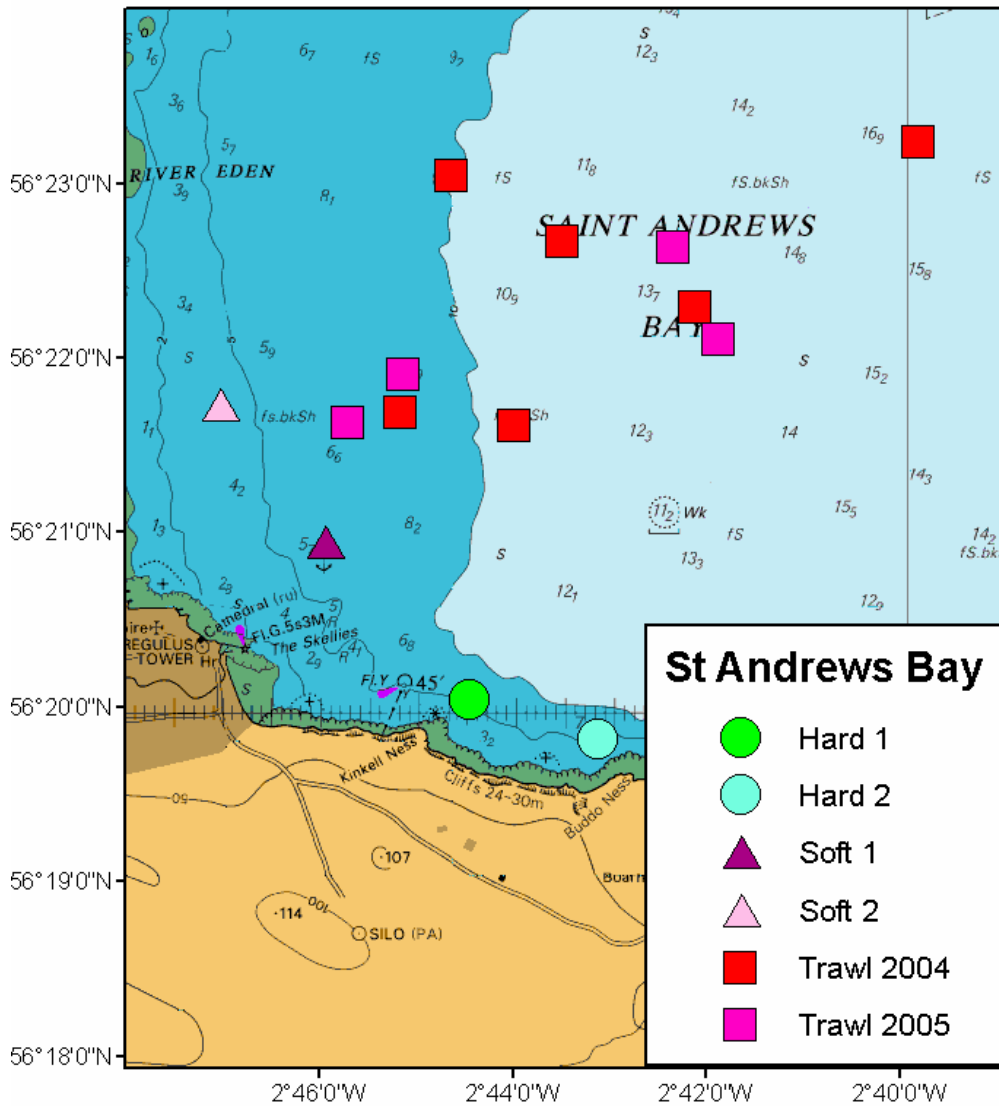
Figure A1.3. Chart of St Andrews Bay and sampling locations of the habitat survey on hard and soft substrate and trawl positions from where the cod were collected for the common environment experiments in 2004 and 2005.

2   Clyde Sea

The Clyde Sea area encompasses the inner and outer Firth and the sea lochs connecting into the Firth in the North (Figure A1.4). The areas sampled were on the eastern edge of the Firth, between 2 miles north of Ardrossan in the South (55º40.30'N 4º52.51'W) and the southerly tip of Great Cumbrae Island in the North (55º44.42'N 4º54.59'W). To the east of the mainland, north of Ardrossan the seabed slopes gradually to approximately 25 metres and then slopes steeply into the channel of the inner Firth of Clyde to over 100 metres. The area at the southern tip of Great Cumbrae Island is steeply sloping

bedrock and gravel to 32 metres. The substrate in this area of the Clyde Sea is more varied than that of St Andrews Bay (Figure A1.5). The softer ground is composed of a mixture of small boulders, pebbles, sand and mud, and the harder ground composed of bedrock, larger boulders, cobbles and occasionally sand. The seabed cover is composed of kelp park, algae, animal turf and sediment. The softer ground contained a higher proportion of sediment and the harder ground a greater proportion of animal turf and kelp.
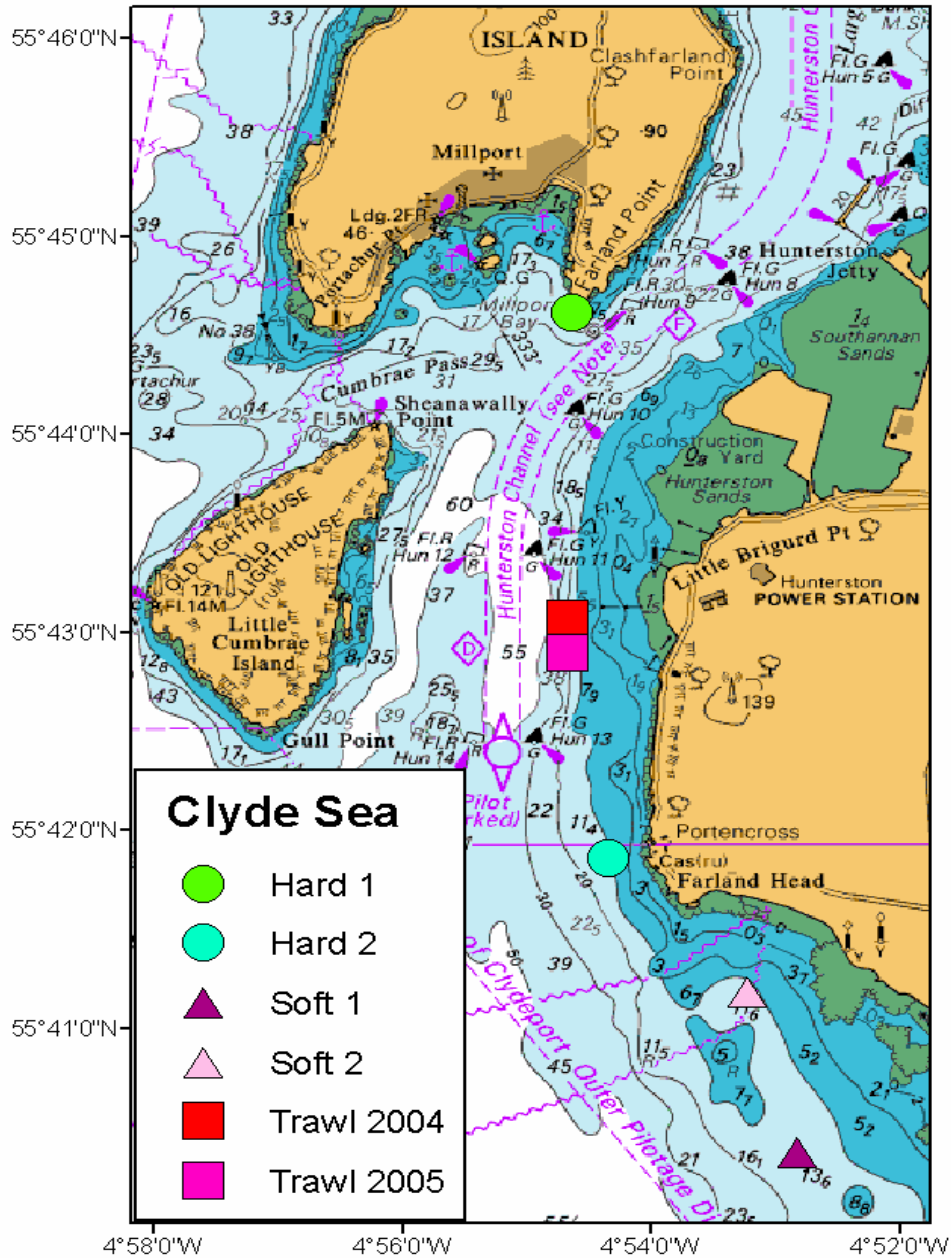
Figure A14. Chart of Clyde Sea, sampling locations of habitat survey on hard and soft substrate and trawl locations in 2004 and 2005 for common environment experiments.
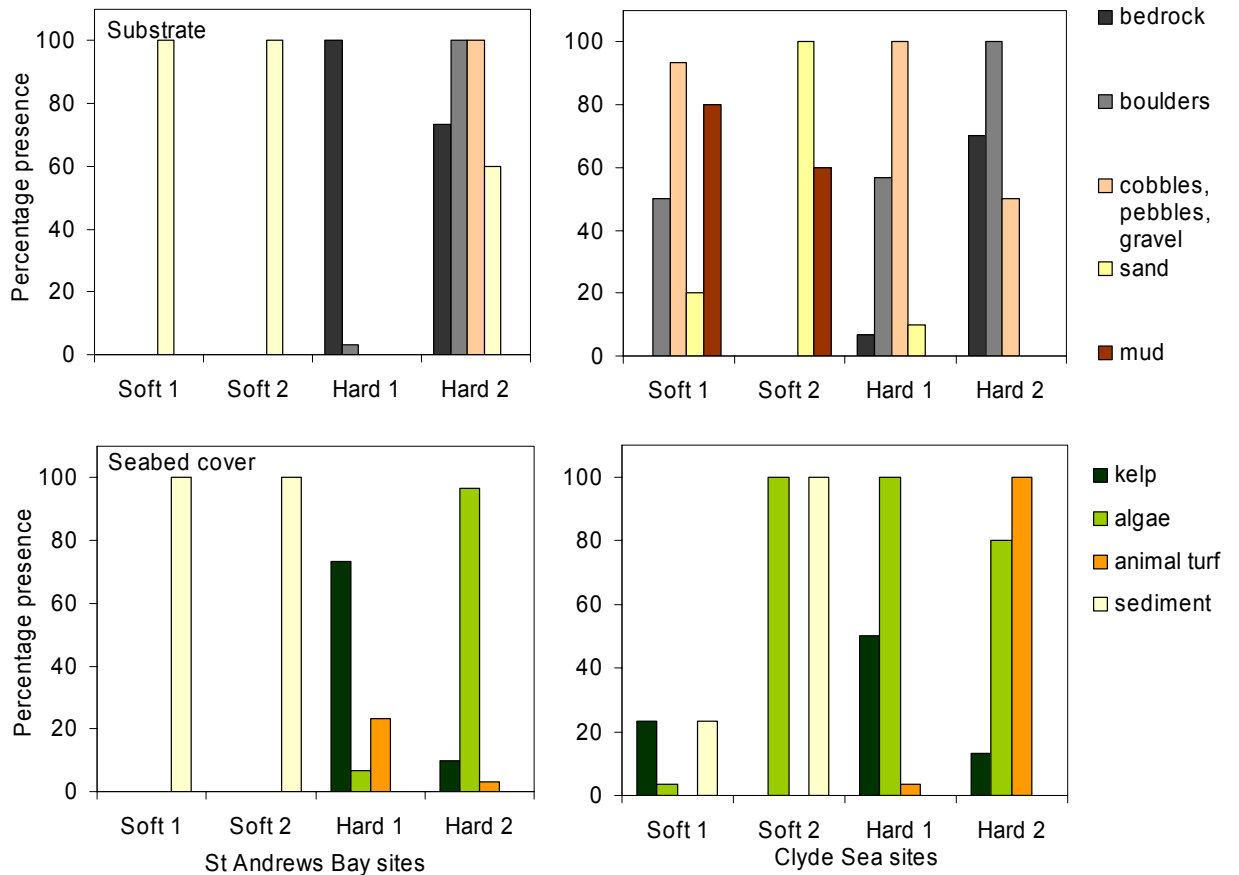
Figure A15. Composition of habitats, substrate and seabed cover, at four sampling sites in each region. Boulders are classified as rocks greater than 30 cm diameter, cobbles (< 30 cm), pebbles (5 – 30 cm). Animal turf refers to sessile invertebrates.

2.3 Lowestoft

No data was collected for this region but based on UK Admiralty Chart (1543), the seabed around Lowestoft, from which the cod were obtained, is an area of gently sloping sand that reaches a depth of approximately 15 metres at 3 nautical miles from the coast (Figure A1.6).

Figure A1.6. Chart of Lowestoft area and position of trawl in 2006 for collection of cod for common environment experiment.

APPENDIX 2

Distribution of haemoglobin genotype frequencies in Atlantic cod from the British Isles

1   INTRODUCTION

The haemoglobin polymorphism in Atlantic cod has been recognised since 1961 (Sick, 1961). Agar gel electrophoresis can reveal the three haemoglobin genotypes; the homozygotes (HbI-1*1 or HbI-2*2) or the heterozygote (HbI-1*2). Such genetic polymorphisms in fishes are thought to enable adaptation to environmental changes, such as temperature or salinity. The functional significance of the haemoglobin polymorphism has received much attention over recent decades. It has been demonstrated that the oxygen affinity of the HbI^2 allele is higher at lower temperatures (less than 10 ºC) (Karpov and Novikov, 1981; Brix *et al.*, 1998; McFarland, 1998), while the HbI^1 allele has a higher affinity for oxygen at temperatures greater than 14 ºC (Karpov and Novikov, 1981; Brix *et al.*, 1998). The optimal temperature for the heterozygote is thought to be between that of the two homozygotes (Karpov and Novikov, 1981). Affinity of haemoglobin for oxygen is essential in the transport of oxygenated blood from the gills to the respiring tissues. Studies of the geographical distribution of haemoglobin genotypes suggest that oxygen affinity is optimised for a given temperature. Frequencies of the HbI^1 allele across the species range vary clinally and are illustrated in Figure A2.1, taken from Petersen and Steffensen (2003), the trend being for higher frequencies in the warmer regions.
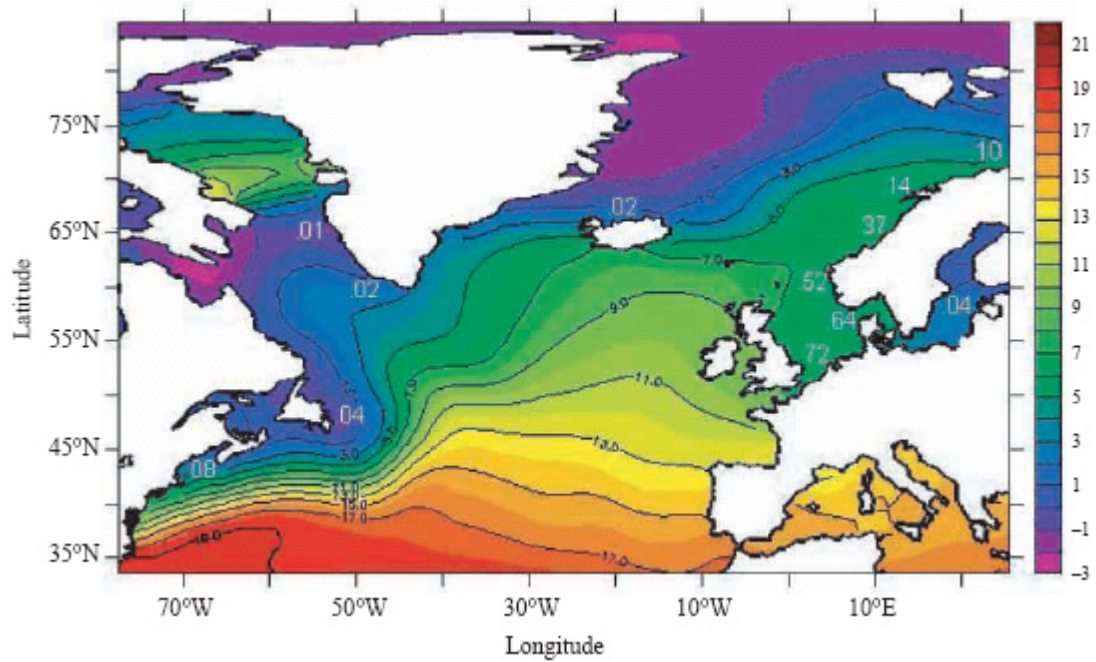
Figure A2.1. Frequency of the HbI[1] allele and spring sea surface temperatures over its geographical range across the northern Atlantic (Sick, 1965; Frydenberg *et al.*, 1965), taken from Petersen and Steffensen (2003).

Moreover, studies of thermal preference in a temperature graded tank revealed a similar trend: those of the HbI-2*2 genotype preferred to swim to the cooler end of the tank (8.2 ℃), while those of the HbI-1*1 genotype preferred the warmer end of the tank (15.4 ℃).

Studies examining the effect of haemoglobin genotype on growth are not consistent and are discussed in Chapter 3. While Mork *et al.* (1984) and Nævdal *et al.* (1992) found a relationship between growth rate and haemoglobin genotype in line with studies of oxygen affinity, subsequent studies have not found such clear evidence (Jordan *et al.*, 2006). Moreover, Imsland *et al.* (2004) found the reverse trend. Cod of the HbI-2*2 genotype had the highest growth rate overall, and had an optimum growth rate at temperatures between 13 and 16 ℃, while the optimal temperature of the HbI-1*1 genotype was at 7 ℃. Haemoglobin genotype has also been found to influence behaviour (Chapter 2) and Salvanes and Hart (2000) found that cod of the HbI-2*2 genotype were more successful in competition for food than other genotypes.

These previous studies led to the prediction that haemoglobin genotype may partly influence phenotypic and behavioural responses. For this reason haemoglobin genotype

was included as a factor in the analyses of growth rate, maturation and behaviour and are discussed in Chapters 2, 3 and 4. It was also predicted that haemoglobin genotype frequencies may vary regionally, as in studies of Frydenberg *et al.* (1965) and studies along the Norwegian coast (Petersen and Steffensen, 2003). The present study investigated the distribution of haemoglobin genotype and allele frequencies in the three study populations, under the prediction that higher frequencies of the HbI[1] allele would occur in cod from the warmer region.

2   METHODS

Cod of the 2005 year class from St Andrews Bay (n = 121) and the Clyde Sea (n = 65) and from the 2006 year class in cod from Lowestoft (n = 28) were sacrificed and screened for haemoglobin genotype according to the methods in Chapter 2, Figure 2.1.

3   RESULTS

Haemoglobin genotype frequencies from each area are given in Figure A2.2, Table A2.1 and allele frequencies in Table A2.2. These indicate an excess of heterozygotes in the Clyde Sea population (F_{IS} = -0.270, *P* = 0.0253) and hence departure from Hardy Weinberg equilibrium. However, there was no evidence that genotypes of cod from Lowestoft or St Andrews Bay differed from the Hardy Weinberg equilibrium. No difference in genotype (G-like test, *P* = 0.406, SE = 0.0092) or allele (*P* = 0.408, SE = 0.01048) frequencies were found between areas.

Table A2.1. Haemoglobin genotype numbers and frequencies in cod from the three study areas; the Clyde Sea, Lowestoft and St Andrews Bay.

| Area | Genotype numbers and frequencies | | | |
	HbI-1*1	HbI-1*2	HbI-2*2	Total
Clyde Sea	(5) 0.08	(39) 0.60	(21) 0.32	65
Lowestoft	(4) 0.14	(13) 0.46	(11) 0.39	28
St Andrews Bay	(29) 0.24	(49) 0.40	(43) 0.36	121
Mean	(12.7) 0.15	(33.7) 0.49	(25) 0.36	214
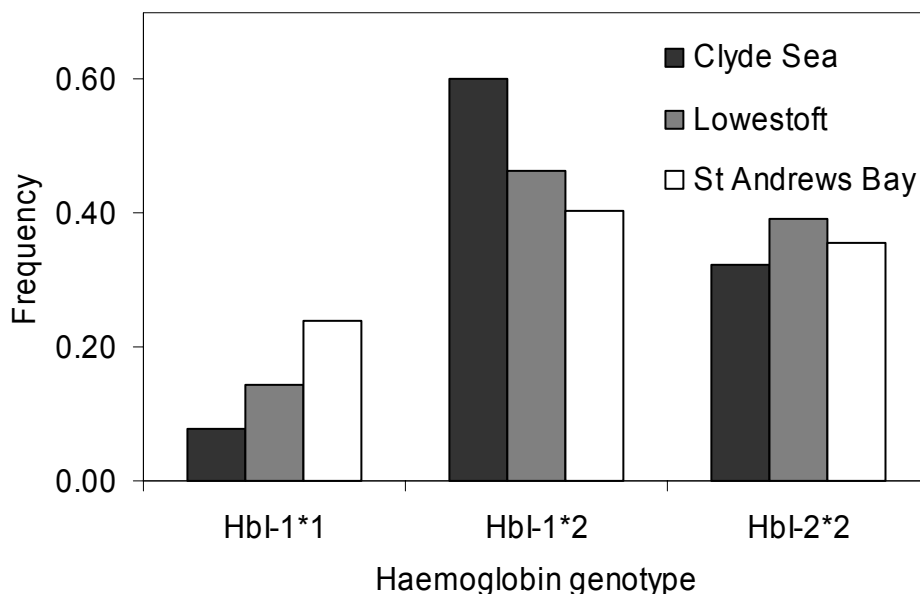
Figure A2.2. Haemoglobin genotype frequencies of cod from the three study areas.

Table A2.2. Allele frequencies of cod from the three study areas.

Area	Allele frequencies	
	HbI[1]	HbI[2]
Clyde Sea	0.38	0.62
Lowestoft	0.38	0.63
St Andrews Bay	0.44	0.56
Mean	0.4	0.6

3   DISCUSSION

Large-scale geographical studies of haemoglobin genotype frequencies have provided clear evidence for population differentiation by haemoglobin genotype along temperature gradients. For example, Petersen and Steffensen (2003) found higher frequencies of the HbI[1] allele in warmer areas. The present comparatively small-scale study over areas of an intermediate temperature, in terms of existence of clear genotype preference (< 8 or > 15 ºC), provides no such evidence for population differentiation.

Along the coast of Norway and into the southern North Sea, where a cline in haemoglobin genotype frequency has been found in relation to temperature (Petersen

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and Steffensen, 2003), the differences in temperature were of a greater magnitude than the temperature differences between areas reported in this study. Spring sea surface temperatures ranged from 3 ºC in northern Norway to 8 ºC in the southern North Sea. In contrast, the difference between spring sea surface temperatures in the present study areas is at most 1.9 ºC, although differences in summer temperatures would be greater, thus selection pressures between the three areas in relation to temperature would be comparatively lower. Also the range of sea temperatures described here is intermediate in terms of the temperature preference of the two homozygotes and thus there may be no strong selection for either homozygote.

Frequencies of the HbI[1] allele are of a similar order to that documented in the compilation of studies in Petersen and Steffensen (2003) for the northwest Atlantic (Figure A2.1). Interestingly, however, the HbI[1] allele frequencies observed here were considerably lower than that documented in Petersen and Steffensen (2003) in a nearby region of the southern North Sea (0.38 in the Lowestoft cod compared to 0.72 previously documented in the southern North Sea). This apparent decrease in the frequency of the HbI[1] allele since 1965 is not in accordance with suggestions of Petersen and Steffensen (2003), who predicted an increase in the HbI[1] allele in response to rising sea temperatures. The low sample numbers of cod from Lowestoft (n = 28), however, means this result should be interpreted with caution, but could indicate that selection pressures for haemoglobin genotype are not only limited to temperature. The temperature preference of genotypes has also been found to vary with oxygen concentration. For example, the HbI-1*1 genotype has a preference for a lower temperature in hypoxic conditions. Further samples should be taken to verify this potential decrease in allele frequency.

In conclusion the present study adds to the understanding of haemoglobin genotype frequency distribution in Atlantic cod and suggests no evidence for population differentiation between the study areas.

APPENDIX 3

Effects of egg incubation temperature on survival, prevalence and types of malformations in vertebral column of Atlantic Cod (*Gadus morhua*) larvae

S.D. Fitzsimmons[1]* and M. Perutz[2]

[1]*North Atlantic Fisheries College, Port Arthur, Scalloway, Shetland Isles, ZE1 0UN;*
[2]*FRS Marine Laboratory, PO Box 101, 375 Victoria Rd., Aberdeen, AB11 9DB.*

Abstract

Survival, prevalence and types of malformations in vertebral column of Atlantic cod (*Gadus morhua*) larvae were assessed at hatch for six different egg incubation temperatures from 6 to 11°C, which reflected the natural temperatures experienced during the spawning season in the Shetland Isles. There were no significant differences in mortality between eggs incubated at different temperatures. A total of 152 out of 249 hatched larvae (61%) exhibited one or more out of four main types of vertebral malformations - kyphosis, lordosis, scoliosis, and severe vertebral curvature. Mean prevalence of malformations ranged from 58% to 66% for different incubation temperatures, and from 51% to 78% for each group of eggs. There were no significant differences in sum of prevalence of malformations between different groups and temperatures, but significant difference between types of malformations at different temperatures. The prevalence of malformed larvae with severe vertebral curvature increased significantly as egg incubation temperature increased.

Introduction

Malformations of fish are prevalent in many species and are particularly important in species cultivated for commercial aquaculture (Fraser et al., 2004). Malformations can have serious economic implications for both hatchery operators and on-growers, and can lead to problems ranging from lowered performance to downgrading at harvest (Barahona-Fernades, 1982; Andrades et al., 1996). The prevalence and types of malformations have been shown to be specific to the hatchery rearing conditions, but there are also general malformations associated with rearing protocols in many hatcheries (Boglione et al., 2001). Use of rearing and feeding protocols of warm-water species has been adopted, however this may not be appropriate for gadoids and lead to future problems (low survival, poor growth, malformations).

Few studies have investigated causes and types of malformations in cold water marine species such as Atlantic cod (*Gadus morhua*) mainly because culturing these species on a large commercial scale has not been successfully achieved until recently (Brown et al., 2003). Olsen et al. (2004) suggested that up to 60% of hatchery reared cod in Norway had problems associated with malformations, and thus it is recognised as a major problem.

*Corresponding author's email: stuart.fitzsimmons@ghd.com.au

Malformations in other cultured species have been well documented in freshwater species such as salmon and trout (Kvellestad et al., 2000; Sadler et al., 2001), and warm-water marine species such as sea bass and sea-bream (Barahona-Fernades, 1982; Andrades et al., 1996; Afonso et al., 2000; Koumoundouros et al., 2002; Boglione et al., 2003), and barramundi (Fraser et al., 2004).

Different causes of malformations that can affect all stages from egg to adult for many different species have been suggested, including nutrition (Bell et al., 1996; Roy et al., 2002; Brown et al., 2003; Olsen et al., 2004), pollution (Muramoto, 1981; Kennedy et al., 2000), adverse temperatures (Ali and Lindsay, 1974; Weigand et al., 1989; Polo et al., 1991; Buckley et al., 2000), gas super-saturation (Chapman et al., 1988), and other diseases (Treasurer, 1992; Kent et al., 2004).

There is very limited research in this area for cultured Atlantic cod, therefore understanding the effects of initial rearing temperatures on cod larval quality may have important consequences in rearing procedures for commercial gadoid aquaculture. This study describes the effects of incubating eggs at temperatures normally occurring in winter and spring on initial survival, prevalence and types of malformations in vertebral column of larvae at hatch.

Materials and methods

Eggs were collected from local Shetland wild broodstock (8 females and 4 males) held in a 24m³ (5kg/m³) circular tank of 1.8m depths. Water at 6.0ºC ± 0.5, filtered through a 60μm was delivered to the tank at a constant flow of 60l/min giving a theoretical retention time of 6.6 hours. The broodstock were fed to satiation on sausages prepared from EWOS marine broodstock booster diet three times per week.

The cod spawned naturally between 2nd February and 28th April 2005. Viable fertilised eggs floated to the surface and were collected each morning using a custom-made 50μm mesh filter from the outflow of the surface drain of the tank. Six groups of eggs were collected on 27th, 28th February, 1st, 2nd, 5th and 7th March 2005.

After collection, the eggs were disinfected in peracetic acid (Vetroxide - Pharmaq) at a concentration of 4000ppm in seawater for 45 seconds before being transferred to sterile seawater. Each egg was pipetted onto a microscope slide for examination using a sawn off pipette together with 0.75ml of seawater before being transferred to well plates. Seawater was filtered through a 1μm filter and UV sterilised. Handling of eggs was minimised to avoid mechanical damage.

144 eggs were selected from each group that were divided equally between the six temperature treatments. Overall a total of 36 groups and a total of 864 eggs were incubated. The eggs were placed in 24 chamber well plates (volume = 3ml) with 1 egg per well. The well plates were put inside plastic boxes and submerged in controlled water baths (901 paxton chilled with ICES TAE-08 chiller for 6°C, Techne Junior TE-8J for 6.5°C, 8°C, 9°C, Griffin & George BJL-400-110F for 10°C, LMS Ltd 305 refrigerator for 11°C) inside the well plates. A temperature-logging device recorded temperature every 5 minutes (Lotek LTD1110) and every hour (Vemco mini-

logger). The six different temperatures obtained were 5.9°C ± 0.1, 6.6°C ± 0.1, 8.1°C ± 0.3, 9.3°C ± 0.1, 10.6°C ± 1.1, 11.0°C ± 0.3. Variation in temperature for 10°C was caused by initial fluctuations that affected only two groups, and this was corrected immediately.

Eggs were examined daily until one day post-hatch using a light, backlit microscope in a room kept at 6°C to assess developmental stage using Thompson and Riley's 1981 index. Malformations were assessed immediately at hatch only for larvae that were living (heartbeat observed). Malformations were classified as described by Boglione et al. (2003). One day post-hatch, the larvae were measured and photographed at a magnification of 3.2 using a digital camera (Nikon Coolpix 4500) under a light microscope (Zeiss 2000-C) to assess types and quantities of skeletal malformations.

Results for survival and prevalence for different types of malformations are expressed as means. Data expressed as percentages were arcine transformed before analysis. One-way ANOVA with Tukey's post-test was used to determine significant differences ($P<0.05$) between groups and temperatures. All statistical analysis was performed using PRISM (Graphpad, 1999).

Results
Survival
A total of 249 out of 864 larvae hatched (29%) for all groups and incubation temperatures. Mean survival varied from 21% to 35% for different temperatures, and 15% to 65% for different groups (Table 1). There were significant differences in survival between groups of larvae (ANOVA: df=5, F=3.37, $P<0.05$), but no significant differences between different incubation temperatures.

Malformations in vertebral column
The prevalence of malformations observed for each incubation temperature for all groups of larvae is shown in Table 1. A total of 152 larvae (61%) exhibited some type of malformation

Temperature (°C)	6	6.5	8	9	10	11
Eggs incubated (N)	144	144	144	144	144	144
Survival (at hatch) %	34	24	35	26	21	34
Larvae examined for malformations (N)	49	34	50	37	30	49
Normal %	41	38	36	43	34	42
Kyphosis %	27	15	28	19	23	22
Lordosis %	24	35	28	22	23	18
Scoliosis %	4	3	6	8	10	2
Kyphosis / lordosis complex %	4	6	0	0	0	0
Severe vertebral curvature %	0	3	2	8	10	16
Sum of vertebral malformations (%)	59	62	64	57	66	58

Table 1. Survival (%) and prevalence of types of vertebral malformations (%) of Atlantic cod larvae at hatch for different incubation temperatures.
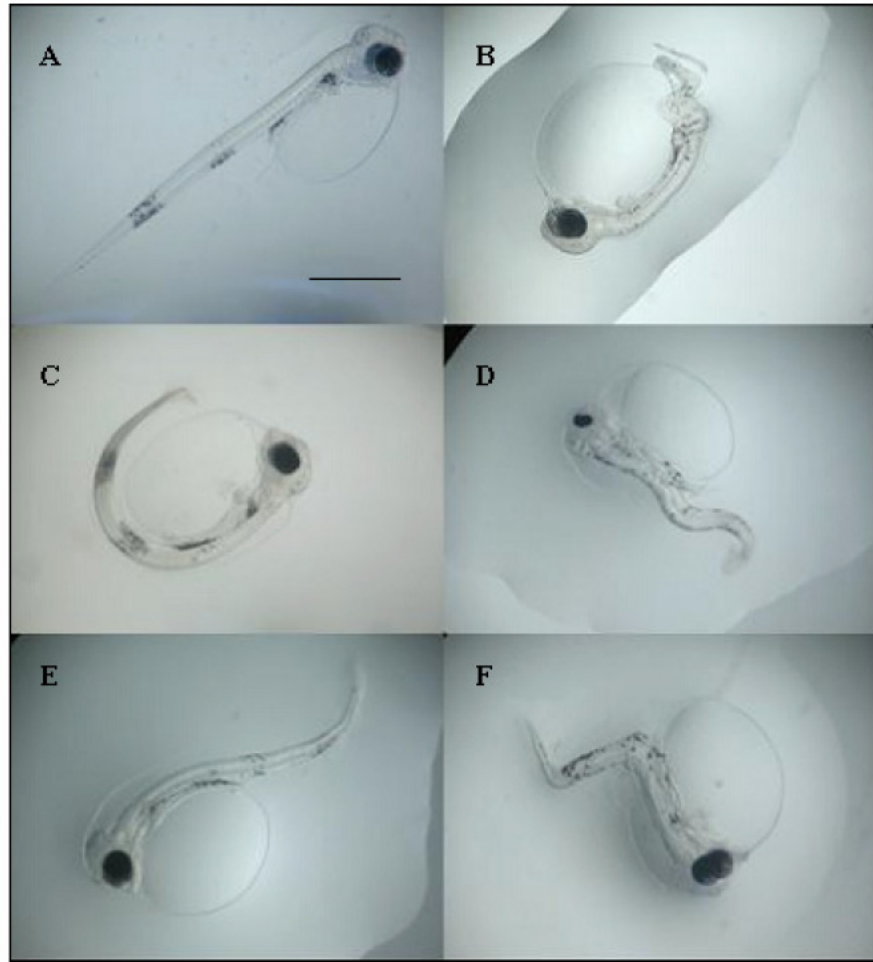
Figure 1. A – normal larvae without signs of vertebral column malformation; B – severe vertebral curvature; C – kyphosis; D – scoliosis; E – lordosis; F – lordosis / kyphosis complex. Scale bar 1mm shown in A.

at hatch. Prevalence of malformed larvae ranged from 57% to 66% for different incubation temperatures, and 51% to 77% for different groups of eggs. One-way ANOVA indicated that there were no significant differences between prevalence of malformations between groups of eggs and incubation temperature.

Types of malformations observed for all temperature treatments included kyphosis (V shape), lordosis (Λ shape), scoliosis (lateral malformation), kyphosis / lordosis complex, and larvae with severe vertebral curvature. Larvae with severe vertebral curvature resulting in shorter tails were considered as individuals exhibiting extreme curvature not shortened vertebral columns. The number of

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individuals having kyphosis, lordosis, scoliosis and kyphosis / lordosis complex did not differ significantly between incubation temperatures. However the prevalence of larvae with severe vertebral curvature with shortened tails increased significantly at higher temperatures (ANOVA: df=5, F=14, P<0.001). The most prevalent anomaly for all temperatures was larvae with malformations of the vertebral column in the vertical plane (kyphosis and lordosis).

Representative examples of types of malformed larvae observed at hatch are shown in Figure 1. The severity of malformation ranged from slight to complete curvature of the vertebral column. Several larvae showed a kyphosis / lordosis complex, where the vertebral column was malformed in several directions. All larvae with types of malformations observed were considered to be non-viable in terms of rearing for aquaculture purposes. Larvae with severe vertebral curvature were alive at hatch (heart beat observed) but showed severe impairment of movements compared to normal or slightly malformed larvae.

Discussion

Malformations in cultured fish, such as Atlantic cod, have a significant impact upon commercial aquaculture (Olsen et al., 2004). Further understanding of rearing protocols, such as incubation temperature, is necessary to reduce the effect of malformations on larval quality and maximise survival rate. Brown et al. (2003) and Thomson and Riley (1981) suggested that survival rates of eggs to hatch was relatively un-affected by incubation temperatures between 6 - 12°C, and cod eggs

developed abnormally and died at temperatures above 12°C. Results from these trials confirmed that although there were significant differences in survival rates between different groups of eggs, there were no significant differences in survival rate for the range of temperatures tested. The differing survival rates may be indicative of varying egg quality between different spawning female broodstock.

The prevalence of malformations in this study (over 50%) was comparable to results stated by Olsen et al. (2004) for reared cod. Four types of malformations were observed in larvae, among which kyphosis and lordosis were most prevalent. The majority of the malformed larvae were observed with only one type, but several were found with combinations of kyphosis and lordosis. Although some larvae displayed very slight lordosis or kyphosis, they potentially could cause problems during on growing. Interestingly though, larvae with severe vertebral curvature increased with increasing temperature, with eggs incubated at 11°C (close to upper thermal limit for survival) having the highest incidence of severely malformed larvae. This type of vertebral malformation was the only type significantly influenced by incubation temperature, and may suggest that although overall results showed no obvious effects of temperature on prevalence of vertebral malformations during egg development, there were subtle thermal influences on types of vertebral malformations at higher temperatures.

The high prevalence of malformations at hatch, regardless of incubation temperature has negative implications on commercial

production with regards to larval quality, and subsequent growth rates and production losses. Since the survival and prevalence of malformations was independent of incubation temperatures up to 11°C, other unknown factors in these trials were contributing to malformations observed at hatch (genetic, broodstock nutrition). This suggests that the range of different incubation temperatures tested could be used to incubate cod eggs for commercial purposes without significantly affecting survival and prevalence of malformations. However, these results also suggest that egg incubation temperature should be kept between 6 – 8°C to minimise the severity of malformations of cod larvae at hatch.

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